MANIPULATING PIG PRODUCTION

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SYMPOSIUM - Seasonal Infertility in the Pig

This symposium examines the main reasons suggested as causing or contributing to the complex jig-saw of seasonal infertility. Undoubtedly there is a temperature or photoperiod component, and the first two papers deal with these areas. The third paper reviews the role of a generalized stress response in causing seasonal infertility, and finally, the role of the boar, which is often neglected when looking for causes of seasonal infertility, is reviewed.

CONTRIBUTED PAPERS/POSTERS

NUTRITION

REVIEW

REVIEW

SYMPOSIUM - Comparison of Methodologies to Estimate Amino Acid Availabilities for Pigs

CONTRIBUTED PAPERS/POSTERS

NEW TECHNOLOGY AND GENETICS

REVIEW

Immunophysiology is the name given to the rapidly developing field of manipulating physiological processes by means of the immune system. Immunophysiology takes advantage of the flexibility and specificity of immune responses to modify the action of selected hormones. This review briefly covers aspects of animal production which may be enhanced by immunophysiological methods, with special reference to the pig industry and its problems.

REVIEW

Transgenic pigs have been produced by microinjection of single or 2-cell zygotes with a transgene construct comprising the porcine growth hormone. This paper reviews this preliminary research. The availability of transgenic pigs commercially will be dependent on developing transgenes whose level of expression is under the strict control of the producer. This degree of control has been achieved in laboratory studies with mice and the first litters of transgenic farm stock with these improved transgenes are awaited with considerable interest.

CONTRIBUTED PAPERS

HEALTH

SYMPOSIUM- The Control of Pre and Postweaning Diarrhoea in the Pig

Colibacillosis is a disease of intensificaton in both animals and humans. While the epidemiology of neo-natal colibacillosis is quite clear, the factors associated with postweaning colibacillosis are not. For instance, it is not known what factors turn a postweaning mortality rate of less than 1% into one of 10-25% almost overnight. This symposium presents a multidisciplinary approach to the problem of colibacillosis. Topics covered include epidemiology, pathogenesis and immunity of pre and postweaning diarrhoea and the effects of diet on postweaning diarrhoea. The paper on "Alternative approaches" represents a break away from the traditional approach to the problem and may be the type of lateral thinking needed to provide a solution.

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MANIPULATING PIG PRODUCTION

PREFACE

The idea for a conference on scientific aspects of pig production arose from informal discussions by scientists in Australia who expressed enthusiasm for an association that would draw together the many facets of pig research. Whilst Australia is well served with various local and international scientific societies, these societies cater for either specific disciplines across all species, or more broadly for all species. Researchers working with pigs have seen the need for a more specific society that would bring together those people whose interests are largely confined to the pig. Such a society will encourage a much more in-depth examination of research findings and problems within the pig industry than is currently possible.

The inaugural conference of the newly formed Australasian Pig Science Association has assembled speakers and delegates from a wide variety of scientific disciplines to provide an excellent forum for scientific and social interaction amongst participants. It is hoped that the conference and this resultant publication will provide an impetus for further research in important areas of pig production and ultimately, an improvement in overall pig productivity.

R. H. King
President-elect
A.P.S.A.

OBJECTIVE ASSESSMENT OF WELFARE IN THE PIG: CONTRIBUTIONS FROM PHYSIOLOGY AND BEHAVIOUR

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INTRODUCTION

The inherent problem in objectively assessing welfare from any standpoint is the inadequacy of objectively defining the abstract concept of welfare.When definitions commonly include "well-being", "suffering" or other subjective "feelings", the problem, in the final analysis, is impossible to solve. However, the lack of ability to define the problem does not remove it from being real and hence it is currently necessary to use a 'best estimate' approach. This approach requires measurement of parameters that are arguably indicators of welfare and it is generally accepted that the best indicators include health, behaviour, physiology and productivity. Numerous scientists have attempted to address the measurement of welfare using these criteria (see Sybesma,1981; Baxter et al.,1983; Smidt, 1983; Zayan,1985).

The changes associated with the stress response have been widely used as physiological indicators of welfare (Dantzer et al.,1983; Dantzer and Mormede, 1983a; Moberg,1985) due to the reasonable belief that if stress increases welfare decreases. However, the problem still remains, and has been frequently raised (eg. Dantzer et al., 1983), as to what level of stress places welfare at risk. A thorough understanding of the concept of stress makes a reasonable start to answering this question and studies with pigs allow some physiological limits to be suggested for acceptable and unacceptable welfare.

Behaviour was originally the major discipline contributing to welfare research. However, it still remains difficult to identify species specific behaviours and behavioural responses in test situations that indicate reduced welfare.

It is the aim of this paper to emphasize the contributions physiology and behaviour have made to the assessment of welfare in the pig and to indicate how an holistic approach has increased our understanding of the pig's welfare related responses. To achieve these aims, the paper will present an overview of some of the physiological and behavioural concepts used in welfare research, the contribution of stress and behaviour to the objective assessment of welfare, the degree of physiological change associated with changes in welfare and some problems using these measures to assess welfare.

CONCEPTS

Stress

There are numerous excellent articles and chapters in books that give different points of view of the concept of stress (McDonald,1979; Friend,1980; Dantzer and Mormede,1983a; Levine,1985).While the following may not be complete, there is some agreement on the synthesis of ideas presented.

As commonly defined in biology, stress is the sum of the non-specific responses to environmental disturbances (stressors) (Selye,1946). Within this definition "non-specific" requires further explanation. There are two types of responses to stressors. Firstly, there are specific responses which may be behavioural or physiological. For example, in response to cold, animals may huddle, shiver, show an increase in metabolic rate and a change in blood flow away from the skin, while to restraint, animals will show a different set of specific responses; they may struggle,

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vocalize and increase heart and respiration rates. Secondly, there are non-specific responses which are independent of the nature of the particular stressor. Therefore, irrespective of the stressor (fighting, restraint, transport) there is a stereotyped pattern of physiological responses involving many systems of the body including the nervous, lymphatic, circulatory and hormonal systems. In practice these non-specific responses can be used to determine the intensity of a stressor and one consistent non-specific response is an increase in corticosteroid concentrations, the so-called stress hormones from the adrenal cortex.

One other aspect of the non-specific responses that requires consideration is their time course of action. The first series of three events is an immediate response (taking just seconds) and is an adrenalin dependent mechanism which results in changes in blood flow and a production of glucose from liver glycogen for an immediate energy supply. These responses prepare the animal for fear, flight or fight responses (see Canon, 1939). This initial reaction lasts for only a short period of time and if the stressor is not removed, a second series of events occurs. This is the short term or acute response (lasting minutes to hours) and is a corticosteroid dependent mechanism.

A major function of this second series of events is to provide glucose from non-carbohydrate sources, particularly protein (from muscle or food), as the glycogen available in the liver for conversion to glucose is limited. During this stage, provided the intensity or duration of action of the stressors is not excessive, a steady state is achieved in which the increased metabolic demands are met by an increased metabolic performance, fuelled by mobilization of energy reserves or increased food intake. This physiological state of stress disappears on removal of the stressor with no ill effects on the animal other than a depletion of energy reserves. It should be obvious that this is an effective mechanism whereby animals can cope or adapt to changes in their environment and as such it is a normal everyday event that occurs on a regular basis and is of obvious benefit, although there is a cost (energetic) involved. At this level of response, stress should be considered as an adaptive mechanism. If the stressor continues the response continues to the third series of events, which is the long term or chronic response (days, months or longer). Again, this series of events is a corticosteroid dependent mechanism, but whereas in the acute phase the effects are potentially beneficial, in the chronic phase while still allowing the animal to cope, they also have detrimental effects with long term effects on health. This is a pathological state of stress and the effects on health such as ulcers, hypertension, arteriosclerosis and a suppression of the immune system may be permanent even if the stressor is subsequently removed. Ultimately, death can arise from these stress related diseases.

It is this final stage of the stress concept that has given rise to considerable vagueness and imprecision about the whole concept, as people feel free to ascribe any undefined health problem to stress, and there is even discussion as to whether these ultimate consequences of stress ever occur.

From Australian biology we have a very useful example of stress and its consequences (Lee et al., 1977). The brown marsupial mouse (*Antechinus stuartii*), a small carnivorous marsupial, has a very synchronized and dramatic life history, the most dramatic feature of which is a total annual mortality of males prior to the birth of the next generation. This mortality is stress related and the main stressors are altered behaviour patterns. As the breeding season approaches (it lasts for about 2 weeks), males become very aggressive towards one another and spend long periods of time searching for females. As a result of these behaviours, there is a whole array of physiological changes including an increase in total corticosteroid concentrations, a decrease in transcortin concentrations (a protein which binds corticosteroids and makes it biologically inactive) and consequently a sustained increase in free (biologically active) corticosteroids during the period of mortality. This sustained increase in free corticosteroids is evidence for a chronic stress response. Some consequences in this species are a loss in body weight, changes in plasma sodium and glucose concentrations, anaemia, haemorrhagic ulceration of the digestive tract and a suppression of the immune system, resulting in a variety of pathological states and mortality.

Having shown that stress can have dramatic consequences, the obvious question is "Does it occur in domestic species such as the pig where many potential stressors e.g. food, climate and disease, have been controlled?" This will be discussed below.

Abnormal behaviour

The overt expression of reduced welfare may often take the form of abnormal behaviour. The presence or absence of such behaviour can be considered to be the applied ethologist's equivalent to the physiologist's use of plasma corticosteroids as an indicator of animal well-being.

A vast number of behaviour patterns may be classified as abnormal on the grounds that they are exceptional, irregular or unusual. But the accepted meaning of "abnormal" in this context is a persistent, undesirable action, shown by a minority of individuals in the population, which is not due to any obvious damage to the nervous system and which is not confined to the situation which elicited it (Dawkins,1980). Thus the specific behaviour pattern involved may be normal, such as biting, but it is used in an undesirable or even destructive manner. In pigs, three main classes of abnormal behaviour have been recognised in intensive systems:

(1) Vices, which are destructive behaviour patterns resulting in injury or damage to the performer or pen mates. These behaviour patterns may be originally derived from motivational systems concerning aggressive, feeding, grooming or exploratory behaviour. Obvious examples are ear - and tail - biting. There is universal agreement that these severe forms of abnormal behaviour are indicative of reduced welfare since they lead to physical injury, and on occasions, death.

(2) Stereotypies, which are usually defined as morphologically identical movements which are repeated regularly, are unusual, and have no apparent function (Odberg,1986). Examples are bar biting, sham chewing (also referred to as vacuum chewing or champing), rhythmic snout rubbing, head weaving, etc. The well-being of animals performing stereotyped behaviour is open to dispute as some authors have argued that the animal is responding to a barren environment by creating its own stimulation or arousal. The aetiology of stereotypies is complex and will be discussed in more detail below.

(3) Apathetic behaviours such as motionless standing and sitting have been recognized as abnormal behaviour (Wiepkema,1983; Broom,1986).

Unlike the preciseness of the physiological concept of stress, the underlying causation of abnormal behaviour is complex and varied. Thus vices may develop as a response to boredom (van Putten,1969), stereotypies may be a response to restraint by a tether (Cronin,1985), frustration of feeding behaviour (Rushen,1985), or boredom from understimulation in a barren environment (Kiley-Worthington,1977), and apathetic behaviour may be a reflection of "learned helplessness" (Fox,1984).

The occurrence of abnormal behaviour is generally acknowledged to indicate the presence of discord between animal and environment. This should alert us to the possibility that welfare may be at risk and that a more detailed examination is necessary.

Feelings

A major problem with welfare assessment is that it is quite possible that an animal may be showing no overt signs of distress in the form of abnormal behaviour patterns, and even no internal sign such as elevated plasma corticosteroid concentrations, and yet still be feeling "unhappy". How can we begin to objectively measure the mental experiences of animals? Clearly, this is a formidable problem for contemporary applied ethology. Griffin (1976) has pointed out that our mental experiences include not only images and intentions, but also feelings, desires, hopes, fears, and a wide variety of sensations such as pain, hunger, rage and affection. All such entities have been generally considered outside the scope of contemporary science because they are private and only observable by the person who experiences them and describable to others only by introspection. Even Griffin (1976) in his book "The Question of Animal Awareness" concentrated on images, intentions and awareness of objects and relationships in the outside world, rather than on subjective feelings. His reason for this choice was that he saw more realistic hopes of developing objective methods for gathering satisfactory data about the former than about what psychologists have called "raw feels".

Despite the problems of measurement, there does seem to be some consensus among

ethologists that animals, at least at the phylogenetic level of birds and mammals, are conscious of what is happening to them, but that the extent of this awareness is unknown (Griffin,1976; Dawkins,1980). Because these feelings are not directly accessible, indirect methods of measurement are required, based on the assumption that animals may express their feelings in their behaviour, for example in a preference test or when subjected to operant conditioning. These procedures are discussed later in the review.

ASSESSMENT OF WELFARE

Stress and welfare

There is clear evidence that the pig responds to short term (acute) physical and psychological stressors with an elevation in plasma corticosteroid concentrations. For example, chasing, electric shock or cold (Baldwin and Stephens,1973), heat (Aberle et al.,1974, Marple et al.,1974, Lundstrom et al.,1975), novelty (Dantzer and Mormede, 1981), transport and mixing (Barnett et al., 1984a) and restraint (Becker et al.,1985) result in elevated corticosteroid concentrations. However, one of the major perceived welfare concerns for pigs is the system of housing adult pigs. Depending on the system, they may be confined as an individual in a single pen (stall or tether stall) for most of their reproductive lives. To identify a housing system as inadequate requires evaluation of long term (chronic) responses and here the evidence is more recent.

Over the past seven years comparisions have been made of a number of housing systems for adult female pigs: Neck-tethers in partial stalls; individual stalls; group housing outdoors or indoors with differing group size and space allowance (Barnett et al.,1984b, 1985, 1986, 1987a, 1987b; Hemsworth et al.,1986a). This research has clearly demonstrated that pregnant pigs housed in certain types of individual housing (tether stalls of a design that permit aggressive interactions between neighbours to occur) show evidence of a chronic stress response (a sustained elevation of free corticosteroid concentrations) compared to either other types of individual housing (tether stalls and stalls of different design that minimize aggressive interactions) or various less confined housing systems (groups). The consequences of this chronic stress response are a change in plasma glucose and urea concentrations (Barnett et al.,1985) indicative of an energy mobilizing mechanism to adapt to the housing system, and a decreased reactivity of the immune system (Barnett et al.,1987b) suggesting immunosuppression. Similarly, Cronin et al.(1986a) found an increased metabolic rate in tethered compared to group housed pigs. Together these data indicate a measureable cost to the animal in adapting to some individual housing systems and current thinking interprets these findings as suggesting a risk to welfare. This is not to suggest that animals are unable to cope or adapt, and this would be patently untrue as individual housing is widely and successfully used, but there is still a cost to the animal and its welfare is potentially at risk in some individual housing systems. Recent experiments (Barnett et al.,1987a, 1987b) have identified apparently unresolved aggression between neighbouring pigs as a major factor in the aetiology of the stress response associated with some designs of individual housing. Modifications to accommodation design so that aggressive interactions resulting in retaliation are reduced results in free corticosteroid concentrations that are similar to other indoor (Barnett et al.1987a) and outdoor (Barnett et al.,1985) housing systems.

Other findings of relevance from these studies were no physiological differences between pigs housed in groups either indoors or outdoors (Barnett et al., 1984b,1985), a chronic stress response associated with housing in pairs (at both the recommended space allowance of 1.4m^2/pig [Anon.,1983] and 3.7m^2/pig) compared with groups of 4, 6 or 8 (Barnett et al.,1984b,1986) and a chronic stress response in groups of 6 with a space allowance of 1m^2/pig versus 2 or 3m^2/pig (Hemsworth et al., 1986a). In response to individual housing in neck-tethers neither parity number (1st or 2nd pregnancy) nor previous experience of tethers ameliorated the extent of the chronic stress response (Barnett et al.,1987b).

Another series of studies that involve stress and welfare in pigs have examined the relationship that develops between stockpersons and the pigs in their care, the human-animal

relationship. Animal husbandry provides opportunity for periods of intense interaction between humans and animals and the quality of this relationship can have a marked effect on animal behaviour and physiology. Considerable progress has been made with pigs in understanding the behavioural patterns of humans that influence the quality of the human-animal relationship and the marked effects of the relationship on pigs' physiology, behaviour, production and welfare (Hemsworth et al., 1981,1986b,1987; Gonyou et al.,1986).

The influence of the nature of handling by humans during rearing on behaviour growth and plasma corticosteroid concentrations was examined in an experiment on individually housed gilts (Hemsworth et al.,1981).Two handling treatments, termed unpleasant and pleasant, were imposed to produce pigs showing different levels of fear of humans (high and low levels of approach behaviour by pigs to humans in a standard test). Gilts which were fearful of humans had a lower growth rate (669 v. 709 g/day from 11 to 22 weeks of age on *ad libitum* feed) and feed conversion efficiency (3.8 v. 3.5) than pigs that were less fearful of humans. In addition, the more fearful treatment had elevated mean free corticosteroid concentrations both after a 2 min exposure to a human and when visually isolated from humans, indicating both acute and chronic stress responses. These elevated corticosteroid concentrations were sufficient to increase plasma glucose and protein concentrations and decrease plasma urea concentrations (Barnett et al.,1983), suggesting a significant metabolic cost.

This study has been extended to examine the effects of both nature and quantity of handling by humans on behaviour, reproduction and corticosteroid concentrations in group housed gilts (Hemsworth et al.,1986b). Similar handling treatments (unpleasant and pleasant) to those in the previous study were imposed to produce groups of gilts showing different levels of fear of humans. In addition, a third treatment (control), involving little contact with humans other than routine husbandry practices, was included. The gilts were mated by fertile boars at their second oestrus.While there were no differences in the sexual behaviour of the gilts, the pregnancy rate at 50 to 60 days post-mating for gilts in the unpleasant, pleasant and control treatments was 33.3%, 87.5% and 55.6%, respectively. Blood samples indicated that gilts showing high levels of fear (unpleasant handling treatments) had a chronic stress response when visually isolated from humans compared to the other two treatments. In addition, across all treatments, free corticosteroid concentrations immediately prior to mating were higher in gilts that were subsequently identified as non-pregnant compared to pregnant. These data suggest that a chronic stress response interferes with reproductive processes.

Other experiments on the human-animal relationship of relevance to the topic of stress and welfare in the pig have shown that the regular release of some behaviour patterns of humans, which intuitively appear to be quite innocuous to pigs, reduced the approach by pigs to humans (i.e. the patterns were threatening to pigs) (Hemsworth et al.,1986c). The adverse effects of these behaviour patterns on the growth rate of pigs were similar to the unpleasant handling treatment that resulted in high levels of fear of humans by pigs (Gonyou et al.,1986). Also, an inconsistent handling treatment (pleasant and unpleasant handling imposed on 80% and 20% of occasions, respectively) produced a chronic stress response and adverse effects on growth rate that were similar to the level found by imposing the unpleasant handling treatment at the same rate on all occasions (Hemsworth et al.,1987).

Abnormal behaviour and welfare

i) Vices

Tail-biting, and to a lesser extent ear-biting, are apparently widespread vices amongst growing pigs in intensive piggeries. Most producers attempt to control the problem by docking the tails of newly born piglets. The efficacy of this treatment is largely undocumented, although anecdotal reports suggest that it may reduce the incidence of tail-biting in some cases. If it is effective, the treatment probably works by reducing the stimulus value of a long, wiggling tail as an object to be seized by other pigs. Depending upon the length of the tail removed, it may also reduce the susceptibility of the tail to damage, but of course without influencing the underlying

behavioural problem, biting. An amazing number of factors have been cited as possible causes of tail - biting, including crowding, boredom, lack of bedding, poor ventilation, disease and nutritional factors (Fraser,1987).

Applied ethologists have paid little attention to the problem, mainly because it has proved impossible to experimentally reproduce conditions which reliably lead to an outbreak. For example, Gadd (1967) conducted an extensive survey of tail biting and suggested that nutrition seemed to be involved in about two-thirds of all cases, and that high energy, low fibre, vegetable protein diets were particularly suspect. In response to this survey, Ewbank (1973) attempted to induce tail-biting by feeding the type of diet indicated, but failed. Thus our understanding of this problem has advanced little in the last 15 to 20 years.

The most plausible scenario for an understanding of this behaviour comes from a combination of van Puttten's (1969) early observations with Fraser's (1987) recent experiments with blood-covered models of tails. An outbreak of tail-biting should be regarded as ocurring in two distinct phases. In the first pre-injury phase, van Putten has suggested that tail-biting develops from redirected exploratory behaviour, exacerbated by a barren environment. The pig's natural tendency to bite and root objects in the environment is directed at other pigs. Bites to the ears tend to provoke retaliation whereas tails are a safer target. This biting leads to the appearance of an accidental wound, which is the beginning of the second phase. The bitten animal vigorously waves its tail which attracts further biting of the bloody tail by other pigs. In this second or injury phase, Fraser suggests that an attraction to blood may explain why the appearance of a wound can lead to such a rapid escalation of tail-biting. Pigs provided with canvas tail models chewed considerably more on blood-covered models than plain ones, although the response was highly variable between individuals. Thus, some pigs may be persistent biters and others may not show this behaviour at all. Thus, this strong but variable response could explain why a minor wound could lead to an unpredictable increase in tail-biting among pigs. Other factors implicated in tail-biting (e.g. poor ventilation, crowding, barrenness) could affect the pre-injury phase by influencing exploratory behaviour of the pig. At this stage the provision of straw or other objects may be sufficient to redirect the exploratory behaviour of the pig away from other pigs. But once the initial wound appears, straw may be ineffectual in controlling a tail-biting outbreak. Dietary factors may be more important at this stage, especially if the pig's attraction to blood turns out to be a response to dietary quality.

ii) Stereotypies

The occurrence of stereotyped behaviour is often used as an indicator that the welfare of confined animals is impaired and the work of Cronin et al. (1986a) has indicated there can be a significant metabolic cost associated with stereotypies. In intensively housed pigs, stereotypies are most commonly observed in stalled or tethered dry sows.

It is difficult to obtain reliable information on the incidence of this behaviour in commercial herds, but in Rushen's (1985) study six sows out of a sample of 30 sows in a 55 tethered sow herd showed consistent, stereotyped snout rubbing, which is equivalent to a herd proportion of 20%. Blackshaw (1986) believes that this piggery has a lack of serious stereotypies, because of the frequent interaction with students and staff. Hence, it is quite possible that a figure of 20% represents the bottom of the range for tethered sows. At the other extreme, Cronin (1985) reported that in a semi-commercial Dutch piggery, all sows in a sample of 117 tethered sows performed stereotypies, although the proportion of time spent performing them was highly variable. Hence, it is quite possible that a figure of 100% represents the top of the range.

Three possible explanations for stereotyped behaviour in confined sows have been proposed. Rushen (1984,1985) has suggested that the frustration of feeding motivation associated with fixed interval feeding periods rather than under-stimulation underlies stereotypies in pigs. He found an association between the performance of stereotyped behaviour and feeding periods. Head waving, bar biting and snout rubbing were most common before feeding, whereas manipulating the drinker and long bouts of rubbing were most common after feeding. However, Baxter (1986) has pointed out that the pigs in Rusher's study were probably underfed and received about 91% of their minimum daily energy requirements. Therefore excessive hunger may at least in part

account for the occurrence of stereotyped behaviour.

The traditional explanation for the occurrence of stereotypies in pigs is boredom. They develop in animals reared in monotonous environments. Fraser (1975) reported that tethered sows without straw performed a great variety of stereotyped oral activities which were greatly reduced by the provision of straw that could be chewed and manipulated throughout the day. The possibility that ingestion of straw was responsible for the behavioural changes was ruled out when the provision of additional chopped up straw in the diet resulted in no change in the performance of stereotyped activities.

A third explanation is that stereotypies are a response to restraint. Cronin (1985) has suggested that stereotypies develop as a result of the frustration/conflict of being restrained, and the consequent loss of controllability over the environment. Stereotypies developed after sows had passed through a number of distinct stages including escape, inactivity and outwardly directed behaviour until the final stereotypy was acquired. The stereotypies that developed contained components that were directed at features in the sow's external environment such as chains, drinkers and bars, and appeared to be derived from aggressive acts. However, older sows performed more self-directed stereotypies, which suggested that the continued performance of these behaviour patterns may be due to lack of stimulation.

The function of stereotypies is not clear, although endogenous opiates have been implicated in their performance. Sharman (1983) has reported that snout rubbing can be induced in pigs both by drugs which stimulate, and by drugs which block, receptors in the brain. Cronin et al. (1986b) found that sows injected with the specific opiate antagonist, naloxone, performed stereotyped behaviour at reduced levels in the two hours after administration and that eight of the nine tethered sows tested ceased the performance of their stereotypies in the short term. Thus, in a similar manner to the stress response, stereotypy performance may be regarded as a coping mechanism. However, as with the stress response, the problem is in deciding at what level of stereotypy performance welfare is at risk. Broom (1983) suggested that if the stereotypy occurred for 10% of the animal's waking life then conditions could be described as bad for the individual. Wiepkema (1983) has suggested that welfare is at risk when less severe abnormal behaviours such as stereotypes occur in more than 5% of all animals. While these limits are well below our earlier estimate of 20 to 100% of individuals per average herd, they are not based on objective measurement and further research is required to effectively use stereotypies to objectively assess welfare.

(iii) Apathy

Fraser (1975) has suggested that motionless sitting in sows may represent a state of drowsiness. The behaviour was almost eliminated by the provision of straw for bedding, with the behaviour occurring in a brief interval just before the sow lay down. With no bedding, the floor surface may provide the sow with less incentive to lie, and so the behaviour persists for long periods. Thus a high incidence of standing or sitting motionless may indicate a lack of physical comfort and a reluctance to lie down. Van Putten and Dammers (1976) have suggested that early weaning and rearing in barren environments may influence this behaviour. Piglets in cages spent more time sitting like a dog than piglets kept together with the sow in a pen with straw.

Another possible explanation for this behaviour is a phenomenon which has received little attention in pigs, that of learned helplessness. When an animal learns that normally adaptive responses such as escape are futile, it has attained a state regarded by Seligman (1975) as learned helplessness, which is similar to the clinical state of depression in humans. Thus, Cronin (1985) has described how the initial responses of sows to tethering are often violent escape attempts followed by a period of lying idle and immobile for long periods. If apathetic behaviour is a response to a lack of environmental control, providing animals with operant feeding devices or increasing the predictability of daily events by provision of safety signals could alleviate the problem (Fox, 1984), such as using a bell to signal a husbandry procedure. Carlstead (1986) has experimentally manipulated predictability of feeding in growing pigs and demonstrated that unreliable feeding signals produce frustration-induced aggression.

(iv) Comparison of behaviour in two environments

This method of assessing welfare, by comparing the frequency of various behaviour patterns in two different environments, must be used with extreme caution if inferences are to be drawn about welfare. Thus, the demonstration that animals perform different behaviour patterns at different frequencies in different environments is to be expected, and it is important not to conclude that a particular environment is detrimental to welfare if a behaviour pattern does not occur. For example, Stolba (1981) studied the behaviour of pigs kept in a large semi-natural enclosure with the behaviour of similar groups in small paddocks and pens. He found significant differences in the frequency of occurrence of all the behaviour patterns recorded. For example, pigs in the enclosure showed more locomotion, sniffing, feeding and chewing whereas pigs in a partially slatted pen showed more exploration and manipulation of their pen mates, agonistic interactions and grooming. These differences should alert us to the possibility that welfare may be at risk, but we then need to examine the causation and function of these behaviour patterns in detail to understand the meaning of differences in their frequency of occurrence. To date this has not been done and thus the usefulness of these types of comparisons to assess welfare are limited.

Other examples of this approach are van Putten's (1984/85) comparison of the effects of three levels of light on the behaviour of growing pigs. In the dark pen there was more lying, less social behaviour and more tail-biting. However, tail-biting damage was greatest in the brightly lit pens and least in the semi-dark pens. Laimers and de Lange (1986) compared the pre- and post-farrowing behaviour of free and tethered gilts and found obvious differences for nest-building behaviour, comfort behaviour and maternal attention.

Variants of this approach to welfare assessment are comparisons of the frequencies of behaviour of different groups of pigs in a reference environment. Robert and Dallaire (1986) compared the behaviour of stress-susceptible and normal juvenile pigs in pens. Stress-susceptible animals drank more often and investigated the pen and their peers by naso-ear and naso-anal contacts more often, but were lying less often. Taylor and Friend (1986) compared the behaviour of pigs previously housed for six weeks on either pasture or concrete floors. Comparisons were made in an "open field" test, the field being a 3 x 12m enclosure on pasture. The concrete floor pigs spent more time chewing, grazing and standing than did the pasture pigs. The conclusions drawn by Taylor and Friend illustrate the blatant misuse to which findings of this type can be put. They suggested that an increased specific-action potential for grazing behaviours occurred in response to maintenance in an indoor environment.. In Lorenzian terminology, the grazing behaviour of the concrete floor pigs "dammed up". This seems patent nonsense as comparative information on the frequency of occurrence of behaviour in an open field gives no insight into the underlying motivational mechanisms controlling grazing behaviour, let alone adequately controlling for the effects of previous exposure to pasture.

(v) Experimental stress

This method of welfare assessment was originally developed in chickens. The idea is to record the behavioural and physiological responses of animals exposed to experimentally imposed frightening or frustrating situations. These responses can then be compared with those of animals in commercial conditions and inferences about welfare drawn. For example, Duncan and Filshie (1979) gave chickens auditory frights (a sudden loud noise) and visual frights (blowing up a balloon near the chicken's head) and recorded behaviour and heart rate. Heart rates suggested that so-called "docile" birds were in fact more fearful than their overt behaviour suggested.

Dantzer has used this technique extensively in pigs. Pigs respond to aversive stimulation with classical fear responses. Pigs conditioned to associate an auditory stimulus with an electric shock showed a typical withdrawal reaction with vocalization and crouching as if in anticipation of the shock; they also frequently defaecated and urinated (Dantzer and Baldwin, 1974). Pigs in a shuttle-box would quickly learn to avoid an electric shock by jumping over a barrier between the two compartments (Dantzer, 1977). Both of these responses, defaecation/urination and avoidance, are typical of mammals placed in frightening situations. However, when pigs were given previous exposure to inescapable shock their performance on a continuous avoidance sched-

ule in the shuttle-box was impaired compared with control animals (Mormede and Dantzer, 1977). Clearly, prior exposure to aversive stimulation has influenced their behaviour.Such a response is consistent with an interpretation in terms of learned helplessness. Thus the pigs exposed to shocks they cannot control, learn that their behaviour does not control their environment and show fewer avoidance responses to further shocks. It remains to determine whether learned helplessness occurs as a generalized response to fear inducing stimuli encountered by pigs housed either in commercial conditions or whether it only occurs as a laboratory phenomenon in response to electric shock.

Frustration in the pig has been investigated in greater detail. Frustration occurs when an animal is motivated to perform a behaviour but is unable to do so. This may be because the appetitive behaviour is physically thwarted or the object of the consummatory behaviour is unavailable. Thus, a hungry animal may be frustrated by offering food under a glass plate or by withholding food. Typical responses shown by animals in these sorts of situations are responses to normally inadequate stimuli (e.g. feeding movements directed towards other objects), displacement activities and aggressive behaviour (Hinde, 1970). Dantzer et al. (1980) have experimentally induced frustration in pigs by extinguishing an operant response. In other words, pigs that had previously learned to press a panel with their snouts to obtain a food reward were no longer rewarded. Pigs tested alone quickly stopped responding, became very restless, attempted to escape, rubbed their noses on the cage floor and scratched the floor with their feet. Dantzer and Mormede (1985) have interpreted these behaviour patterns as displacement activities. Plasma corticosteroid concentrations also increased significantly following extinction. When two pigs were tested together they developed aggressive behaviour accompanied by increases in plasma corticosteroid concentrations. However, although this indicates the presence of frustration-induced aggression, the appropriate control condition of simply pairing pigs together was not tested. In another experiment, Arnone and Dantzer (1980) attempted to overcome this problem by testing pigs in pairs that had been previously acquainted. During extinction, aggression developed between unacquainted pigs but not in acquainted pigs.Thus, frustration induced aggression does not seem to occur during extinction.

If pigs do find frustration aversive then it should be possible to modify their response with tranquillizing drugs. Dantzer (1977) confirmed that diazepam increased resistance to extinction in pigs previously trained to perform an operant response for food, compared with control pigs.

Dantzer and Mormede (1983b) have also frustrated pigs using a different experimental paradigm. Hungry pigs were submitted to intermittent food deliveries with a chain hanging adjacent to the food trough. Pigs developed stereotyped nibbling on the chain in the intervals between food deliveries. Pigs that nibbled the chain had lower plasma corticosteroid concentrations at the end of the experimental session than pigs that had no chain available to them. Dantzer and Mormede have suggested that displacement activities are an effective way for the animal to cope with the aversive situation, perhaps by allowing it to switch attention to stimuli other than those that are the source of the problem. Similarly, Cronin and Barnett (1987) have shown a negative correlation between level of stereotypies and corticosteroid concentrations in tethered sows, perhaps suggesting the efficacy of stereotypies as a coping mechanism.

Tests with other experimentally imposed stressors have attempted to replicate to some degree the conditions found in commercial situations. Algers and Jensen (1985) investigated the effects of continuous exposure to fan noise at a level of 85 dB on the nursing-suckling interaction. High noise levels seemed to disrupt the normal nursing-suckling pattern and this may have led to a lower milk gain for the piglets. Ingram et al. (1983) and Sharman et al. (1985) have simulated the effects of transport on pigs by exposing them to noise and vibration. Although operant conditioning tests suggested that noise and vibration were aversive to the pig, there was no increase in the secretion of catecholamines from the adrenal medulla, suggesting that the pigs' adaptive responses did not necessitate physiological mechanisms.

vi) Preference tests

Although the previous method of welfare assessment allows us to identify the responses

of the pig to fear and frustration, it gives us little idea of what the pig feels about these motivational states. One method which might give us indirect evidence is to allow the animal to choose between different environments or aspects of them. Preference tests were first applied to domestic animals by Hughes (1975) and Dawkins (1976) working with laying hens. The earliest tests examined the response of birds to different floor types. Similar tests have been done with pigs, again with most attention given to floor type. Pouteaux et al. (1983) tested groups of four weaner piglets placed for one hour in a square pen which had a different floor type in each quadrant. The four perforated floors tested were expanded metal, plastic-coated expanded metal, fibreglass slats and moulded plastic. Time spent standing was relatively constant for all floors, but pigs spent more time lying on the plastic-coated expanded metal than on the other three floors. The authors concluded that time spent lying was the best single indicator of overall preference for a floor type.Blackshaw (1981) found that piglets weaned at four weeks of age into pens with a solid and wire mesh floor in different proportions invariably chose a solid floor to lie on. In pens also provided with a box the piglets chose a wall to huddle against. Mwanjali et al. (1983) tested miniature pigs in an hexagonal multiple choice pen with cubicles containing sawdust, straw, concrete, concrete slats, perforated metal, rubber or plywood floors. Not surprisingly, piglets reared on straw spent most time lying in the straw cubicle. Fraser (1985) gave groups of three pigs aged 4-8 weeks access to two sides of an experimental pen, one with straw bedding and one with a bare concrete floor. He found no consistent preference for the bedded or unbedded side. However, preference appeared to be influenced by environmental temperature. At 18-21° C pigs selected the bedded side for resting, while at temperatures of 25-27° C they selected the bare concrete. Van Rooijen (1981) gave pregnant sows continuous access to pens with two compartments containing either earth- earth, concrete-concrete or concrete-earth floors. When both floors were of the same material pigs spent most time in the inner compartment, adjacent to the neighbouring pig. When earth-concrete floors were tested pigs spent most time on the earth floor, regardless of its location.

Other environmental factors which have been examined in choice tests with pigs include air velocity, air temperature and floor temperature. Geers et al. (1986) tested piglets in a pen in which the floor temperature of two halves could be manipulated and the air temperature and velocity above the floor varied. Pigs preferred a lying area with an enhanced air velocity when air temperature was 14-25° C and floor temperature was greater than air temperature. However, apart from this study there have been no attempts to study the choice behaviour of pigs when simultaneously exposed to more than one environmental variable.

(vii) Operant conditioning

Operant conditioning has often been touted as a highly promising method of objectively assessing animal welfare (Dawkins, 1980; Duncan and Dawkins, 1983; Baldwin, 1983). It is a procedure commonly used by experimental psychologists where an animal learns to perform a response in order to obtain a positive reinforcement (reward) or avoid a negative reinforcement (punishment). The technique could provide a window on animal feelings if we assume that animals experience subjective feelings of pleasure in the presence of rewards, and distress in the presence of punishments (Dawkins, 1980). The method has been extensively applied to pigs because they readily learn to perform operant responses such as panel pressing or lever lifting. Baldwin and Ingram (1967) investigated behavioural thermoregulation in pigs. They found that pigs soon learned to press panel switches with their snouts to obtain three-second bursts of radiant heat from infra-red heaters suspended above their cages. When pigs aged 8-12 weeks were exposed to a range of temperatures from 10 to 40° C, the rate of pressing to turn on the heaters declined markedly in the range 20 to 30° C, which was equivalent to the thermoneutral zone for pigs of this age. Presumably the pigs were cool at 20° C, but more comfortable at 25° C. Baldwin and Meese (1977) looked at the illumination preferences of pigs. Light onset was reinforcing for pigs, but they would only work to keep it on for 0.5% of the time. Light offset was not reinforcing. In light-dark preference tests, pigs kept themselves in the light for 72% of the time, but would not perform operant responses to obtain prolonged illumination. In more recent experiments, Baldwin and Start (1985) have shown that pigs kept in darkness will work to obtain 40 sec periods of illumination, but when placed in continuous light they would not respond to obtain 40 sec periods

of darkness. Thus, pigs do respond to light and not merely a change of stimulus.

These early experiments with operant conditioning evaluated the reinforcing properties of fairly simple changes in the physical environment.. However, Duncan and Dawkins (1983) suggest there is no reason why more complex questions cannot be asked, such as "Will an animal work in order to gain access to a particular environment in which it can perform a certain behaviour pattern?", and "Will it work in order to avoid being frustrated in a particular way?". As a bonus we can also ask "How hard will it work?". Van Rooijen (1984) has described an apparatus for "operant preference tests" where pigs were given the opportunity to move between two compartments of a pen with or without straw by pressing on a plate which opened a gate. He found that pigs raised on straw worked significantly harder to enter the compartment with straw than the one without straw. No significant difference was found for pigs raised on a bare floor.

Hutson (unpublished) has made extensive use of operant methods to measure the responsiveness to earth of weaner piglets and the motivation of farrowing sows to utilize straw. Groups of weaner piglets in a flat-deck cage could lift a lever which gave access to an earth trough, an empty trough or had no effect. Group lever lifting performance was unaffected by earth in the trough, but at least one individual, the "worker piglet", operated the lever more than the others to gain access to earth. Once the lid of the trough was opened other piglets were attracted to the earth and spent more time using the earth trough than the empty trough. Previous experience of earth appeared to modify the lever lifting behaviour. In one experiment a worker piglet did not emerge, and in another the worker operated the lever for earth at a reduced rate. The number of piglets using the earth trough and the amount of time spent utilizing it was reduced by prior exposure to earth. Hutson concluded that earth was a mild reinforcer to weaner piglets and would sustain a low rate of responding on an operant schedule, and that a large component of its reinforcement value is the novelty of the material. In contrast to these results, pregnant sows did not appear to find straw reinforcing. Sows farrowing in a pen could lift a lever to gain access to a box of straw. The sows showed an increase in activity prior to farrowing which coincided with increased manipulation of the lever and the appearance of nest-building behaviour patterns such as pawing. However, the response to straw was variable, with some sows ignoring it altogether. Control pigs showed similar increases in activity, lever manipulation and pawing behaviour when the straw box was empty. Clearly, the sows had a low motivation towards utilization of straw, which may indicate that it is not important to the sow as a key stimulus for nesting behaviour.

PHYSIOLOGICAL CHANGE AND THE RISK TO WELFARE

The series of experiments described earlier on housing systems and the human-animal relationship have shown that either housing systems or small amounts of threatening behaviour by humans, imposed either regularly or irregularly, can produce chronic stress responses as evidenced by a sustained elevation of plasma free corticosteroid concentrations and subsequent or concurrent detrimental consequences e.g. a change in growth rate, pregnancy rate, immunoreactivity or metabolism. However, the question that was asked earlier still remains: "At what level of increase in free corticosteroid concentrations is welfare at risk?",

The data from these studies, some of which is presented in Table 1, can help answer this question. The table, compiled from studies on housing systems and handling, shows a range of corticosteroid responses of -4 to +64% for the various comparisons made. The consequences of the increase in free corticosteroid concentrations are either physiological, measured in terms of immunoreactivity or plasma glucose, urea or protein concentrations, or production related, measured in terms of growth rate, sexual behaviour or pregnancy rate.

Physiological consequences occur when increases in free corticosteroid concentrations of >48% occur and do not occur in those studies where there were increases in free corticosteroid concentrations of 22-39%. Similarly, growth rate depression occurs when free corticosteroid concentrations increase by >48% and pregnancy rate and sexual behaviour can be affected with an increase of >41%. The data are not perfectly clear cut; in the study comparing a group of

2 v. 8 pigs (Barnett et al., 1986), a 63% increase in free corticosteroid concentrations occurred with no significant effect on sexual behaviour. However, mating rate was low across all treatments in that study and it was considered that space allowance may have been limiting in all treatments and may have masked any sexual behaviour response to group size.

Table 1. Percentage increase in free corticosteroid concentrations affected by housing system or handling, and some consequences.

Comparison	% increase in*	Consequences: + = increase - = decrease	Reference
Housing Experiments			
tether (type a)* v. group	63	+ glucose, - urea	Barnett et al., 1985.
stall (type a)* v. group	22	no change	
paddock* v. group	34	no change	
tether (type a)* v. group	57	+ immunosuppression	Barnett et al., 1987b.
tether (type a)* v. group	64	not measured	Barnett et al., 1987a.
tether (type b)* v. group	-4	not measured	
stall (type a)* v. group	11	not measured	
group of 2* v. 8	63	no effect on sexual behaviour[x]	Barnett' et al.,1986
group of 2* v. 4	21	no effect on sexual behaviour[x]	
group of 2* v. 3-6	39	no change in glucose, urea or protein	Barnett et al., 1984b.
space allowance of $1m^2$* v. 2 m^2	53	- sexual behaviour	Hemsworth et al., 1986a.
space allowance of $1m^2$* v. 3 m^2	44	- sexual behaviour	
Handling Experiments			
unpleasant* v. pleasant	48	- growth rate, + glucose[y], - urea[y], + protein[y]	Hemsworth et al., 1981.
unleasant* v. pleasant	41	- pregnancy rate	Hemsworth et al., 1986.
unpleasant* v. pleasant	56	- growth rate	Hemsworth et al., 1987.
inconsistent* v. pleasant	63	- growth rate	

* denotes the treatment (in comparison column) in which the increase in free corticosteroids occurs.

[x] space allowance may have been limiting.

[y] see Barnett et al., 1983 for details of metabolite concentrations

A change in plasma concentrations of glucose, urea or protein indicates a significant metabolic cost to the animal, immunosuppression indicates a potential health risk and depressions in growth rate, pregnancy rate or sexual behaviour indicate a production loss. All these types of

changes are associated with a sustained increase in free corticosteroid concentrations of >40%. Thus, with the limited knowledge available, it is reasonable to suggest a risk to welfare when a sustained elevation of >40% in free corticosteroid concentrations is evident. Thus, in the studies shown in Table 1, welfare is at risk when adult female pigs are housed in neck-tether stalls (of type a), when adult female pigs are housed with $1m^2$/pig and on occasions when housed in pairs, and when handled such that they show a high level of fear of humans. Conversely, welfare is not at risk in individual stalls of the design used in the experiment, in group housing systems either indoors or outdoors, in neck-tether stalls of type b, in groups of 3-8, when housed with a space allowance of $2m^2$ or $3m^2$/pig and when they show low levels of fear towards humans.

A similar question arises with the interpretation of behaviour data: "At what level of change in behaviour (stereotypies, vices, learned helplessness etc.) is welfare at risk?". Unfortunately the available literature on behaviour does not permit compilation of a similar table to Table1 for behavioural change and associated risks to welfare.

LIMITATIONS IN USING BEHAVIOUR AND PHYSIOLOGY TO ASSESS WELFARE

Physiology

(i) Terminology

Most of the criticism of the concept of stress and its application to the assessment of welfare arises because of confusion over terminology, particularly a definition of stress and the cavalier attitude of ascribing undefined problems to stress. Defining stress (in biological terms) to everyone's satisfaction is fraught with almost as many difficulties as defining welfare and some of these problems and limitations have been addressed (Selye, 1975a,1975b; Dantzer and Mormede,1983a; Moberg, 1985; Levine, 1985; Reite, 1985). In this paper Selye's1946 definition has been used (see section on the Concept of Stress) and it is worth restating part of it again i.e. 'the sum of the non-specific systemic reactions ....'. The definition emphasizes two important points. Firstly, the one of non-specificity which has been discussed and secondly, the sum of the responses. This means that while elevated corticosteroid concentrations may be a consistent feature of the stress response they do not represent the totality of the response. Thus, while a sustained elevation of corticosteroid concentrations provides *prima facie* evidence of a chronic stress response, the stress response and elevated corticosteroid concentrations are not synonymous (although they are often implied to be); the latter is just part of the former. Included in the totality of the physiological responses to stress can be a host of changes including changes in endocrine function (such as changes in thyroid, adrenal and pituitary activity), cardiovascular function (such as increases in blood pressure and cardiac output), metabolism (such as increases in blood glucose and free fatty acids), the immune system (such as changes in the proportion of white cell types) as well as others not mentioned or yet to be determined. In our research (JLB) with the pig we have concentrated on the pituitary- adrenal axis to indicate the presence of a stress response, rather than some of the other parameters, as there is a large body of literature on this parameter (see Selye, 1976 who cites and/or abstracts 7,700 references out of a collection of 110,000 on the subject) and it has been used to good avail to identify stress and welfare problems in the pig. However, irrespective of the parameter used, it can only provide *prima facie* evidence of a real or potential risk to welfare. An actual risk to welfare can only be identified on the basis of the magnitude of the response and the detrimental consequences of elevated corticosteroid concentrations, or some other parameter. If a particular situation results in a loss of body protein, a reduced reproductive performance, a sustained increase in basal metabolic rate or a suppression of the immune system, it is reasonable to suggest that the welfare of animals in that situation is at risk. This is the approach used with the pig (see Table1 and discussion in previous section).

Another aspect of the concept of stress and the three stages (alarm reaction, stage of resistance and stage of exhaustion) of the General Adaptation Syndrome (Selye,1946) that has received some criticism is whether the phenomenon of exhaustion occurs. This doubt has arisen

because death of animals can occur despite high levels of circulating corticosteroids (eg. Panaretto and Vickery,1970; Bradley et al.,1980). However, it is a misconception to think of the stage of exhaustion as suggesting underactivity of the adrenal cortex and consequent low concentrations of circulating corticosteroids. In Selye's (1946,1976) accounts of the General Adaptation Syndrome, it is the ability to resist (i.e. collapse of the stage of resistance) that is exhausted and the characteristics of the stage of exhaustion are similar to the alarm reaction and include hyperactivity of the adrenal cortex.

(ii) Measurement

Two criticisms are often raised in regard to the measurement of physiological criteria. The first is deciding how much of a change in a physiological parameter indicates reduced welfare; this point has already been discussed (see section on physiological change and the risk to welfare) and considerable advances are being made. Secondly, there are often implied criticisms because of the difficulty of the techniques themselves, necessitating indwelling cannulae to obtain blood samples with minimal disturbance to the animal and sampling problems associated with diurnal rhythms of the parameter being measured.

These problems present a challenge to the scientist and provided that the detailed background work is undertaken, they can be overcome and eventually become routine techniques. For example, when our laboratory (JLB) started work with the pig it was known that there was a marked 24 h rhythm in plasma corticosteroid concentrations (Whipp et al.,1970; Bottoms et al.,1972; Baldwin and Stephens,1973; Edqvist et al.,1980) but many factors known to influence this rhythm in other species had not been examined in the pig. For example, the circadian rhythm in plasma cortisol in man and sheep is composed of a number of secretory episodes which vary both in frequency and amplitude throughout the day (Krieger,1975; Fulkerson,1978; Fulkerson and Tang, 1979). This circadian rhythm can be influenced in the mouse and rat by season (Haus and Halberg,1970) and the anticipation of feeding (Moberg et al.,1975; Morimoto et al.,1977). Also, there is an inverse relationship between plasma corticosteroid and transcortin concentrations in man (Angeli et al.,1978) and the level of this binding protein may influence the biological activity of plasma corticosteroids during the day. Therefore, prior to any welfare research with the pig it was a priority to understand the effects of some of these factors and to determine a blood sampling regime to give meaningful estimates of corticosteroid concentrations.

The data obtained from an experiment by Barnett et al. (1981) suggest that a suitable blood-sampling regime for determination of valid estimates of corticosteroid secretion in pigs would be as follows. Firstly, treatments to examine the effects of short-term stressors should be carried out in the afternoon rather than in the morning because the between animal variation in the hormone levels is less and the hormone levels are more stable. Secondly, to define the period of high corticosteroid activity and changes that occur in corticosteroid concentrations during other times of the day, blood samples taken at 1 h intervals are sufficient as they are well correlated with samples taken at a more frequent sampling interval.Thirdly, because of the lack of a circadian rhythm in the maximum corticosteroid binding capacity, the average of five samples gives a value which can be used to determine free corticosteroid concentrations at any time of the day. The protocol routinely followed in our laboratory is blood sampling at 1 h intervals between 0700-1700 h or 0800-1700 h in summer and winter, respectively, using indwelling venous cannulae. The cannulation technique is based on the methods of Christison and Curtin (1969) for cannulating the jugular vein and Takken and Williams (1981) for cannulating the cephalic vein. The technique involves implanting a silicone cannula into a vein, under full surgical anaesthesia, and exteriorizing it at the back of the neck where it is stored in a zipped pouch and held in position with an adhesive bandage that forms a collar around the pig's neck. The cannulae require daily maintenance (flushing with anti-coagulant) and have remained patent in experiments lasting 34 days for pigs in individual pens and for 14 days in group pens. It is good practice to cannulate all pigs within a group rather than part of a group to minimize destroyed cannulae.

(iii) Acute versus chronic responses

As discussed earlier in this paper, differential welfare of pigs can be a consequence of different housing systems or level of fear of humans and is in part evidenced by a chronic stress

response. However, there are also numerous examples of acute stress responses in the pig and on occasions there is an erroneous tendency to treat any acute stress response as evidence of suffering and reduced welfare. Again, as discussed in the section on "Concepts", it is to an animal's advantage, and hence a normal biological occurrence, that it has the ability to cope with adverse or novel situations through effective energy mobilising mechanisms. Acute stress responses occur in the pig to such diverse stimuli as electric shock (Baldwin and Stephens, 1973), novelty (Dantzer and Mormede, 1981) and transport and mixing (Barnett et al.,1984a). However, it has been argued (Rushen, 1986) that since supposedly pleasant as well as unpleasant stimuli can produce an acute stress response e.g. coitus in rats (Szechtman et al.,1974) and perhaps voluntary exercise in man (Sutton and Casey,1975) could be described as not being unpleasant, great care must be taken not to interpret all acute stress responses as signifying reduced welfare. Notwithstanding this limitation, it is possible to use the duration and intensity of the acute stress response to address specific issues of concern by identifying the severity of different stressors and to determine management procedures that minimize acute stress responses. For example in sheep, shearing is a more severe stressor than yarding (Fulkerson and Jamieson,1982), fast shearing is less stressful than slow shearing (Kilgour and De Langen,1970) and herding with dogs is more stressful than herding by people (Thurley and McNatty,1973). In calves surgical castration produces a greater adrenal response than castration by application of 'elastrator' rings (Fell et al.,1986).

This approach of attempting to minimize stress may be of some benefit in identifying improved management techniques but it must be remembered that acute stress responses are short term responses, do not generally have long term detrimental consequences and thus are difficult to interpret in terms of welfare.

(iv) Psychological versus physical stressors

There is much argument over the relative importance of physical and psychological factors in activating the pituitary-adrenal axis and the implications this has for the stress concept (Moberg,1985; Levine,1985; Dantzer and Mormede,1985). These authors cite their own work and others' experiments (e.g. Mason,1968,1975) that show that less readily identifiable factors (e.g. emotional states) appear to be equally (or more) potent stimulators of the stress response than the more easily recognizable physical factors considered by Selye in his conceptualization of the stress response. However, while this means there may be uncertainty as to the precise nature of the specific stressors, as has been emphasized in this paper and by others (e.g. Moberg,1985), it is the consequences of the stress response that are important to welfare and not the cause(s) of the response. Thus while the argument may have heuristic value in understanding the complex concept of stress, it is of little practical value to the welfare debate.

Behaviour

(i)Terminology

One problem with the use of abnormal behaviour as an indicator of welfare is similar to the problem with stress terminology. We need to ensure that we are all talking about the same thing (Odberg, 1986). Are the frustrated feeding movements recorded by Rushen (1984,1985) in hungry sows before and after feeding in the same category of behaviour as the self-directed and environment-directed behaviour patterns observed by Cronin (1985) in sows at other times of the day? Are these behaviour patterns stereotyped to the same degree (i.e. do they have the same form from one action to the next) and are they repeated at the same rate? Are any differences in structure related to differences in underlying causation? Keiper (1970) has suggested that the stereotypies of caged canaries, route tracing and spot picking, have different causation. Route tracing was a response to the physical restrictions imposed on movement by caging, whereas spot picking appeared to reflect some form of sensory or motor deprivation. Thus we need to undertake a rigorous study of stereotypies in pigs with experimental manipulation of the four major variables which have been implicated: Level of feeding (hunger), feeding schedule (frustration), sensory deprivation and restraint. We suggest that a fifth and frequently overlooked variable, age at weaning, should also be examined. Pigs are weaned at three to four weeks of age in most modern

commercial piggeries, whereas the weaning age of free-ranging domestic pigs is between 14 and 17 weeks (Jensen,1986). Clearly there is a possibility that pigs are weaned with gross motor and sensory deficits because the opportunity for normal suckling behaviour has been curtailed. Conceivably, this deficit could be expressed in the acquisition of stereotyped oral behaviour patterns.

(ii) Comparisons in one or more environments

The least promising behavioural method of welfare assessment is by comparisons of behaviour in one or more environments. The inference to be drawn from any differences in frequency of behaviours is that one environment is better or worse than the other. Clearly this is not the case, and as Beilharz (1982) has pointed out, intensively confined animals will adapt genetically to the environment in which they are kept. Thresholds for the performance of behaviour patterns are readily altered by artificial selection and harsh environments will lead to rapid genetic change. It is especially disappointing when frequency differences are used in support of a particular model of motivation, as they give no insight into the precise type of control which is involved. In the Lorenzian model of motivation, a behaviour pattern is considered to be controlled by action-specific energy which accumulates during periods of non-performance. The animal inevitably becomes motivated to perform the behaviour if the appropriate releasing stimulus is absent or the immediate environment prevents its expression. In this situation vacuum activities may occur or the behaviour may be released by an inappropriate external stimulus. Lorenz's view of motivation is no longer widely accepted by ethologists (Dawkins, 1983; Huntingford,1984), partly because it contains no provision for proprioceptive feedback, but especially because we can now recognise the diversity of control systems operating in different motivational systems (Hutson, 1986). Thus a pig confined in a barren stall is not *a priori* a collection of thwarted drives. Deprivation experiments of the type used by Taylor and Friend (1986) cannot justify a Lorenzian model for the control of grazing behaviour by the pig, because of the large number of uncontrolled variables. Do deprived pigs respond to grass because of their "insatiable curiosity" or do experienced pigs fail to respond because they are bored? Is there feedback from the performance of grazing and is it a result of blood metabolites from the ingestion of grass, the performance of nibbling behaviour or the visual stimulus (sign stimulus?) of grass?

(iii) Preference tests

The use of preference tests to objectively assess welfare has been the subject of some controversy (e.g. Duncan,1978; Hutson,1984), but despite the frequent reiterations of their problems, they continue to be mis-used. The main criticisms are that (1) preference in a choice test is relative and gives no indication of the absolute status of the choices, (2) previous experience affects choice, (3) the type of test, either continuous access or T-maze, can influence choice, (4) the type of response required, such as locomotion or bar pressing, may influence choice, (5) animals do not always choose wisely in the long-term interests of their welfare, (6) animals make non-exclusive choices, where the minority choice could be as equally important to welfare as the major choice and (7) the choices put to the animal may be inappropriate to the animal's welfare requirements (Duncan, 1978; Hutson, 1984).

As an example of the problems with this method, consider the results of Pouteaux et al. (1983). They found that pigs preferred to stand and lie in one quadrant regardless of floor type. The preferred quadrant was the one farthest from the door and was therefore the best location for observing the entry and exit of the experimenter. Van Rooijen (1981) found that pigs in a T-maze offered a choice between a pen with food and a pen with no food preferred one side of the T-maze regardless of whether it contained food or not. However, these position preferences were eliminated in two pigs by reducing the amount of time they were held in the start box. Thus, the strong position preferences of pigs, when considered together with their reactability towards the experimenter and the criticisms outlined above, makes preference testing of pigs a very dicey business indeed.

A further illustration of the limitations of relying too heavily on preference test data comes from Gravas' (1979) study of the effects of different floor surface treatments on the behaviour of farrowing sows and their piglets. Sows spent more time lying on rubber-covered than epoxy-

covered or concrete floor surfaces, whereas wounds on the front legs of piglets were larger in diameter and deeper on rubber floors than on concrete floors. Thus, although the rubber floor may be more comfortable for the sow, it leads to increased injury of the piglet. A choice test of floor type using either time spent standing or lying as a measure of preference would have difficulties in detecting this apparent conflict of welfare interests.

(iv) Operant conditioning

Operant conditioning techniques still appear to hold considerable promise for welfare assessment in pigs. Dawkins and Beardsley (1986) encountered problems using operant methods to investigate whether access to litter was reinforcing to hens. Hens did not peck more often at a key to gain access to a goal box with or without litter, and in a choice test did not learn to peck the key which opened the litter goal box. But when the operant response required was changes from key-pecking to crossing a photoelectric- beam, the hens showed that they found litter reinforcing, but only after a large number of trials. Clearly, the operant task chosen appeared to influence the hen's performance in the test apparatus. This type of problem has not been encountered with pigs, since they are enthusiastic performers of operant responses for a variety of sensory reinforcers in addition to food, and are very sensitive to variations in schedule (e.g. the ratio of responses to reinforcements). Dawkins and Beardsley (1986) seemed a trifle disenchanted when they commented that "operant conditioning tests are no more than preference tests in which the animal has to learn to work for whatever it prefers". This seems an unnecessarily harsh judgement which misses one of the fundamental points of operant work. That is, that it allows an animal to indicate a level of response to a variable independently of a choice situation, which may, as in Dawkins and Beardsley's experiments, interfere with clear expression of preference. Furthermore, Julian Huxley has warned that we must beware of all such "nothing-buttery"."Whenever anybody says or implies that something is 'nothing but' something else or is explicable 'merely' in terms of its elements or origins, we can be quite sure that he is wrong" (Huxley, 1964).

SUMMARY

This chapter is not intended to be a comprehensive review of all physiological and behavioural methods of assessment of welfare. Instead it emphasizes the contributions behaviour and physiology have made to the assessment of welfare in the pig and discusses some of the real and imagined limitations of these approaches. While the physiological approach has concentrated on the stress response as mediated by free corticosteroid concentrations, this is not to imply this is the only or best methodology to use. It is just the approach we have taken. Other hormonal systems are responsive to stressors in a number of species and changes have been identified in catecholamines, thyroid hormones, growth hormone, prolactin, endorphins, etc. However, our understanding of the significance of some of these changes is poor and thus there are even more difficulties in interpreting these data in terms of welfare. It is highly probable some of these systems will make a significant contribution to the assessment of welfare (and stress research) in the future.

Although all behavioural methods may have their limitations, we believe the best approach to objectively assess welfare is a multi-pronged attack using both behavioural and physiological methods, to experimentally manipulate fear, frustration and boredom, combined with rigorously controlled preference testing and operant conditioning techniques. However, it must be borne in mind that the scientist's overall aim is not to develop the world's best diagnostic welfare test, but to gain an understanding of pig behaviour and physiology, especially their function in the interaction of the pig with its environment. Although there has been some attempt in this review to separate the physiological and behavioural contributions, this has not always been successful or warranted. For example, the aetiology of the chronic stress response observed in pigs housed in certain types of tether stalls is behavioural in origin, stereotypies are associated with changes in corticosteroid concentrations and may have a physiological (ß-endorphin) reinforcement, and frustration produces both behavioural and physiological changes. It is probably these types of studies, i.e. those using a multidisciplinary approach, that have contributed most to our understanding of animal responses and welfare and this is the approach that should be pursued in the future.

Intensive animal production has developed rapidly in the past 20 years with the main goal of cost effective production. The developments have generally been *ad hoc* with no thorough understanding of the consequences for the animal and this has raised some community disquiet. It is the role of the animal scientist interested in animal welfare to objectively assess welfare so that the validity of current practices can be tested and to suggest modifications, when required, to improve animal welfare. This research is continuing, species specific, expensive and challenging.

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RELATIONSHIP BETWEEN ADRENAL RESPONSIVENESS AND GROWTH RATE

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One way to reduce the impact of a pig's stress response to its environment, and thus ensure that its welfare is not at risk, and that potential productivity is maximized, is to select pigs that are physiologically and genetically best suited to the environment. It has been shown that large repeatable differences in adrenocortical responsiveness to a standard dose of adrenocorticotrophic hormone (ACTH) occur between pigs of the same age, weight,sex and genetic origin (Hennessy et al.,1984).

This trial was designed to relate adrenal responsiveness to growth rate and feed conversion efficiency (FCE). To test for potential response to stress approximately 1200 two-to-three week old pigs were bled by jugular venipunture, tagged, weighed and injected intramuscularly with 6.75 i.u. synthetic ACTH. A second blood sample was taken one hour later. Serum cortisol concentrations were measured using a validated radioimmunoassay. The individual males and females that fell in either, the highest, median or lowest 15% of the ranked post-ACTH cortisol levels were used for the growth and FCE study. The pigs were weighed at weaning and randomly allocated within response groups into pens of 22 to 23. They were reweighed at 9, 15 and 21 weeks of age. The feed given to each pen between 9 and 21 weeks was recorded and pen FCE calculated. The results are summarised in the table.

Table 1. Summary of growth and feed conversion data. Data are the mean ±s.d. for 4 pens of about 22 pigs.

Reponse	Weight in kg at			Total gain (kg)	FCE
Group	3 wks	9 wks	21 wks	9 to 21 wks	g gain/kg feed
FEMALES					
Low	4.44 [a]	27.13 [a]	78.02 [a]	50.88 [a]	354.50 [a]
	±0.32	±1.16	±3.19	±2.59	±31.14
Median	3.98 [b]	25.62 [b]	74.61 [b]	48.85 [b]	319.92 [b]
	±0.40	±1.54	±3.77	±2.39	±40.82
High	3.60 [c]	23.56 [c]	69.39 [c]	45.73 [c]	314.54 [b]
	±0.42	±1.74	±2.98	±1.63	±25.82
MALES					
Low	4.51 [a]	27.94 [a]	82.73 [a]	55.04 [a]	371.69 [a]
	±0.20	±0.63	±1.39	±1.84	±48.19
Median	4.07 [b]	25.97 [b]	77.73 [b]	51.77 [b]	347.89 [b]
	±0.47	±1.51	±2.56	±1.91	±39.31
High	3.70 [c]	24.45 [c]	74.88 [c]	50.59 [b]	333.32 [b]
	±0.24	±0.66	±1.02	±1.23	±33.53

* Within sexes different letters in the same column denote significance at $P<0.01$

In summary, there are positive benefits associated with pigs that have a low potential to respond to stress. It is suggested that the conditions in intensive piggeries may be perceived by the majority of growing stock as stressful. The pig's reaction is to evoke a stress response, resulting in the secretion of cortisol. This response is associated with reductions in growth rate and FCE. The greater the stress response the greater the decrease in production. It follows then that selecting animals that have a low potential to respond to stress will lead to an increase in productivity and to an improvement in the welfare status of the individuals in that unit.

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PITUITARY AND ADRENAL RESPONSES TO CORTICOTROPHIN RELEASING FACTOR IN PIGS

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Differences in adrenocortical response to a standard dose of adrenocorticotrophic hormone (ACTH) have been demonstrated in pigs of the same age, weight, sex and genetic origin (Hennessy et al., 1984). Furthermore, the magnitude of the adrenal response was consistent and repeatable in individual animals. Since the adrenal response to ACTH is only one facet of an animal's response to stressful stimuli, we were interested to know whether the pituitary might attenuate the differences in adrenocortical response to ACTH seen between individual pigs. The present experiments were conducted to examine the pituitary and adrenal responses to corticotrophin releasing factor (CRF) in pigs with either a high (H) or low (L) adrenocortical response to exogenous ACTH.

Eight Large White x Landrace female pigs (4 H- and 4 L-responders), aged 10 weeks were selected from a commercial piggery on the basis of their adrenocortical response to exogenous ACTH. Plasma cortisol concentrations one hour after injection with 25 i.u. ACTH were: H-responders, 524±32 (mean ± s d) nmol/l; L-responders, 243±27 nmol/1. Under halothane anaesthesia each of the 8 pigs had a catheter placed in a lateral abdominal vein.

Synthetic human CRF (Peninsula Laboratories, California, U.S.A.) was given as an intravenous bolus at dose rates of 0, 0.01, 0.05, 0.1, 1.0, and 2.0 µg/kg bodyweight. Blood samples (4ml) were collected at known times before and after injection of CRF, and were placed in cold tubes containing ethylenediaminetetracetic acid and 0.1 M N-ethylmaleimide. Plasma cortisol concentrations were measured by a validated radioimmunoassay (RIA). ACTH was measured using a commercial RIA kit (Diagnostic Systems Laboratories Inc., Texas, U.S.A.).

There was no significant difference between the H- and L-responders for either the basal plasma concentrations of ACTH (18.0±2.9 vs. 20.2±3.4 pg/ml) or cortisol (63.5±5.6 vs. 53.7±3.8 nmol/l), and neither group showed a response to the control injection. CRF elicited a dose-related increase in ACTH and cortisol in both H- and L-responders. Plasma ACTH concentrations at the same dose rates of CRF were not significantly different between the two responder groups. However, the peak concentration of plasma cortisol after 1.0 µg/kg CRF injection was markedly higher in the H-responders (185.6±7.5 nmol/1) than in the L-responders (103.8±10.2 nmol/1). Thus, while CRF produced a lower maximal adrenocortical response than did exogenous ACTH the relative differences in response between the H- and L-responders were similar following both CRF and ACTH stimulation. This suggests that the differences in adrenocortical response to exogenous ACTH, seen between individual pigs, are not attenuated by variations of pituitary response. It also suggests that these differences in adrenocortical response to ACTH are an accurate reflection of that animal's perception of, and response to, stressful stimuli.

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DO DAY OLD PIGLETS NEED TO HAVE THEIR TEETH CLIPPED ?

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It is well documented (Hartsock and Graves, 1976) that piglets fight to establish a teat order following birth. Teeth clipping prevents injury to littermates and the dam's udder but there is only anecdotal assessment of this procedure (Taylor, 1985). The objectives of the study were to observe the effects of needle teeth clipping on (i) the behaviour of piglets; (ii) damage to the sow and litter; and (iii) growth rate of the piglets.

This study was undertaken during summer at the Queensland University Veterinary Science farm 90-sow piggery, Pinjarra Hills, Australia. Large White, Landrace crossbred sows and litters were grouped as follows, with 4 litters in each group: (a) Clipped Group, Small Litters (CS); (b) clipped group, large litters (CL); (c) unclipped group, small litters (US); and (d) unclipped group, large litters (UL). Small litters had less than 8 piglets and large litters had 8 or more piglets per litter.

The sows and litters were observed in the piggery's farrowing area in farrowing pens between 0900h and 1100h. Observations occurred on the morning of birth or the next morning for 60min including a nursing period, and on day 7 for a further 60min. During this period the room temperature averaged 29°C. The behavioural patterns; nursing times, damage scores and growth rates of all groups were recorded, analysed and compared. Damage scores were rated from1-4 (no scratches on body or face of sow or piglet to severe wounds on body or face).

Only in the case of agonistic behavioural patterns (fight, bite, push, and repeated pushing) was there a significant interaction between teeth clipping and litter size ($P<0.01$). Table 1 shows means from split plot analysis. The CL showed the most agonistic behaviour followed by the US, UL and CS groups. The nursing behaviour, damage scores of the sows and litters, and growth rates were not affected by teeth clipping or the litter size. Since the piglets nursed normally (about once/ hr) their weaning weight did not significantly alter. Also, as the overall growth rates indicated no differences between the teeth clipping regimes, the decision to clip or not to clip the teeth did not affect productivity. The husbandry procedure of teeth clipping does not stress the piglets enough to affect their growth and behaviour.

During late winter to early spring a north Queensland piggery left the needle teeth of 40 litters (435 piglets) unclipped. Minor marking occurred in most litters and 6 litters had bad marking. Four of these sows had teats chewed off, and two of them lost all teats.

If litters can be easily monitored, clipping could cease in the absence of excessive agonistic behaviour. Where fighting occurs, teeth should be clipped. As the procedure does not appear to be unduly stressful many farmers may prefer to clip routinely in case fighting begins several days later.

Table 1. Table of means from split plot in time analyses of agonistic behaviour, nursing behaviour periods and damage scores of sows and litters

Measurement	CS	CL	US	UL	Mean C	Mean U
Agonistic behaviour/h	2.88	7.88	6.73	4.89	5.38	5.82
Nursing periods (min)	3.31	3.62	4.25	3.37	3.46	3.81
Damage score sow	1.00	1.50	1.12	1.37	1.25	1.25
Damage score litter	1.87	2.62	1.62	1.87	2.25	1.75

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AN ASSOCIATION BETWEEN PLASMA CORTICOSTEROIDS AND PERFORMANCE OF STEREOTYPIC BEHAVIOUR IN TETHERED SOWS

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The ability of pigs to manipulate a chain when frustrated is associated with a reduction in plasma corticosteroid concentrations (CS) (Dantzer and Mormede,1983), and presumably is an adaptive mechanism whereby detrimental consequences of elevated CS are obviated. Large variation has been observed in levels of stereotypic behaviours (SB) in tethered sows (27 min. to 10.7 hours per day; Cronin and Wiepkema, 1984) and the following experiment was to test the hypothesis that high levels of SB are associated with low CS.

Sixteen tethered and 15 group-housed (groups of 7 and 8) sows were studied. The behaviour of individual sows was monitored via direct observation for 20 min. each week, in weeks eight, nine and ten of the experiment. The sows were then cannulated and four to five days later bled at hourly intervals from 0800-1700h.

The tethered sows were more active than the group-housed sows (mean $\pm$ s.e.; 54.4$\pm$5.14 and 31.4 $\pm$ 4.09% of observation time, respectively; t=3.48, P<0.01), particularly due to increased performance of SB (26.5 $\pm$5.52 and 8.6$\pm$2.29% of observation time, respectively; t=2.93, P<0.01) and drinking (4.6$\pm$1.33 and 1.7$\pm$0.68% of observation time, respectively; t=1.91, P>0.05). Free CS (mean $\pm$ s.e.) for sows in the two housing treatments were 3.5 $\pm$ 0.13 and 2.3 $\pm$ 0.14 ng/ml, respectively (t=6.38, P<0.001; tethered and group-housed sows).

Within the tether treatment, the level of SB plus drinking performed by individual sows was negatively correlated with free CS (Table 1). (In the group treatment, only total activity was correlated with CS; r=+0.51, P<0.05, n=15). These data support the hypothesis that the level of performance of SB (and drinking) by tethered sows was inversely correlated with the free CS. This finding raises several questions relevant to animal welfare. Of particular interest is whether welfare is at risk if sows engage in high levels of SB and consequently reduce CS, and whether high SB and high CS can occur concurrently. Further, it is not known if SB are a consequence of high CS or if they are independent in origin, and if so how ß-endorphins may be involved in their development as suggested by Cronin et al.(1986).

Table 1. Correlation coefficients for behaviours and free corticosteroid concentrations in tethered sows.

Stereotypies	Stereotypies plus drinking	Total Activity
-0.55(P<0.-0-5)	-0.63(P<0.01)	-0.77(P<0.01)

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SEASONAL VARIATION IN ADRENAL RESPONSIVENESS TO ACTH CHALLENGE IN THE PIG

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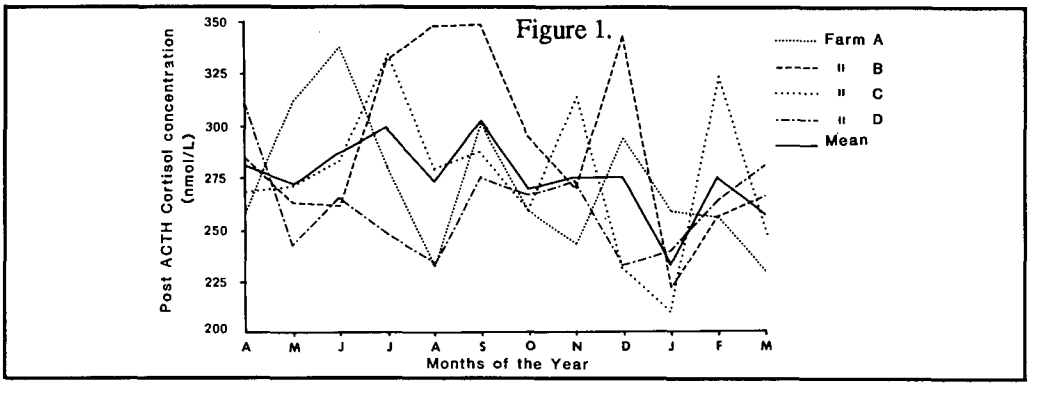
This trial forms part of an overall programme aimed at developing a "Dynamic Stress Test" kit for the pig industry to assist in the selection of pigs most adapted to their environment. The existence of diurnal variation in basal plasma cortisol concentration has been well documented in the pig (Barnett et al., 1981). These authors also showed that the diurnal pattern of cortisol was influenced by photoperiod. Kaneko et al. (1981) showed that the responsiveness of the rat adrenal gland to adrenocorticotrophic hormone (ACTH) varied diurnally. Given this diurnal variation in the adrenal gland's activity and that it can be influenced by photoperiod, this trial was established to determine whether there was a seasonal pattern inherent in the ability of the pig to respond to an homogeneous stress challenge. This information was needed to allow further development of a diagnostic kit for use as an aid in selecting breeding stock that are physiologically most suited to their environment.

Forty-eight animals per month, on each of four geographically isolated commercial piggeries in Victoria were tested over a period of twelve months. Equal numbers of male and females in the 55-65 kg weight range (approximately 16 weeks of age) were used in the experiment. Eight boars were chosen from each of three pens and eight gilts were chosen from each of another three pens. Each pig was bled via jugular venipuncture, to determine basal adrenal activity, followed by an intramuscular injection of 25 i.u. synthetic ACTH. A second blood sample was taken one hour later to measure potential adrenal activity. Serum cortisol concentrations were then analyzed using a validated double antibody radioimmunoassay, and the results collated and analyzed.

The mean response to ACTH seen on each farm for each month is shown in Figure 1. A hierarchical analysis of variance revealed highly significant differences in adrenal response between farms, months and sex ($P<0.001$), and a significant month by farm interaction ($P<0.001$), but the variation between months was not correlated with the environmental temperature.



In conclusion, the results failed to show a clear seasonal trend in adrenal responsiveness to ACTH. However, the considerable variation in the mean adrenal response of small groups (n=8) of pigs will need to be taken into account when developing the on-farm "Dynamic Stress Test".

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EMBRYONIC LOSS IN THE PIG: AN ENIGMA

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Embryonic loss is an elephant. It is the same elephant as described by each of a group of blind men. One blind man who grasped the leg in his arms said it was like a tree, another felt the trunk and said it was a large fire hose,a third touched the tusk and described it as a spear and the fourth thought it was a rope as he held on to the tail.They were each correct but they were each wrong. Embryonic loss in the eyes of the nutritionist is a result of improper feeding practice.The veterinarian declares that subclinical endometritis or an infectious organism is the cause, while an injection of the proper combination of hormones will cure it, according to the endocrinologist. An undesirable set of genes that can be selected against is responsible proclaims the geneticist. Maternal-embryo histoincompatability explains it, says the immunologist.The cytogeneticist finds chromosomal aberrations and deduces that these errors are at fault. Each may be correct but possibly each is also wrong.The elephant of embryonic mortality may be even more complex when viewed individually by the many research workers who have studied it over the years, or it may be many factors acting through a relatively common mechanism to produce one result, loss of some embryos.

There have been many reviews of embryonic mortality. Some reviews have attempted to objectively report observations of researchers while avoiding being a proponent for a particular point of view. Others have expressed a bias, which in some cases has provoked discussion and further research (Hanly,1961; Bishop,1964; Hunter,1966; Zimmerman,1972; Scofield et al.,1974; Bazer et al.,1982; King,1985; Pope and First, 1985; Wilmut et al.,1986). For several years, in an attempt to narrow the field, I tried to determine what might not cause embryo death. In this treatise I will attempt to explain why some factors are not likely to be major causes and why some are more likely than others to be implicated in a significant part of embryonic loss. An explanation will be offered as to how some factors might act to cause the loss of embryos. We will also consider the possibility of reducing embryonic loss; can something be done?

So that there will be a clear understanding of the limits of the discussion, I will define embryonic loss as that part of prenatal loss that takes place while the embryo is differentiating, in contrast to foetal loss which occurs during the period of growth after differentiation. For the sake of the discussion I will arbitrarily consider loss before day 30 of gestation in the pig as embryonic and loss after day 30 as foetal, although such a sharp distinction cannot be made on a physiological basis. Embryo losses are usually calculated on the basis of the proportion of ovulation points or embryos transferred that are not represented by live embryos at 30 days. This assumes that the number of corpora lutea at ovulation and at day 30 are the same and that each represents one potential embryo. There are possibilities of errors in these counts, so prudence dictates that absolute numbers of corpora lutea or numbers of embryos classified as normal at transfer be viewed with healthy scepticism. Any researcher who claims to have made no mistakes in counting or classifying has not examined enough ovaries and embryos to recognize the possibilities of such errors.

One of the reasons for attempting to distinguish embryonic from foetal loss, out of the total prenatal loss, is that there appears to be different causes for losses in the two stages. As the discussion progresses it is important to keep this distinction in mind. Embryonic loss is characterized in several ways. In polytocous animals, such as the pig, the percentage of the potential litter lost can vary from 0% to possibly 100% but the mean is about 30% regardless of the number of embryos (Hanly, 1961). Deviations from the mean occur under many seemingly unrelated conditions or for no apparent reason. What is not clear at this time is how embryonic survival can be affected by several factors that are apparently so unrelated. Is it possible that there is one integrative factor that can have a major effect on embryo survival? A very practical question centres on the possibility of preventing embryonic loss and thereby salvaging the embryos and increasing

litter size.

About 20% of litters have no loss. It appears that embryo loss is not a requirement for each litter. The death of one embryo is not necessary so that another one may live. There is no evidence to suggest that an embryo will be more likely to survive in one segment or position of the uterus than in another. Some embryos survive to day 30 and others perish. Embryonic loss is ubiquitous, is lowly repeatable and heritable, usually affects only part of the litter and is neither predictable nor infectious. It can be influenced by the male, the time of insemination relative to ovulation, semen handling, diet and environmental temperature. Any satisfactory explanation must account for these known factors. To perhaps oversimplify a solution to the mystery I will speculate at the beginning, that "The butler did it, with some help", and then try to justify the statement as the discussion proceeds. The butler in this case is asynchrony in development between members of a litter and between mother and embryos. The importance of synchrony between stage of the uterus and stage of the embryo for survival of the embryo has been known for some time.

The idea that embryonic mortality may be due to asynchrony of develpment between members of a litter or between embryos and the uterus is not new (Doyle et al.,1963; Webel et al.,1970; Pope and First,1985; Wilmut et al.,1986; Liehman and Fulka,1986; Techakumphu et al.,1987). The evidence leading to asynchrony as a major contributing factor in embryonic loss is quite strong. Resultant asynchrony provides a unifying explanation for such different indirect causes as influence of semen extenders or maternal diet. The embryo is dynamic, changing almost hourly morphologically, metabolically and in the genetic control for development (Hunter,1974; Anderson,1978; King and Ackerley,1985; Gadsby et al.,1980; Nieder et al., 1987). The uterus in turn must be dynamic to recognize the presence and accommodate the changing needs of the embryo (Bazer,1975; Geisert et al., 1982). The uterus must respond to signals from the embryo and the embryo must in turn respond to signals from the uterus (Cook and Hunter,1978; Flint et al.,1982). We will assume that most of these signals are chemicals, mediated through hormones, enzymes and other compounds and various combinations of these chemical mediators (Heap et al.,1981; Ford and Stice,1985). Intrinsic to the success of the entire embryo-maternal interaction is the need for synchrony between the stage of the embryos and the stage of the uterus. An embryo that fails to send or respond to a signal or does so at an inappropriate stage of uterine development will perish. An embryo that cannot compete successfully with its siblings for limited resources will also perish.

The proportion of embryos lost seems, in general, to be uninfluenced by the number of embryos. This holds true whether the number is smaller than usual (Dziuk,1968; Webel and Dziuk,1974; Hagen and Kephart,1980) or if it is larger than normal. Increasing the number of embryos by superovulation, transfer of super numbers of embryos (Pope et al.,1972), adding embryos to the existing litter (Dziuk,1968) or by a spontaneously greater number of eggs ovulated as in hyperprolific strains or prolific pigs of Chinese origin (Cheng, 1983) has not significantly changed the proportion of embryos lost (Hunter, 1966; Zimmerman,1972; Webel and Dziuk,1974; Gautherie,1976). By virtue of the fact that there were more embryos to start with, the absolute number lost would be greater but the proportion remains the same. The observation that the proportion of embryos surviving to day 30 stays the same with very large numbers of embryos or with small numbers would lead one to think that loss of embryos is unlikely to result from a quantitative deficiency of some factor, nutrient or compound. This view is also supported by noting that smaller litters with a greater proportion of total resources available to each embryo have the same losses as usual sized litters. When many embryos are confined to a restricted segment of the uterus the survival rate is no different from that when embryos have more of the uterus available than usual (Dziuk, 1968). If a deficiency of some kind existed as a limiting factor to embryonic survival then additional embryos would not survive to day 30. Because a larger than normal number of embryos can survive to day 30 it seems reasonable to look for causes other than number of embryos or lack of uterine support to explain embryonic loss. A larger number of embryos per uterus, uterine horn or segment does create a relatively crowded condition but this crowding does not influence embryonic survival. It is important to remember that insufficient space and unequal spacing can affect foetal survival and crowding does have a profound effect on foetal survival

(Webel and Dziuk,1974; Dziuk,1985). A number of experiments modifying the space available per embryo has shown conclusively that up to about day 30 in the pig the number of embryos or the proportion of the uterus available to each embryo has little influence on the proportion that are lost. An equally large number of experiments have shown that the addition of embryos to an existing pregnancy or production of more than the usual number of embryos by exogenous hormonal or genetic selection for a large number of ovulations has not consistently produced larger litters (Legault and Gruard, 1976; Johnson,1984). On the basis of this evidence we can conclude that intrauterine crowding contributes very little to embryonic loss but does have a profound affect on foetal loss. Elimination of foetal crowding as a major factor in embryonic loss or part of the elephant, does not solve the question of what are possible causes for embryonic loss, nor does it help us understand how these causes can be integrated to provide a unifying explanation, or to further the analogy, a whole elephant.

Infectious organisms can and do influence survival of some or all of the foetuses in a litter. Such effects can be curbed by proper preventive practices (Scofield et al.,1974). The loss of foetuses from an infectious disease or toxic condition should not be confused with loss of part of a litter at the embryo stage (Wrathall,1984). Because only part of the litter of embryos is lost, because embryonic loss is so ubiquitous and because there has been no organism or infectious condition identified with embryonic loss, there seems to be few compelling reasons to consider the condition to be easily alleviated by a vaccine, prophylactic treatment, hygiene or an antibiotic.

Embryonic loss after isolation, mircroscopic examination and transfer of only apparently normal embryos is little different from that noted after a usual pregnancy in which no sorting of embryos takes place. To the extent that embryos may be determined to be normal at the stage of transfer, these observations would indicate that the proportion of early losses that are due to detectable oocyte abnormalities is quite small (Webel et al.,1970; Pope et al.,1972.,Polge,1982). Results of examination of recovered oocytes by present microscopic methods also indicate that relatively few oocytes can be predicted to be abnormal and certainly not enough to account for the 30% loss seen (Fechheimer, 1968; Hunter, 1976).

It seems unlikely that embryonic loss can be resolved into a one-item-cause-one-result-phenomenon. A more plausible consideration might be that several factors impinge through a common mechanism to produce a common result. After all, death of all warm blooded animals is due eventually to lack of oxygen to the brain.This lack can be brought about by many means just as embryo death may have many paths.

VARIATION IN RATE OF DEVELOPMENT OF EMBRYOS

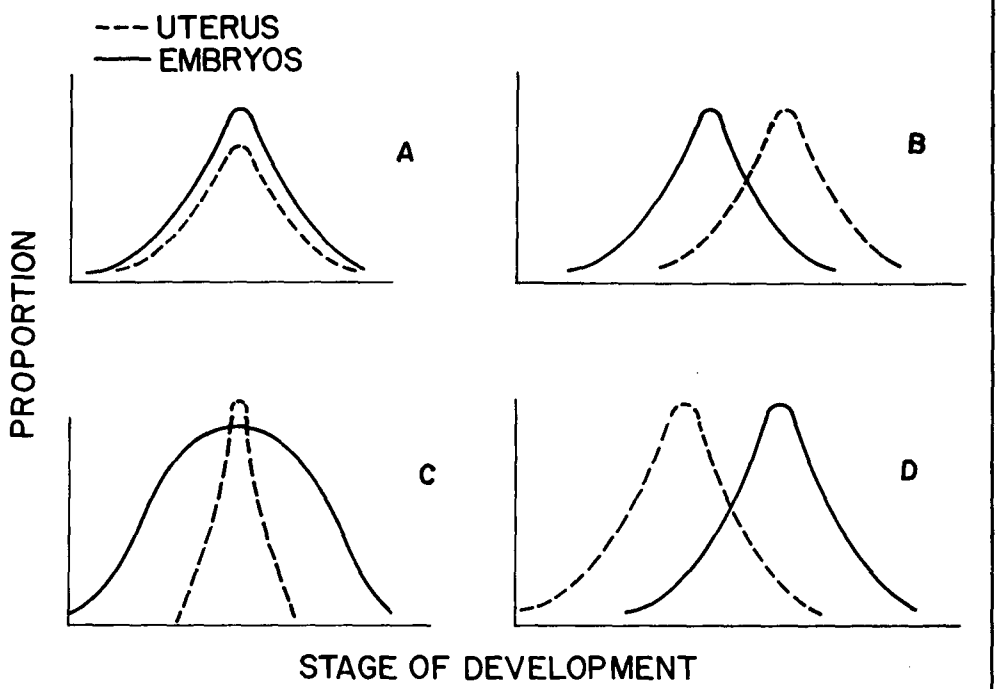
Is there evidence that embryos of the same age vary in their stage of development? Yes, the stage of development of embryos can vary considerably within a litter. At about day 12 of gestation when implantation and rapid growth of the trophoblast is taking place, some embryos are partially elongated whereas others are spherical (Hunter,1974; Anderson, 1978; Polge,1982). It seems to be more the rule rather than the exception that stage of development among embryos is unequal.Embryos from different females mated at the same time and presumably ovulating near the same time can be at different stages of development. Rates of development have been shown in the mouse to be genetically controlled (Whitten and Dagg, 1961; Kaleta,1977; Warner,1986; Warner et al.,1987). There is much evidence for variation in stage of development at days 10 to 12 in the pig. Genetic control of variation in the rate of embryo development in the pig has not been documented but there is no reason to think that genetics do not influence rate of development. Genetics of blood groups has an association with litter size (Rasmussen,1983), although the causes of the relationship were not established. The role of immunological incompatability is unresolved (Koch, 1985).

As stated earlier, asynchrony between members of a litter and between the mother may be a link between seemingly unrelated factors. How can it be that the stage of development of embryos is not uniform within a litter? How can the uterus develop asynchronously with the embryos? Both the embryo and uterus undergo dramatic changes almost from the time of fertilization. The

phenomenal growth of the trophoblast from a spherical structure a few mm in diameter at 11 days to an elongated one, 20 to 30 cm long at 13 days, attests to the rapidity of change even in morphology at that critical point. It is at about day 12 when the embryo elongates, signals the uterus of its presence, stops its intrauterine movement and establishes its home for the next 100 days (Dziuk,1985). Embryos in a litter that have developed at the 'normal' rate would elongate and pre-empt the available space leaving no spot for 'slow' embryos. Slow embryos would find the uterus inhospitable. The 'fast' embryos in turn may have arrived in an inhospitable uterus that is not in synchrony with them and are lost. In either case the embryos that did not survive were not necessarily defective, they were just out of step. This concept mitigates against the hypothesis that dead embryos were genetically defective (Bishop,1964). An analogy that may apply would be cross fostering a pig at birth into another litter of pigs born 48 hours earlier.The teats are already claimed, the colostrum is about gone, the mother may not recognize the newcomer and its new litter mates are already hours and a few jumps ahead. It will likely not live. There was nothing intrinsically defective in this pig, it was at the right place but at the wrong time just as an extra early or late embryo could be at day 12.

Synchrony between embryos within a litter and between embryos and the uterus need not be within minutes of each other for survival of the embryos because changes are not likely to occur in minutes. Differences can be as much as 48 hours, but when asynchrony becomes greater than 48 hours, embryonic loss increases to nearly 100%. A little asynchrony is tolerated, too much is not tolerated and synchrony gives optimal survival. Both the uterus and the embryo can make a little adjustment. Only those embryos that match the uterus will survive. This concept is depicted in Figure1.

Figure 1. Schematic presentation of synchrony between embryos (-) and uterus (----). Perfect synchrony (A) with possibly little loss of embryos. Uterus further developed than embryos (B) with loss of that proportion of embryos falling outside the range of synchrony. Embryos ahead of uterus (D) with loss as in B. Embryos with a wide range of development (C) both ahead of and behind a uterus with a narrow range.



With this concept in mind any skewing of the curves would also reduce embryo survival. Anything that would advance or retard development of either the uterus or some or all of the embryos would influence the proportion of embryos that survive. Because there is little evidence, to my knowledge, that one part of the uterus differs much from another part in its stages of development, there should be less of a problem as a result of variation within the uterus. The development of the entire uterus might be advanced or retarded by fluctuations in production of hormones from the ovary, exogenous hormones from the embryo, or by changing rates and paths of metabolism of hormones thereby changing concentrations of critical materials. Development of the uterus in this case includes all that is implicated by uterine environment. There are several possibilities of mechanisms by which some apparently normal embryos die and others survive within the same litter.

It is usually conceded that nearly all eggs are fertilized. This assumption may be uncertain and needs careful scrutiny but it is the present state of knowledge. Gametes have finite lives. Some boar sperm may live in the female genital tract for 48 hours. The ovulated oocyte must be fertilised within 10 hours or it will produce an embryo that will probably die (Hunter, 1976). The aged oocyte may be penetrated by more than one sperm with the result that the embryo dies. Polyspermy seems to be much more frequent when inseminations occur after ovulation or when the number of sperm in contact with the eggs is large at the time of penetration. The real problem under conditions of late mating, or when the normally restrictive utero-tubal junction is bypassed by surgery, or the restrictive effect is mollified by administration of progesterone, is the presence of too many sperm at the site of fertilization (Day and Polge,1968; Hunter,1973). Abnormally high levels of progesterone will cause the egg to enter a site in the oviduct or uterus with a very high number of sperm. It matters little whether the progesterone is from an endogenous source, as from a luteinized follicle produced by an excess of gonadotropins, or from an exogenous source. The effect will be the same, polyspermy. Not every egg will be polyspermic as a result of an excess of sperm but the proportion will be higher than that following a mating which precedes ovulation and where the timing and numbers of sperm are normal. An egg that is fertilized within an hour or so after ovulation will not be synchronous with an egg fertilized 10 hours later even if the late one is not polyspermic. This 10 hour difference may be critical if each embryo is competing for limited timed resources and must be synchronous with the uterus. The stage of development of the uterus may coincide with that of some embryos but is either too early or late for others.

Insemination too late is definitely not conducive to maximum embryo survival (Hunter,1967). Equally adverse is an insemination either before oestrus or at the very onset (Boender,1966). Timing of insemination too early has also been found to increase embryonic loss and reduce litter size. When the period from insemination to ovulation is about 13 hours, fertilization and litter size are maximum.When insemination is more than 13 hours before ovulation, fertility and litter size decline (Martin and Dziuk,1977). One explanation proffered for the adverse effect of early insemination is that the sperm are too old by the time they must fertilize the egg. Another explanation may lie in the fact that there are so few sperm at the site of fertilization that sperm-egg collisions are so infrequent that fertilization of all the eggs extends over a considerable period. The embryos in the same litter resulting from this extended fertilization are then at several different stages. This extended period of fertilization may also occur under any condition that effectively reduces the number of sperm at the site of fertilization at the time of ovulation. These conditions could include *in vitro* storage of sperm, deep freezing of sperm, the effect of components of semen extenders, the fertility of the male, and the concentration of sperm inseminated (Dziuk,1959; Maurer and Beier,1976; Christenson,1986; Robl and Dziuk,1987). Results of these studies could lead one to believe that the timing of insemination is a tenable explanation for embryo loss, since the interval from insemination to ovulation has a marked influence on the conception rate, embryonic loss and litter size.

Clearly, inseminations occurring at times in the oestrous cycle far removed from the time of ovulation will be infertile. As deviation from the optimal time for insemination relative to ovulation decreases, fertility will increase and embryonic survival will increase. Any condition that leads to inseminations at times other than the optimal one will have an adverse effect on embryonic

survival. Such conditions might include inaccurate detection of oestrus, forced matings and single matings at the very onset or end of oestrus. The uterus develops at a rate determined by the development of the corpus luteum from the time of ovulation and really cannot adjust completely to variations in embryos resulting from variation in the interval to fertilization. The uterus goes through the same changes up to day 9 in both pregnant and nonpregnant gilts. Some embryos may be synchronous with the uterus in cases of prolonged fertilization and some may not. Earlier work (Krzanowska,1964; Kaleta, 1977) also shows how mouse embryos can vary in the stage of development due to the slow penetration of sperm from certain males. If the period from ovulation of the first egg to ovulation of the last egg were extended over a period of 8 or 10 hours and fertilization occurred shortly after ovulation in each case, the result would also be variation in stage of development. The uterus could not be at the correct stage for all embryos resulting from eggs ovulated and fertilized over an extended period.

The same relationship holds for number of sperm at the site and time of fertilization. Double insemination of gilts with semen from two boars whose offspring were distinguishable, showed that the boar used 6 hours earlier than the optimum interval produced fewer offspring than the boar used nearer the optimum interval (Martin and Dziuk,1977). Studies of other species such as the rabbit also show the importance of time of insemination and presumably the numbers of sperm.

The proportion of sperm from each male in an insemination of mixed semen gives a very consistent and predictable proportion of offspring from each male. Not every sperm from each male is equally effective in fertilizing when compared to sperm from a competing male. Insemination of a mixture of equal numbers of sperm from two males will invariably produce litters in which the proportion of offspring fom the males will not be equal. In many cases, the proportion will be very skewed (Roche et al.,1968). If the proportion of sperm is adjusted to account for the apparent disparity, the proportion of offspring will be equal (Martin and Dziuk,1977). As an example, when a mixture of sperm numbers are in a 1:1 ratio from males A and B and resulting offspring are in a ratio of 2A:1B, changing the ratio of sperm to 1A:2B will produce a 1:1 ratio of offspring. This indicates strongly that even though the absolute number of sperm from each male are equalized as in the first example given, sperm from male A are twice as likely to be the first to fertilize an egg in competion with sperm from male B. This same relationship for males carries over to embryonic loss. Males whose sperm do not compete well have greater embyronic loss (Boender,1966). The greater embryonic loss can be attributed to fewer effective sperm at the site of fertilization, with the consequent extended period of fertilization resulting in embryos not synchronous within a litter and not synchronous with the stage of development in the uterus (Krzanowska,1964).

Litter size in pigs can be affected noticeably by the boar. The effect of the boar could be a reflection of the proportion of his sperm that can effectively fertilize eggs (Boender,1966; Olivier and Legault,1967). On the basis of the evidence presented thus far it is not possible to sort out the effect of chromosomal aberrations, gene defects or genetic control of rate of development from the effect of an extended period of fertilization (Hunter, 1976). Whatever the case the results will be similar.

Does asynchrony just happen or are there some possible explanations? Embryonic losses are greater when sperm have been stored for some time, when sperm have been stored under adverse conditions of extender or temperature, when males produce low numbers of sperm or when inseminations are done at a greater interval from ovulation than one that produces optimal survival. All of these factors can increase the period from fertilization of the first egg, soon after ovulation, to fertilization of the last egg to as much as 8 or 10 hours. This variation in the moment of initiation of development may persist to those critical times at implantation and establishment of pregnancy. Observations on mice (Robl and Dziuk,1987) show that reducing the concentration of sperm reduces the rate at which eggs are fertilized and the strain of the male can also have an effect. Similar observations have been made in pigs. A reduced concentration of sperm reduced the number of sperm on eggs and retarded stage of development (Baker et al., 1968).

Fertilization is a phenomenon governed by numbers of sperm and time. The greater the concentration of sperm capable of fertilizing an egg at the site of fertilization, the shorter the time

to fertilization of all eggs. Any treatment or condition that reduces this concentration of sperm capable of fertilization will prolong or delay the interval from ovulation to fertilization.

SHIFTING OF THE STAGE OF DEVELOPMENT OF THE UTERUS

The question still remains how factors that affect the newly pregnant gilt can influence the proportion of embryos that survive. Because there have been few, if any, studies documenting changes in the uterus that have a known effect on survival of embryos, we can only rely on circumstantial evidence to develop a hypothesis rather than a postulate. Progesterone is required but the concentration can be much lower or higher than the normal level and still maintain pregnancy (Ellicott and Dziuk,1973; Webel et al.,1975; Thomford et al.,1984). Reducing the concentration of progesterone in the plasma by ovariectomy and replacement at day 5 or attempting to reduce it by removing many of the corpora lutea, has not necessarily disrupted pregnancy until the concentration falls to a level below that seen in abortion.

There is a great deal of variation between pregnant gilts in the absolute concentration of progesterone in early gestation. There is no universal and consistent increase in embryo survival with a greater concentration of progesterone or with exogenous progesterone (Michael et al.,1975; Wildt et al.,1976; De Sa et al.,1981; Lawson and Cahill, 1983; Moore,1985). Only in certain specific isolated cases has there been a relationship between these concentrations and the proportion of embryos surviving (Rampacek et al., 1975; Archibong et al., 1987). Whatever changes might be wrought by external environment on the uterus through the action of progesterone and other steroids, have for the most part, escaped detection. Oestrogen is produced by the pig blastocyst and would seem to be important in embryo survival (Heap,1981). There is an association between the presence of embryos and oestrogen but there is little evidence that a certain concentration or production of oestrogen will ensure embryo survival. That the uterus, the endometrium and uterine secretions are under the influence of oestrogen and progesterone, has been well documented for many years. Exactly what the steroid requirements are at any stage to induce optimum survival of embryos has not yet been determined. This is a very indeterminate part of the elephant of embryonic loss but it is certain to be present and to be important.

If we consider that concentrations of steroids can influence the uterus and this in turn will affect embryonic loss, how could those environmental factors such as diet influence steroids? Whereas plane of nutrition and certain dietary ingredients have been found to influence embryo survival in some cases, the results have not been consistent (Friend et al.,1981; Cole, 1982; Knott et al.,1984). It may be that the situation is analogous to a scientific study of the cause of inebriation. The study revealed that ice plus vodka, ice plus whiskey, ice plus rum and other iced drinks caused the condition. It was then deduced that since ice was the obvious common ingredient, ice was responsible. We may thus far be studying the ice in our efforts to discover the relationship between dietary ingredients and embryonic survival. Plane of nutrition and specific dietary components have been found to influence the activity and level of steroid metabolizing enzymes (Campbell and Hayes, 1974; Clinton et al., 1977; Thomford and Dziuk, 1986; Thomas et al., 1987). These same sorts of diets have also been shown to influence hormone concentrations and fertility (Jordan and Swanson,1979; Dyck et al.,1980; Cole, 1982; Thomas et al., 1987). Although at this time the relationship between the effect of certain diets and reproduction in general and embryonic loss specifically is only associative, further work may reveal a cause and effect relationship. Certain diets and xenobiotics have been found to have an effect on the number of ovulations (Thomas et al.,1987). The common characteristic among these factors was their ability to induce steroid-metabolizing enzymes. A lessening of the inhibiting effect of steroids as a result of more rapid metabolism of steroids would allow more gonadotrophins to be secreted. This in turn would increase the number of follicles produced and ovulated. Because pregnancy depends on steroids for maintenance, any disruption of the gradual changing concentrations of progesterone, oestrogen, their derivatives and possibly other closely related compounds by extraordinary rates of metabolism would result in embryonic loss. The shift in steroid concentrations would shift the rate of development of the uterus away from synchrony relative to the embryos (Bazer et al., 1968; Schacht

and Foote,1978; Levasseur,1984). The mechanism by which this might act has not yet been fully elaborated although a number of studies have been done which implicate concentrations of steroids with a shift in uterine function (Bazer et al.,1968; Bazer,1975; Cook and Hunter,1978; Flint et al., 1982; Heap et al.,1981; Dalton and Knight,1983; Lawson and Cahill, 1983; Wilmut et al., 1986; Pope et al.,1987). Under these circumstances the uterus could be the cause for asynchrony with the same result (dead embryos) as when rate of development of embryos shifted. In addition to diet, any environmental influence that changes the rate of production or metabolism of steroids could influence embryonic survival (Wetteman and Bazer,1985).

CAN OR SHOULD SOMETHING BE DONE?

One might wonder why a loss of about 30% of embryos occurs in a wide variety of species and conditions. If this loss has a genetic basis, why has evoluntionary selection not reduced the loss? Is there some advantage to a certain amount of asynchrony with the consequent death of some embryos? Or, is there a disadvantage in very precisely timed events in development? After promoting asynchrony as a cause of embryonic loss, one would think that the writer would state no to both questions. Upon further thought, the answer might switch to yes for both questions. Survival of species depends on adaptability and flexibility. If a species had a very rigid and narrow synchrony requirement for the early complex embryo-maternal relationship, no member would meet the requirements and all would be lost. If, on the other hand, there were no requirements for synchrony, the reproductive cycles and gestations would be chaotic, and all would be lost. To go from the general to the specific, let us first deal with the uterus. A pig uterus that would allow embryos of a very specific stage of development to implant only during a period of a very few minutes at a precise interval after ovulation would support very few embryos to term because only rarely would the necessary events be synchronous. Under usual conditions, the embryos in a litter have a sufficient range of stage of development that they survive in a uterus that can accommodate the majority of embryos but eliminates the extremely precocious and the laggards. In the long run more offspring may result from a population when some loss occurs in most litters as a result of asynchrony from a range in the stage of development rather than all be lost from a too concise requirement for synchrony. The uterus responds to ovarian hormones by a steady progression of changes. It would be unusual if during the 30-day period after fertilization when embryonic loss occurs, that changes would progress at exactly the same rate in every pig and at each successive oestrous cycle. There is likely to be variation in rate from mother to mother and from time to time in the same mother. Survival of each embryo in a precisely synchronous world would of necessity require that each embryo be matched perfectly with each mother. This is very unlikely with the normal variation in the genetics and environment that influence the rate of development of both embryo and mother. An embryo that is slow in a uterus that develops rapidly will be lost as would the fast embryo in a slow uterus. However, that same slow embryo would survive in the slow uterus as would the fast embryo in the fast uterus. It seem reasonable that there would be an advantage to a litter whose members were perfectly synchronous with each other especially if the uterus could adjust its stage of development to accommodate all embryos by perfect synchrony.

Having stated this as an obvious suggestion, I will immediately recant and modify it. If all embryos were perfectly synchronous with each other and they were in a uterus that was even slightly asynchronous with them, all would be lost. Therefore there may be an advantage to some variation in rate of development of embryos with variation in the uterus. If the number of synchronous embryos were greater than the total resources that the uterus could accommodate, each embryo would claim an equal but inadequate share of those resources or space. All would eventually be lost, if not as embryos then certainly as foetuses. A little like a lifeboat with one more occupant than its carrying capacity. Again, it may be in the best interests of the mother if some embryos have an advantage over others within a litter so that at least some survive nearly every time. Selection for a genetic strain with precise and narrow synchrony of embryos that are in precise and narrow synchrony with the mother may reduce embryonic loss provided the mother is not influenced by variations in her environment, including diet. Even though such strains may have

lower embryonic loss only when matings occur within strains, such strains would not necessarily be useful in crosses with other strains because of the different rate of development between strains. Crosses between two such dissimilar strains would likely have higher than normal losses because the hybrids would not necessarily be synchronous with either mother. Even though there may be a significant genetic component associated indirectly with embryonic loss, it may not be reasonable or even possible to select directly for reduced loss (Johnson,1984). Because of the known effect of certain combinations of blood type of parents on litter size and rates of embryo development, thorough screening and careful planning could prevent some apparent losses (Warner et al.,1987). As more information becomes available mating plans might include these considerations (Rasmusen,1983).

If embryonic death is a final result of multiple indirect causes, most of which are poorly understood, then it follows that present recommendations to alleviate the loss would be poorly based. Thus, continued efforts should be exerted to try to understand the complexities of embryo-maternal relationships to provide a sound basis for recommendations. This basic information must include a reasonable encompassing explanation for the great diversity of factors which are known or suspected to have an influence on embryonic loss. This is no small task but without this information, attempted therapies are little more than shots in the dark. One treatment in one gilt may be helpful by chance whereas the same treatment in a different gilt or at the next pregnancy would only make matters worse. Too often the positive response to a treatment is published while the negative results are not. Perhaps in some cases when many tests are done the observer is led to conclude from one or two tests that the conditions imposed were responsible for the result when it possibly was just chance alone.

On the basis of existing evidence it appears that some conditions can increase embryonic loss but few really reduce it consistently. Regardless of treatment about 30% of embryos are lost. Perhaps at this stage of our knowledge, the best we can do is to avoid those conditions that apparently increase loss while trying to understand the conditions that lead to the survival of all embryos in nearly 20% of litters. It may well be that embryo survival requires a certain amount of flexibility to accommodate the variation between individuals and the uterus and that this flexibility is intrinsic to some loss. These views need not be deemed pessimistic but realistic at this time.

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A SYMPOSIUM: SEASONAL INFERTILITY IN THE PIG.

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INTRODUCTION

Seasonal infertility is a recurring and serious economic problem to the Australian pig industry. It is common in all types of Australian piggeries to see a fall in the fertility of both sows and gilts during late summer to early autumn. The onset, duration and severity of the infertility vary between piggeries (they may even vary between units on the same piggery), and from year to year. Because the decrease in fertility occurs mainly during the hotter months, it has often been referred to as "seasonal or summer infertility". While there is undoubtedly a seasonal or temperature component, it is not, as shall be shown, the only factor contributing to the onset of seasonal infertility.

The main feature of "seasonal infertility" as seen in Australia is a lower farrowing rate, i.e. a reduction in the number of mated sows and gilts that produce a litter to that mating. Affected animals either show a delayed return to service (>24 days) or a failure to return to service. Some of these pigs would be classified, by the farmer, as "not in pig" if pregnancy diagnosis was carried out. The decrease in fertility is most common in late summer to early autumn, but there are piggeries that have the clinical symptoms of seasonal infertility present for 12 months of the year (Hennessy and Williamson, 1984), or where the infertility only occurs in the cooler months of the year (Johnston, 1979).

While the main feature of seasonal infertility is a decrease in farrowing rate, other reproductive problems do occur on some herds on a seasonal basis. These are namely:

* longer weaning to mating interval
* delayed puberty and/or poor oestrous expression in gilts
* decrease in litter size
* increase in abortion rate
* increase in stillbirths and/or mummified foetuses

Before getting into the main papers of this symposium it is useful to highlight the complexity of seasonal infertility. An appreciation of the true complexity of seasonal infertility will help in understanding why there has been little real progress in trying to reproduce the symptoms of seasonal infertility in the laboratory, and in trying to alleviate the problem both here in Australia and overseas.

EARLY RESEARCH

The first reports defining seasonal infertility in Australia appeared in 1978. Paterson et al. (1978) and Love (1978), independently reported on the incidence and characteristics of a seasonally occurring drop in the farrowing rate in a commercial piggery in Western Australia and in New South Wales, respectively. These early reports concentrated on the role of heat stress. It was suggested that heat stress was the main factor causing either a hormone imbalance or resulting in whole litter loss.

The idea that heat stress is the major cause of seasonal infertility is still held in wide acceptance today. It is however a far too simplistic view of what is, in reality, a very complex problem. For example, as mentioned, there are piggeries that have the clinical symptoms of seasonal infertility present for 12 months of the year, or where the infertility is only seen during the cooler months of winter and early spring. Clearly in these cases heat stress is not involved. Another interesting observation is that some piggeries do not see any drop in fertility throughout the year. Yet these units are of the same general design and in the same general climatic environment as units

that regularly suffer seasonal infertility.

THE COMPLEXITY OF SEASONAL INFERTILITY

The first indication as to the complexity of seasonal infertility came from the study of Williamson and Hennessy (1975). Using the vaginal biopsy method of pregnancy diagnosis they showed that pigs suffering seasonal infertility could be divided into two main groups. Those that could have been pregnant when tested at 20 to 22 days after mating, and those that definitely were not pregnant at this time. Williamson et al. (1980) showed that at least three types of infertility could be detected by measuring plasma progesterone concentrations in sows 18 to 21 days after mating (see Table 1). They found that some infertile pigs had high progesterone concentrations at this stage. These pigs may have been pregnant when tested, or they may have had luteinized ovarian cysts. Those pigs with low progesterone at this time were definitely not pregnant, but when the progesterone concentration of these pigs was measured about 10 days later, another type of infertility became evident (Table 1). Some of these pigs had high progesterone concentrations, indicating that they were cycling but had not been detected in oestrus. However the majority of pigs with low progesterone at 18 to 21 days also had low concentrations at 30 days, indicating that they were not cycling and that the ovaries had become inactive. This was confirmed at postmortem examination.

Table 1. Plasma progesterone concentration in sows subsequently shown to suffer from seasonal infertility. A: Initial blood sample taken 18 to 21 days after mating to a fertile boar. B: Second blood sample taken 30 days after mating in some sows having low progesterone in the initial sample. (Data from Williamson et al. 1980).

	Total	High Progesterone ≥ 5 ng/ml	Low Progesterone < 5 ng/ml
A: Blood sample taken 18 to 21 days after mating			
Total number non pregnant	136	55	81
Normal return to oestrus (18-24 days)	17	0	17
Delayed (> 24 days) or failed return to oestrus	119	55	64
B: Second blood sample taken 30 days after mating			
Number retested	40	10	30

Johnston et al. (1982) demonstrated that infertile pigs, which had high plasma progesterone 21 days after mating, could be further subdivided into two groups by measuring the plasma concentration of oestrone sulphate (see Table 2). This hormone is produced by the developing embryo, and elevated concentrations can be detected around 28 days after mating. Pigs with high concentrations of both hormones obviously had viable embryos when tested. If those animals with high progesterone and low oestrone sulphate were pregnant then embryonic death must have occurred very early in pregnancy, yet the corpora lutea had not regressed. An alternative is that these animals were never pregnant but were suffering from stress-induced luteinized ovarian cysts.

The point made clear from all this data is that seasonal infertility is a complex problem. These data indicate that it is not simply early embryonic death or an endocrine imbalance, but rather that there are several forms of infertility each manifest in a common clinical symptom. Williamson et al. (1980) suggested that many stressors were combining and contributing to a total and detrimental stress on the pigs. The pig's response to these stressors resulted in either early embryonic death, luteinized ovarian cysts, small ovarian cysts, poor oestrous expression, or an early undetected abortion. Each of these forms of infertility may be present as a single common

clinical symptom of a delayed return or a failure to return to oestrus after mating.

Table 2. Plasma progesterone and oestrone sulphate concentrations, three to four weeks after mating, to a fertile boar, in sows subsequently shown to suffer from seasonal infertility. (Data from Johnston et al., 1982).

CATEGORY	NUMBER	POSSIBLE TYPE OF INFERTILITY
Total	81	
High progesterone and high oestrone sulphate	28	Pregnant when tested and lost litter
High progesterone and low oestrone sulphate	31	Unlikely to be pregnant when tested, possibly cystic ovaries
Low progesterone and low oestrone sulphate	22	Inactive ovaries, or poor oestrus detection

In this symposium we shall look at the main reasons suggested as causing or contributing to the complex 'jig-saw' of seasonal infertility. Undoubtedly there is a temperature or photoperiod component, and the first two papers will deal with these areas. The third paper will review the role of a generalised stress response in causing seasonal infertility, and finally the role of the boar, which is often neglected when looking for causes of seasonal infertility, will be reviewed.

THE INVOLVEMENT OF PHOTOPERIOD IN SEASONAL INFERTILITY

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INTRODUCTION

Seasonal reproduction is a common phenomenon amongst wild animals and occurs to a greater or lesser extent in some domesticated species. Strong natural selection has favoured seasonal breeding to ensure offspring are born at the time of the year when their chances of survival are greatest. The breeding season is determined by the gestation length of the species. Thus sheep and goats, with pregnancies of five to six months, are fertile during the autumn and winter while horses, with an 11 month pregnancy, are fertile during the spring and summer. The primary environmental factor controlling the breeding season is the variation in daylength (Lincoln and Short, 1980).

Daylength may be a factor in the seasonal infertility of pigs. The wild pig is clearly a seasonal breeder oriented to producing one litter per year born in the spring. This review examines the influence of photoperiod on various reproductive characteristics of the pig and relates these changes to the phenomenon of seasonal infertility.

SEASONALITY IN PIGS

The wild pig

The European wild pig (*Sus scrofa* L.), from which domestic pigs are derived, shows a clear seasonal pattern of reproduction in Europe (Mauget, 1982; Mauget and Boissin, 1987). A non-breeding season occurs during the summer and autumn months in both sows and boars. From April to June (equivalent to October to December in the Southern Hemisphere) the frequency of anoestrus amongst unmated sows increases progressively until all are anoestrus from July to September (equivalent to January to March). A uni- or bi-modal farrowing pattern is observed depending on when the sows come out of anoestrus. During the non-breeding season, testicular size and testosterone concentrations in boars are reduced (Mauget and Boissin, 1987) and prolactin concentrations are high in both sows and boars (Ravault et al., 1982).

The progression into the non-breeding season begins during a period of relatively high food availability and is observed even under stable feeding conditions (Mauget, 1982). Mean environmental temperature at the onset of the non-breeding season is only 10 °C. If nutritional or temperature factors are not responsible for triggering the non-breeding season, the most likely alternative is photoperiod, which increases from 12 to 16 hours per day as the incidence of infertility increases.

Seasonal patterns of endocrine secretion in pigs

In species that are distinct seasonal breeders, the most striking hormonal change in response to daylength is that of prolactin. Photoperiod has been shown to be the primary determinant of prolactin concentrations in rams (Pelletier , 1973) and ewes (Munro et al., 1980). Whether the high prolactin concentrations typical of anoestrus impair ovarian activity, as suggested by Walton et al (1980), is not clear. Experimental reduction of prolactin levels during anoestrus does not cause resumption of oestrous cycles (Land et al., 1980) nor influence the onset of the next breeding season (Schanbacher, 1980). The failure to find a discrete role for prolactin does not alter the fact that the elevation of basal prolactin during the non-breeding season provides a good indicator of response to photoperiod.

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In wild pigs there is a clear seasonal rise in prolactin concentrations, corresponding with the non-breeding season, in both sows and boars (Ravault et al., 1982). In domestic pigs no seasonal rise has been found in boars but a trend towards higher concentrations in summer and autumn was identified in Large White sows (Ravault et al., 1982). A seasonal increase in prolactin concentrations during summer and autumn in domestic pigs may be interpreted as an event driven by photoperiod. Anoestrus may be associated with or perhaps even mediated by this rise in prolactin.

Wrathall et al (1986) described a marked seasonal variation in progesterone concentration in sows at the same stage of pregnancy, with autumn levels being lower than at any other time of the year. Luteal production of progesterone bears a close temporal relationship to luteinizing hormone (LH) secretion (Parvizi et al, 1976) so it is likely that the seasonal pattern of progesterone reflects a seasonal pattern of LH secretion. Serum LH and GnRH in the brain were found to be significantly lower in sows that lactated in summer than those that lactated in winter, indicating that insufficient production of GnRH in summer may be a factor in seasonal anoestrus (Armstrong et al., 1986).

The effect of photoperiod on LH secretion has been best described in sheep. During the breeding season a high basal, high frequency, low amplitude pattern of LH secretion is seen while during the non-breeding season a low basal, low frequency, high amplitude pattern is seen (Goodman and Karsh., 1981).

It has been hypothesized that the mechanism responsible for the control of the seasonal pattern of LH secretion in sheep is a change in responsiveness of the systems governing tonic LH secretion to the negative feedback action of oestradiol (Karsh et al., 1980). At the transition into anoestrus, this response increases and remains high until the onset of the breeding season when the response to oestradiol again diminishes.

Paterson (1982) found a seasonal difference in the ability of oestradiol to induce ovulation in anoestrous gilts; responses being lowest during summer and early autumn and highest in winter. A recent study has shown that season also alters the responsiveness of the CNS to oestradiol in pigs, thereby controlling sexual behaviour and LH secretion (Cox et al., 1987). In this experiment ovariectomized sows received a standard dose of oestradiol benzoate intramuscularly in summer, autumn, winter and spring. Concentrations of serum LH were suppressed within six hours of oestradiol benzoate administration in all seasons, then rose to levels which varied according to season. In autumn and spring concentrations of LH often remained suppressed for longer than in summer and winter, possibly indicating an increased sensitivity to the negative feedback of the steroid during these seasons. During autumn, recovery of LH to pretreatment levels following oestradiol benzoate induced suppression was delayed and oestrous behaviour was less sensitive to oestradiol benzoate. During summer, both basal LH levels and pulse amplitude were greater than in other seasons. No seasonal differences in pulse frequency were demonstrated. However, samples were taken for only four hours at 15-minute intervals and more frequent sampling over a longer period may have revealed differences.

Artificial lighting programmes

Claus and Weiler (1985) cited French work in which mature boars were exposed to 10 or 16 hours of light per day. Temperature was kept constant at 15 or 35 °C. Boars subjected to short daylength had an increased total number of spermatozoa per ejaculate compared to those subjected to long daylengths in both temperature groups. It was concluded that photoperiod changes sperm production by influencing the hypothalamo/pituitary axis, whereas high temperature has a direct destructive effect on the germ cells.

In a large German A.I. centre a light programme using the reverse of natural daylength was applied (Claus and Weiler, 1985). The programme resulted in significant improvement in libido, ejaculate volume, spermatozoa per ejaculate and the number of doses obtained per ejaculate during June to September (equivalent to December to March) in boars on reverse (short) light than those on natural (long) light.

Artificial lighting programmes applied to sows have concentrated mainly on the effect of

supplementary light. Constant 16h light applied from the end of pregnancy had no effect on the weaning-to-oestrus interval, conception rate, farrowing rate or litter size compared with a constant 8h regime (Greenberg and Mahone, 1982) or natural photoperiod (Mabry et al., 1982). The only significant difference observed when sows were changed one day after weaning from natural daylength to 24h light, 24h dark or 12h light/12h dark per day (12L: 12D) was a longer behavioural oestrus under long periods of light (Perera and Hacker, 1984).

Thus far, only one lighting programme using a decreasing light regime has been reported (Claus et al., 1984). Light was decreased from 15h 20min by 20 minutes per week beginning about one month prior to the expected increase in the weaning-to-oestrus interval previously experienced in a German piggery. The weaning-to-oestrus interval decreased from 23.6 days under natural photoperiod to 5.7 days under the lighting programme. Although this piggery was small (80 sows) and comparisons were made between different years, the dramatic difference in the weaning-to-oestrus interval implicates photoperiod very strongly as a factor in seasonal infertility.

THE MEASUREMENT OF DAYLENGTH

Two general models exist to account for photoperiodic time measurement:

(1) the 'hourglass' model, whereby some physiological product is produced either during light or dark, allowing the animal to measure light or dark or the ratio of one to the other;

(2) circadian rhythm based model, whereby the response to photoperiod depends on the coincidence of light with an endogenous rhythm of photosensitivity ('external coincidence') or on a specific phase relationship of two (or more) circadian oscillators that are entrained by photoperiod ('internal coincidence').

Experiments in which ahemeral (non 24h) lighting regimes are used have contributed greatly to the idea that photoperiodic time measurement relies on a circadian rhythm rather than a simple measurement of the ratio of light to dark. One such experiment using the Soay ram (Almeida and Lincoln, 1982) found that the rams exhibited maximal reproductive stimulation when a short lighting regime that was a multiple of 24 hours was used (8L: 16D or 8L:40D). When the light differed by 12 hours from 24 hours (8L: 28D), testicular development was greatly retarded. According to the hourglass model, rams would interpret a 8L: 28D lighting regime as a short-day regime; instead, they interpret it as a long-day regime, indicating the inadequacy of the hourglass model. The reverse situation was found in the long-day breeding golden hamster (Stetson and Watson-Whitmyre, 1984), indicating a common use of circadian based systems by both long- and short-day breeders.

Regardless of which mechanism is used to interpret the photoperiodic information received by the animal, it is clear that a physiological system for measuring daylength is necessary. Most of the mechanisms by which photoperiod is transduced into internal signals altering reproduction are shared by both long- and short-day breeders (Hansen, 1985). That is, the retina serves as the sole photoreceptor from which photoperiodic information is relayed to the pineal gland via its sympathetic innervation. At the pineal this information is transduced from a neural to a humoral message. The indoleamine melatonin appears to be the pineal hormone critical to the photoperiodic response in both long- and short-day breeders (Bittman, 1984; Tamarkin et al., 1985; Arendt, 1986). Tamarkin et al. (1985) surmised that a nocturnal rise in melatonin concentrations occurred in all mammals studied to that time. The similar pattern of melatonin secretion in both long- and short-day breeders suggests that the pattern of melatonin secretion is interpreted differently by long- and short-day breeders, rather than having a direct pro- or anti-gonadal effect.

Melatonin profiles in pigs are not clearly defined. Brandt et al. (1986) did not detect circadian fluctuations in prepubertal gilts maintained on 10L: 14D. DeBoar and Hacker (1986) demonstrated an extraordinary rise in daytime concentrations of melatonin in sows maintained on 14L: 10D on day 14 of lactation and day 2 post weaning. In the latter study, the daytime rise in melatonin concentrations was in the order of a fourfold increase over night-time concentrations. A daytime rise was observed in all 18 sows studied (R.R. Hacker, personal communication).

McConnell and Ellendorff (1987) found that plasma melatonin concentrations

increased by two to five times in three out of four sows during the dark phase on a 12L: 12D lighting regime. This nocturnal elevation was of 3.8h duration and peaked 5.5h after the onset of dark. The nocturnal elevation was abolished in these sows upon exposure to 16L:8D and was not reinstated upon subsequent exposure to 8L: 16D. A further experiment using different sows in a different season (autumn vs. spring) showed a nocturnal elevation in two out of four sows maintained on 12L: 12D. The peaks occurred later (8. 5 and 9. 5h after the onset of dark) in these sows than in the sows in the former experiment.

McConnell and Ellendorff suggested that the absence of a nocturnal rise in melatonin concentrations under long and short daylengths may be a factor in the decline in reproductive performance during winter and summer. However, caution must be exercised in interpreting these data as the presence or absence of the nocturnal rise in melatonin concentrations may have been due to the order of exposure to the photoperiods or the relatively short periods of time allowed for readjustment of the melatonin profile.

Ingram and Dauncey (1985) tentatively concluded that the wild pig may have inherently weak circadian rhythms and that under domestication these rhythms may have been further weakened. The small amount of work done thus far with melatonin seemingly supports this conclusion. Perhaps the absence of a circadian rhythm of melatonin under long hours of daylength is a factor in the seasonal infertility of pigs. Too little information is available to draw this conclusion as yet but the information that is available is enough to warrant further research into this possibility.

EXOGENOUS MELATONIN

Durational changes in melatonin secretion appear to be sufficient to drive seasonal changes in Syrian and Siberian hamsters and sheep (Tamarkin et al., 1985). Evidence from other species suggests that melatonin is a neuroendocrine transducer and not in itself an indicator of reproductive activity. However, timed administration of exogenous melatonin can be used to convey 'seasonal messages' and induce an early resumption of oestrous cycles in sheep (Nett and Niswender, 1982; Kennaway et al., 1982a; Arendt et al., 1983). More recently, the use of continuous-release implants have been shown to have a similar effect in advancing the breeding season (Kennaway et al., 1982b; Kennaway et al., 1982/1983; English et al., 1986). A set minimum exposure to long days appears to be necessary before sheep are responsive to the 'short' days caused by melatonin administration (Lincoln and Ebling, 1985). The use of exogenous melatonin has also been shown to cause an increase in fecundity in sheep (Kennaway et al., 1987).

We suggest that the pig is naturally a short-day breeder and the seasonal infertility observed in many piggeries around the world is a reflection of the endogenous non-breeding season. The fact that the summer infertility period occurs largely after the summer solstice (Paterson et al, 1978; Love, 1978, 1981), that is, when photoperiod is decreasing, may seem to contradict our suggestion that the pig is a short-day breeder. However, the delay in pituitary response to daylength in sheep is in the order of two to three months (Evans and Robinson, 1980). If a similar time course is true in pigs and daylength is not inhibitory until very long days, the observation of seasonal infertility after the summer solstice is consistent with our suggestion that pigs are short day breeders.

If the pig is a short day breeder, exogenous melatonin may be a possible way of mimicing short days and overcoming the inhibitory effects of long days. This is not to say that exogenous melatonin is the only solution for seasonal infertility; photoperiod is almost certainly not the sole factor involved in seasonal infertility. Exogenous melatonin will probably not completely negate the endogenous seasonal pattern of breeding activity as animals become refractory to the continued influence of exogenous melatonin (Lincoln and Ebling, 1985). However, the period in which pigs have the potential to become seasonally infertile may be moved. If, as is generally agreed, the coincidence of a number of factors is required for seasonal infertility to occur, moving the underlying photoperiod effect may prevent this coincidence and so prevent infertility.

CONCLUSIONS

To date, despite a considerable research effort, no significant inroads have been made in the study of seasonal infertility. Most studies have been based on the assumption that heat stress is responsible for seasonal infertility. However, experiments in which sows have been heat stressed have not satisfactorily reproduced the characteristics of seasonal infertility seen in the field (Edwards et al., 1968; Mercy and Godfrey, 1986). The failure of cooling to alleviate the problem (Hurtgen et al., 1980) also indicates that heat stress per se is not the cause of seasonal infertility.

The various reproductive inefficiencies that characterize seasonal infertility are not usually dramatic. A drop of 10% in conception rate represents a serious problem to producers but is not easily demonstrated under laboratory conditions. A problem exists reaching statistical significance using parameters such as conception rate, weaning-to-oestrus interval or delayed returns when small numbers of animals are involved. More subtle indicators of reproductive performance such as basal LH concentration, pulse frequency and amplitude may reveal changes that are undetectable using other parameters. In this way, laboratory studies using small numbers of animals may provide the basic information required to understand the problem of seasonal infertility.

THE ROLE OF HIGH AMBIENT TEMPERATURE IN SEASONAL INFERTILITY IN THE SOW

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INTRODUCTION

There are many reports from throughout the world of depressed reproductive performance in pig herds during the summer months. This problem has become known as summer or seasonal infertility. The exact characteristics of seasonal infertility vary but a typical feature is a reduction in pregnancy rate and an increase in the percentage of infertile sows which exhibit extended, irregular return to service intervals after mating (dioestrous intervals).

Ambient temperature and daylength increase during summer and both have been proposed as causative agents in seasonal infertility.It has also been suggested that other stressors such as social, housing and managerial influences are involved in the aetiology of this condition. Indeed when the appropriate combination and/or intensity of stressful stimuli are present a condition identical to seasonal infertility can occur at any time of the year (Hennessy and Williamson, 1984). Clearly seasonal infertility is a complex phenomenon involving a number of factors. In this paper data on the effect of high ambient temperature on reproduction in the sow are reviewed and an attempt made to explain the role of heat *per se* in seasonal infertility.

FIELD OBSERVATIONS

Corteel et al. (1964) observed a fall of about 10 per cent in the farrowing rate of sows during the summer in four French pig herds. Mean daily maximum temperature and farrowing rate were negatively correlated. During the same period there was an increase in the number of sows which returned to oestrus more than 45 days after mating. In Australia several workers have reported that farrowing rate showed a marked seasonal trend and was inversely related to ambient temperature (Stone, 1977; Love, 1978; Paterson et al.,1978).

Analysis of data from 9011 matings over five consecutive years on one farm in Western Australia (Paterson et al.,1978) showed that when the mean maximum temperature in the week of mating exceeded 32°C there was a higher than normal proportion of infertile sows but when mating took place in weeks with a mean temperature below 32°C, return rates were basal. This decrease in fertility was associated with an increased number of sows which had long and irregular dioestrous intervals. Love (1978) reported a similar observation and in this respect the Australian data agree with the earlier work from France (Corteel et al., 1964). However, the ambient temperatures reported in the French work were much lower than in the Australian studies. Both Corteel et al. (1964) and Stork (1979), working in England, found seasonal infertility became a problem when the ambient temperature exceeded 23°C. The higher critical temperature of 32°C observed by Paterson et al. (1978) may indicate adaptation to the hotter Australian environment. It is interesting to note that several workers have reported that physiological indicators of heat stress such as rapid elevation of rectal temperature and respiration rate occur in pigs when ambient temperatures approach 32°C (Teague et al., 1968; d'Arce et al., 1970; Morrison and Mount, 1971).

The duration of seasonal infertility is more protracted in some years than in others and there is variation in its severity among years (Love, 1978, 1981; Paterson et al., 1978). Paterson (1979) found that the years in which the mean weekly maximum temperature exceeded 32°C for long periods were also the years in which summer infertility was protracted. In years when there was no clear-cut period of seasonal infertility, temperatures were either uniformly low or the fluctuations in temperature between seasons were less than in years when seasonal infertility was well

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defined.

There is no doubt that many other factors in the pig's environment may act as stressors and, in combination, can adversely affect reproduction (Hennessy and Williamson, 1984). However, the data reviewed here clearly show that heat *per se* is likely to be the most important component of true seasonal infertility.

The exact reproductive processes which are affected by heat are difficult to define from retrospective record data. Love (1978) suggested that the effect of heat on sow fertility was most severe if it was imposed more than 7 days after mating rather than immediately post mating. Litter size was not affected and he concluded that heat stress during early pregnancy caused total embryo loss in affected sows. If embryo death occurred before implantation, return to service would be expected in the normal range but if death was after implantation the interval may be extended and irregular. Paterson et al. (1978) concluded that sows were sensitive to heat at or around the time of mating because sows mated just before the period of high temperature were unaffected even though most of their pregnancy took place in hot conditions. They concluded that the reduction in fertility was not due solely to fertilisation failure or loss of embryos before implantation because the increase in infertile animals consisted almost entirely of sows with extended cycles. They also argued that it was unlikely that embryo death after implantation on an "all or none" basis was the primary cause of seasonal infertility. Under laboratory conditions exposure to severe heat regimes in early pregnancy increased loss of individual embryos but did not cause total loss in more than one or two sows (Edwards et al., 1968; Teague et al., 1968). In addition, the fertility of sows returning to service after extended cycles was normal in the field studies. Since the fertility of females of other species which remate after losing their conceptus is usually impaired (Edey, 1972; Sawyer and Knight, 1975), these data may be seen as further supportive evidence against total late embryo loss being the major cause of seasonal infertility in the pig.

The decrease in fertility in the summer months must involve fertilization failure and/or loss of fertilized embryos but how high temperatures disrupt the reproductive process is unclear. Paterson et al. (1978) speculated that heat interferes with ovarian function, either directly or via the hypothalamic pituitary axis, altering the pattern of circulating hormones. They suggested that this endocrine imbalance causes temporary infertility by disrupting the normal sequence of events in the oestrous cycle and results in extended and irregular dioestrous intervals. Subsequent studies have clearly shown that ovarian hormones in the sow are affected by high temperatures (Krieder et al., 1978; Hoagland and Wettemann, 1984) and these data strongly support the conclusion that reduced fertility in sows exposed to high temperatures at or around the time of mating is due to an alteration in the endocrine milieu (see next section of this paper).

CONTROLLED EXPOSURE TO HIGH AMBIENT TEMPERATURE

Studies in which sows are exposed to high ambient temperatures at various stages of the reproductive cycle may help us understand how heat affects reproduction. However the problem with most studies which have been done on sows is the relatively small number of animals used and the highly variable nature of reproductive parameters which makes statistically significant differences difficult to achieve. For example, a difference of 20 per cent in farrowing rate which would be highly significant in the field situation did not achieve statistical significance in the study by Mercy and Godfrey (1980) who used 20 gilts per treatment. In addition, most attempts to induce seasonal infertility under laboratory conditions have added little to our understanding of the condition because they have not examined the mechanisms by which heat affects reproductive processes.

High temperature before mating

Teague et al. (1968) housed gilts at 26.7, 30.0 or 33.3°C throughout the oestrous cycle and for 25 days after mating. The percentage of gilts failing to show oestrus tended to increase with ambient temperature (0 vs 2.5 vs 8.8%) but these differences were not statistically significant.

Edwards et al. (1968), whose heat regime (38.9°C for 17 hours, 32.2°C for 7 hours throughout the cycle) was severe enough to kill five gilts and severely depress feed intake and body weight in the survivors, found no effect on the number of anoestrous gilts. Similar results were reported by d'Arce et al. (1970) and Mercy and Godfrey (1980) when gilts were heated throughout the cycle. These studies show that high temperatures during the oestrous cycle have little effect on the proportion of sows which display oestrus but there is evidence that cycle length, duration of oestrus and the timing of ovulation may be affected. Edwards et al. (1968) found the length of the oestrous cycle during exposure to high temperatures was two days longer than the cycle prior to treatment. Pett (1984) found the oestrous cycle of gilts exposed to high temperatures (38°C for 17 hours and 32°C for 7 hours) was extended by an average of 1.4 days if heating commenced 6 days prior to the expected oestrus. If heating commenced 3 days prior to the expected oestrus, cycle length was not affected but only 50 per cent of the gilts were receptive to the boar for more than 1 day compared with 80 per cent at the subsequent oestrus. The timing of the heat treatments corresponds to the time of luteal regression (6 days) and the final follicular phase (3 days) and indicates that these two events may be sensitive to high temperature.

The failure of other workers to observe similar responses may be due to the application of high temperatures throughout the whole cycle commencing soon after ovulation allowing time for a degree of adaptation before the sensitive stage of the cycle was reached. The work of Edwards et al. (1968) supports this suggestion because rectal temperatures of heat treated gilts stabilized after 6 to 8 days.

When Edwards et al. (1968) applied their heat regime to 10 gilts for 5 days prior to oestrus there were no significant differences in oestrous cycle length but the duration of oestrus was not reported. D' Arce et al. (1970) found no effect on oestrous cycle length in gilts housed at 33.3°C for varying periods during the oestrous cycle. Again duration of oestrus was not reported but they did observe the visual appearance of the corpora lutea of heat treated gilts varied greatly from that of control gilts, suggesting that the timing of ovulation had been altered.

Recent research in Western Australia (D.H. Pett, unpublished data) has confirmed and extended the findings of Pett (1984). When gilts were exposed to high ambient temperatures (38°C for 17 hours and 32°C for 7 hours) from day 13 to 18 of the cycle, the decline in plasma progesterone (P) expected at this stage of the cycle was delayed by about 24 hours compared with gilts housed at 20°C. This was associated with the pre-oestrous peak of oestradiol (E_2) and the pre-ovulatory surge of luteinizing hormone (LH) being delayed by 34 hours ($P < 0.05$) and 48 hours ($P < 0.025$),) respectively. The onset of oestrus was delayed by 1.4 days which was identical to the delay seen in the previous experiments (Pett, 1984). When gilts were heated for 5 days from day 17 of the cycle, mean concentrations of plasma P, E_2, and LH were not affected but the interval between the pre-oestrous peak of E_2 and the onset of oestrus was longer in heat treated gilts (14.8 vs 24.6 hours, $P < 0.05$). The preovulatory surge of LH occurred 12 hours after the peak of E_2 in both control and heated gilts. Consequently the LH surge was 2.4 hours before the onset of oestrus in control gilts and 12.6 hours before oestrus in the heated gilts ($P < 0.05$). The length of the oestrous cycle was not affected but 40 per cent of the heated gilts displayed oestrus for less than 24 hours compared with none of the control gilts.

The evidence for high temperatures prior to mating affecting fertility is equivocal. Teague et al. (1968) found pregnancy rate tended to decrease in heated gilts (90.5 vs 84.8 vs 77.5%) but these data are confounded because high temperatures were maintained for 25 days after mating. Edwards et al. (1968) found no significant differences in pregnancy rate, litter size or embryo mortality in gilts heated for a full cycle despite the fact that the cycle length was extended. Among gilts heated for 5 days they found that the mean ovulation rate and number of viable embryos per gilt were both higher in the control gilts but the differences did not reach statistical significance. In the studies of Pett (unpublished data) where the oestrous cycle was disrupted, reproductive performance was impaired. High temperatures during luteal regression reduced pregnancy rate (100 vs 72%, $P < 0.10$) and litter size (9.3 vs 6.0, $P < 0.05$) and increased embryo mortality (21.7 vs 47.8%, $P < 0.10$). In addition, three of the five non- pregnant animals had dioestrous intervals in excess of 25 days. High temperatures during the follicular phase reduced pregnancy rate (71.2

vs 42.4, P <0.05) and litter size (10.5 vs 7.8, P <0.05) and increased embryo mortality (14.6 vs 42.0%, P <0.05).

Data from these recent experiments clearly show that the oestrous cycle of the sow can be disrupted by short periods of high temperatures if they correspond to the time of luteal regression or the late follicular phase. In these cases, reproductive performance may also be affected, probably by changes in the temporal relationships between luteal regression, the LH surge, oestrus and ovulation. An abnormally long interval between the LH surge and oestrus may disrupt the timing of insemination necessary for maximum conception rate and litter size. This problem would be exacerbated by the shorter duration of oestrus displayed by the sows affected by high temperatures.

High temperature after mating

Experiments conducted in environmentally controlled chambers have shown that high temperatures during the first 15 days after mating can depress pregnancy rate, litter size and embryo survival (Tompkins et al., 1967; Edwards et al., 1968; Omtvedt et al., 1971; Wildt et al., 1975). High temperatures between 15 and 30 days post mating (Edwards et al., 1968; Wildt et al., 1975) or 53 and 61 days post mating (Omtvedt et al., 1971) do not appear to lower reproductive performance. Exposure to high ambient temperatures in the final stages of pregnancy (90-115 days) may result in foetal deaths but the experimental evidence for this in the pig is limited to the work of Omtvedt et al. (1971), where the heat regime was severe enough to kill two gilts.

This early work clearly shows that reproduction in the sow may be depressed by high temperatures in early pregnancy but its usefulness is limited in defining the role of heat in seasonal infertility. The patterns for return to service of the non-pregnant animals were not examined because they were slaughtered soon after heat treatment ceased. No attempt was made in these studies to elucidate the mechanisms by which high temperature affects reproduction. Again, too few studies have addressed these questions.

In a series of five experiments, the effect of high temperature on the reproductive variables important in seasonal infertility, pregnancy rate and dioestrous interval, were examined (Mercy and Godfrey, 1980; Mercy et al., 1983; Mercy and Godfrey, unpublished data). Whilst the results of the individual experiments were slightly variable, there was a consistent trend for exposure to high temperatures in the post-mating period to lower reproductive performance. The combined results show that the pregnancy rate of 111 gilts kept at 22-24°C was greater than that of 151 gilts exposed to high temperatures after mating (85 vs 74%, P <.05). The incidence of extended dioestrous intervals (> 25 days) among the non-pregnant gilts was also greater in the gilts exposed to high temperatures (35 vs 51%). The difference in both pregnancy rate and dioestrous intervals are of the same order as those recorded in commercial piggeries experiencing seasonal infertility. They support the hypothesis that high temperatures are a major contributing factor to true seasonal infertility.

High temperature may have a direct effect on gametes, embryos or uterine function or they may have an indirect effect on these by altering the endocrine status of the sow. When Kreider et al. (1978) heated gilts for the first eight days of pregnancy, concentrations of plasma P were increased and plasma E_2 were decreased compared with controls. The ratio of P:E_2 is important in controlling the transport of embryos through the reproductive tract (Chang, 1966) and in controlling the secretion of histotroph on which the survival of the embryos depends (Knight et al., 1973; Bazer, 1975). When Hoagland and Wettemann (1984) heated gilts between days 8 and 16 after mating, plasma hormones were not affected in pregnant gilts. However in non-pregnant gilts, those exposed to high temperatures had lower plasma P between days 13 and 19 of the cycle and their plasma P did not return to basal levels until day 25. These gilts also had higher plasma E_2 on days 10, 11 and 12. Oestrogens are luteotrophic in the pig (Gardner et al., 1963) and exogenous oestrogens given between days 9 and 15 can maintain the corpora lutea and result in extended oestrous cycles (Guthrie, 1975; Kraeling and Rampacek,1977). The extended luteal activity measured in these gilts may have been due to the luteotrophic action of the elevated oestrogen levels on days 10 to 12. There is also evidence linking high levels of oestrogens on days 9 or 10 with

decreased embryo survival rates in sows (Pope and First, 1985). The finding that high temperature increases plasma E_2, which can both reduce embryo survival and extend luteal function, is consistent with the type of reproductive dysfunction observed in seasonal infertility.

While direct effects of high temperature on the embryo or conceptus development have been reported (for review see Wettemann and Bazer, 1985), direct effects alone do not explain the type of infertility seen in heated sows. The literature reviewed, particularly the work of Kreider et al. (1978) and Hoagland and Wettemann (1984) supports the conclusion that high temperatures during early pregnancy alter the reproductive endocrine system, particularly the control of luteal function. As a result, fewer sows become pregnant and there is an increase in the incidence of extended dioestrous intervals among non-pregnant sows.

CONCLUSIONS

Taken together the field studies and the laboratory experiments suggest that seasonal infertility in the sow is primarily due to exposure to high temperature immediately before or within 15 days of mating. While direct effects of heat on the developing embryos may contribute to infertility the data reviewed here support the conclusion that true seasonal infertility is largely due to changes in the endocrine milieu caused by exposure to high temperature at sensitive stages of the reproductive cycle.

SEASONAL INFERTILITY : A STRESS RESPONSE

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INTRODUCTION

Williamson et al. (1980) have shown that seasonal infertility is not due only to early embryonic death (Love, 1978,1981) or to an endocrine imbalance (Paterson et al., 1978) caused as a result of heat stress. It was suggested by Williamson et al. (1980) that factors such as social, managerial, behavioural and nutritional influences, acting as stressors, were also involved. Evidence for the involvement of a generalised stress response in causing seasonal infertility was presented in the study by Hennessy and Williamson (1984). They identified several management factors that were considered to be placing undue stress on the breeding stock. When steps were taken to reduce the influence of these stressors, there was a marked increase in the herd farrowing rate. With the intensification of pig production, there are more social and managerial stressors acting on the breeding herd. Thus, it is not surprising to see stress-related reproductive problems occurring in the modern piggery.

This paper will consider five main areas: A definition of stress; the mechanisms by which stress causes seasonal infertility; the role of environmental factors, husbandry practices and health status in causing seasonal infertility; current research findings; and finally, the prevention of seasonal infertility.

DEFINITION OF STRESS

Stress was defined by Seyle (1973) as "a non-specific endocrine response of the animal in an attempt to adapt to one or a combination of adverse physical or psychological aspects of its environment in order to maintain homeostasis". This reaction is referred to as the General Adaptation Syndrome (G.A.S.), and is characterized by increased activity of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of glucocorticoids from the adrenal cortex. Factors which are perceived by the brain, and stimulate the HPA axis are known as "stressors". An animal's response to stressors may be either acute or chronic. Upon exposure to acute (short term) stressors, glucocorticoids are secreted. One of the effects of cortisol is to increase blood glucose concentration by decreasing peripheral utilization of glucose, increasing protein breakdown and lipolysis, and inhibiting protein synthesis (Moore,1976). These changes in energy supply allow the animal to cope with the situation which confronts it. This response to stressors is a normal event, and enables the pig to adapt to changes in its environment. However a prolonged or persistent exposure to one or more stressors will result in a sustained G.A.S. reaction, leading to the prolonged release of glucocorticoids, which can have detrimental effects on growth, reproduction and health.

EVIDENCE THAT STRESS CAUSES SEASONAL INFERTILITY

One major detrimental effect of glucocorticoids is their influence on the reproductive cycle. Adrenocorticotrophic hormone (ACTH) and glucocorticoids have been used to mimic the response to stressors in the environment. When these hormones were given to sows, during the follicular phase of their ovarian cycle, it resulted in the formation of cystic ovaries (Liptrap, 1973; Scholten and Liptrap, 1978). The action of ACTH in inducing cystic ovaries was mediated through the adrenal cortex (Liptrap, 1973; Scholten and Liptrap, 1978).

Barb et al. (1982) showed that both ACTH and hydrocortisone suppressed the preovula-

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tory LH surge in gilts. This led to the inhibition of follicular development, which resulted in the formation of cystic ovaries. It was proposed that ACTH and hydrocortisone suppressed pituitary responsiveness to LH-RH and/or decreased the secretion of LH-RH from the hypothalamus. In ovariectomized prepuberal gilts, Fonda et al. (1984) observed that the influence of ACTH in suppressing LH release was mediated via glucocorticoids which acted at the hypothalamic level rather than at the pituitary level. In male rats, Ringstrom and Schwartz (1984) demonstrated that cortisol implants resulted in a decrease in pituitary responsiveness to GnRH. Also, elevated cortisol inhibited GnRH secretion from the hypothalamus and this resulted in a reduction in GnRH receptor numbers at the pituitary and reduced the GnRH stimulation of LH release by the pituitary. Schoonmaker and Erickson (1983) found that glucocorticoids can act directly on the ovaries and disrupt the differentiation of FSH-stimulated granulosa cells. They suggested that hyperactivity of the adrenal gland would lead to an inhibition of the normal ovarian funtion.

Weaning piglets at birth may evoke a stress response in some sows and lead to the formation of either large or small ovarian cysts, or to inactive ovaries (Kunavongkrit et al.,1983). Sows with large ovarian cysts had high cortisol and low LH concentrations (Kunavongkrit et al., 1984).

There has been no attempt made to monitor the relationship between elevated glucocorticoids and the different stressors that have been suggested to cause seasonal infertility. The failure to prevent seasonal infertility from occurring when a particular stressor (e.g. heat stress) has been removed, raises the question whether other stressors are still acting on the pig to maintain the elevated glucocorticoid levels that cause infertility.

Heat

The effects of heat stress on ovulation have been reviewed by Paterson and Pett in this symposium. However one aspect which is often overlooked when trying to reduce the heat load is the relative humidity. For example, BeVier and Backstrom (1980) and Hurtgen et al. (1980) found that the introduction of various cooling methods was not effective in preventing the incidence of seasonal infertility. It should be realised that some cooling methods will increase the relative humidity in the surrounding environment. Under high relative humidity, the animal is not able to dissipate its body heat easily, and so it may still be stressed even though the ambient temperature may be lowered.

Daylength

Variations in daylength have been suggested as another factor which may cause seasonal infertility. However, the research in this area has been mainly concerned with the effect of daylength on the weaning to mating interval and on the induction of puberty in the gilt. The role of photoperiod and melatonin in influencing the reproductive process of pigs has been reviewed by Peacock and others in this symposium.

Husbandry practices

Hurtgen and Leman (1980) reported that the number of sows in a group can influence reproductive performance. They observed that sows, which were confined to individual crates after mating, had a higher farrowing rate than sows kept in groups of 6-20 pigs. While a seasonal decrease in farrowing rate was noted for both the individual and group housed sows, the decrease was much greater for sows housed in groups. There are two factors which may contribute to the better reproductive performance of individually stalled sow. Firstly, individual sows may be able to dissipate their body heat more readily, and are therefore less likely to be heat stressed in summer. Secondly, the lack of competition for space, food, water and social dominance in a single stall means that the sows are exposed to a lower level of stress. Barnett et al. (1985) showed that neck tethering of pregnant pigs can induce a chronic stress response compared to other methods of housing. The importance of good stockmanship in relation to good reproductive performance has recently been demonstrated. Hemsworth et al. (986) showed that poor handling of gilts and boars would induce a chronic stress response, which in turn led to a reduced conception rate in the gilts and a reduced sexual development in the boars.

Given that elevated glucocorticoids can induce the clinical symptoms of seasonal infertility, it is not unreasonable to suggest that housing systems and/or stockmanship which cause a chronic stress response may be major factors in the aetiology of seasonal infertility in many Australian piggeries.

Health

Infectious agents have often been lightly dismissed as a major cause of seasonal infertility. There are however, two points that should be considered. Firstly, Rodeffer et al. (1975) demonstrated that porcine parvovirus (PPV) infection in sows around 30 days post mating, could kill all the embryos. The embryos were completely resorbed, without a concurrent regression of the corpora lutea, leading to a delayed return to oestrus. In other words, PPV infection in susceptible stock can cause the clinical symptoms of seasonal infertility. Secondly, elevated glucocorticoid concentrations, in response to chronic stress, have been reported to have a suppressive effect on the pig's immune system (Kelley et al., 1984; Westly and Kelly, 1984). Kovalenko (1974) and Kovalenko et al. (1977) demonstrated that a chronic stress response could cause suppression of the immune system in sows and result in the reinfection of sows which had been previously immunised against erysipelas and swine fever. This raises the possibility that infectious agents, such as PPV, may be more involved in seasonal infertility than previously thought.

CURRENT RESEARCH

Our current research is based on the hypothesis that seasonal infertility is mainly caused by the pig's response to stressors in its environment. It is proposed that when the level of stressors reach a threshold, a stress response will occur. The glucocorticoids secreted in this stress response will then interfere with various aspects of the reproductive process or the immune system. The current study was designed to find whether :

1) The total level of stress acting on the breeding pig population varied with season;
2) there was a relationship between basal plasma cortisol concentrations and subsequent reproductive performance;
3) individual pigs that show a large adrenal response to stress (high responders) were predisposed to stress-related infertility;
4) infertile pigs differed from fertile pigs in their body condition and/or sexual behaviour.

This investigation was conducted on a commercial farm (Farm A) in central Victoria, where historically a 15-20% drop in farrowing rate had occurred over the late summer and early autumn period. Observations were made for 10 weeks in spring (1985) and for 12 weeks over the summer-autumn period (1986). A total of 355 and 625 gilts, and 279 and 263 sows were monitored in the spring and summer-autumn periods, respectively. The pig's body condition prior to mating was calculated using the method of King et al. (1986), and was based on the live weight and backfat at P1 level for the gilts, and backfat at P1 level for sows. To obtain data on the "basal" plasma cortisol concentrations in both gilts and sows before mating, 20 gilts and 20 sows were sampled each week. The pigs were bled by jugular venipuncture within one to two minutes of first approaching the pig. Also, over the summer-autumn period, both gilts and sows were challenged with 50 i.u. of synthetic ACTH injected intramuscularly, and a second blood sample was collected one hour later to determine whether pigs with a high adrenal response to stress were predisposed to stress-related infertility. This test for adrenal responsiveness was also conducted on another farm (Farm B) in NSW for 12 weeks over the summer-autumn period. A validated radioimmunoassay was used to estimate plasma cortisol concentrations.

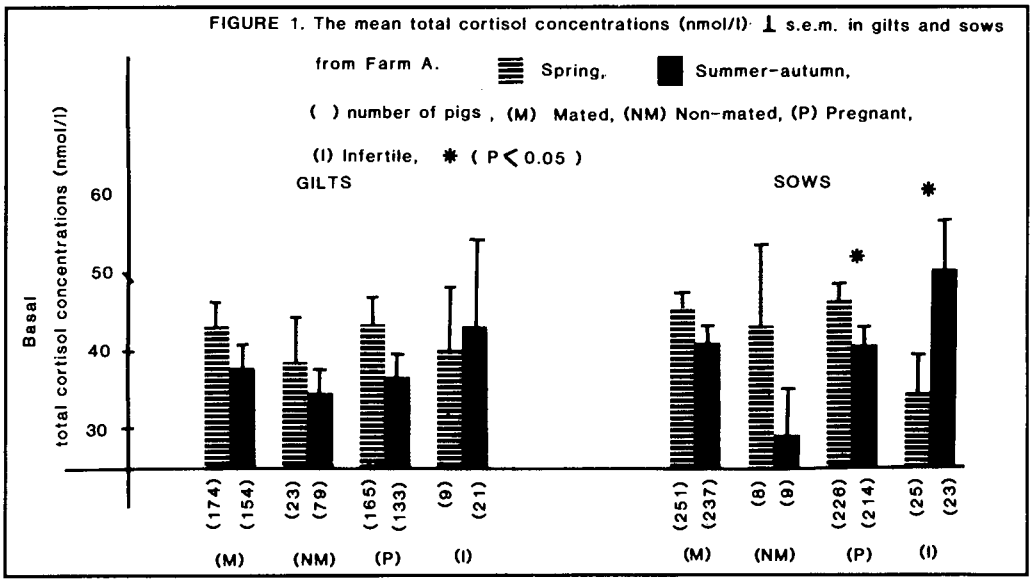
The reproductive performance for Farm A is shown in Table 3. The mating rate for the gilts was markedly lower in the summer-autumn period than in the spring. The reason for this is that the management, in anticipation of a drop in farrowing rate, had increased the number of gilts entering the breeding herd. This caused a near doubling of the stocking density, increased the amount of fighting between the gilts and reduced boar exposure that each gilt received. Also, the

pressure to mate extra gilts meant that gilts of lighter than normal live weight with thinner backfat layer were selected for entry into the boar shed. As a result of this extra pressure, the proportion of gilts failing to reach and/or show signs of puberty was higher in summer than in spring (Table 3). In contrast to previous years, the incidence of seasonal infertility was low. The reason for better than expected performance may be related to the relatively low temperatures during the summer of 1985/86. Therefore the total level of stress acting on the breeding herd would have been lower than in past years.

Table 3. Summary of reproductive performance for gilts and sows at Farm A in the spring (1985) and in the summer-autumn (1986).

	Gilts		Sows	
	Spring	Summer-autumn	Spring	Summer-autumn
No.of pigs entering boar shed	355	625	279	263
Mating rate (%)	91.0	71.0	97.8	97.0
Farrowing rate (%)	91.8	88.0	89.8	90.0
Normal return to oestrus (%)	3.7	5.2	7.4	5.7
Delay/failure to return to oestrus (%)	4.4	6.7	2.7	4.3

Figure 1 shows the basal plasma cortisol concentration for pigs at Farm A. For both gilts and sows, there was a trend for cortisol concentrations to be lower in the summer than the more temperate spring months. This is consistent with the animals adapting to the hotter, more stressful temperatures of summer (Dantzer and Mormede, 1983; Guerrini and Bertchinger, 1982). However, in pigs that were subsequently found to suffer from seasonal infertility, this trend was reversed; infertile pigs had higher basal cortisol concentrations in summer-autumn than in spring. Similarly, Gill et al. (1985) found that barren mares had higher cortisol levels than pregnant mares. This suggests that the infertile pigs had a greater adrenal response to the higher level of stress (overcrowding, fighting, heat load, etc.) acting in summer.



The body condition of both gilts and sows was not different between the pregnant and infertile pigs. This suggests that body condition is not a major factor in the aetiology of seasonal infertility. The sexual behaviour of gilts in the boar shed was assessed using the method of Hemsworth et al. (1982), and it was not related to the gilt's subsequent reproductive performance.

Hennessy et al. (1984) have shown that pigs of the same age, sex and genetic strain varied considerably in their adrenal response to stressful stimuli. The type of response to stress in individuals was repeatable, and the pattern of response in a population was normally distributed. In this study we ranked the individual pig's adrenal response to ACTH from lowest to highest, and then compared the reproductive performance of the highest and lowest 30% of the population. The results are shown in Table 4. For Farm A it was found that there were significantly more prepubertal gilts in the high responding 30% of the herd. The farrowing rate of gilts and sows on Farm A was not different between the high and low responders. On Farm B, where the incidence of seasonal infertility was much higher, the farrowing rate for the low responding gilts was significantly higher than for those pigs with a high adrenal response to stress. In other words, pigs which produced large amounts of cortisol in response to stressful stimuli were more likely to suffer from delayed puberty or seasonal infertility.

Table4. Summary of average basal and post-ACTH cortisol concentrations (nmol/l) and the reproductive performance of the high and low responders from Farms A and B. Data are shown as mean with the S.E. in brackets below the mean (* = P< 0.05)

	Gilts			Sows		
	HIGH		LOW	HIGH		LOW
Farm A						
No. of pigs	72		72	72		72
Basal cortisol	53.2		24.1	53.4		25.9
concentration	(4.6)		(1.8)	(3.3)		(2.5)
Post-ACTH cortisol	328.4		156.4	248.1		103.2
concentration	(7.3)		(2.9)	(6.3)		(2.2)
Mating rate (%)	62.0	*	72.9	98.6		100.0
Farrowing rate (%)	79.9		81.1	88.4		91.6
Litter size (Born alive)	9.2		9.0	11.7		11.5
	(0.32)		(0.30)	(0.34)		(0.28)
Farm B						
No. of pigs	50		50	60		60
Basal cortisol	80.5	*	54.8	57.2	*	39.8
concentration	(6.4)		(4.5)	(3.8)		(3.0)
Post-ACTH cortisol	399.7	*	219.3	317.7	*	161.8
concentration	(8.1)		(4.4)	(8.5)		(3.5)
Farrowing rate (%)	70.0	*	88.0	83.3	*	76.7
Litter size (Born alive)	8.6		8.8	9.4		10.2
	(0.30)		(0.35)	(0.40)		(0.32)

A retrospective analysis of the breeding records of the sows from Farm B (Table 4) showed that the reproductive performance of the pigs classified as high responders was significantly lower than those classified as low stress responders. The low responders had a higher farrowing rate, a higher litter size and a lower incidence of lactational failure (sow fails to suckle its litter). When the data was analysed according to parity (Table 5) the better reproductive performance for the low stress responders was mainly associated with the parity 1 sows. The reproductive performance of the older parity sows was similar for both the high and low responders. This suggests that either sows adapt to the pressure of intensive pig production in that they do not suffer from reproductive disturbances due to high adrenal activity, and/or they are culled from the herd for poor reproductive performance.

These data, together with the basal plasma cortisol data, support our hypothesis that a generalized stress response is involved in the aetiology of seasonal infertility.

Table 5. Summary of the previous reproductive performance of the high and low responding sows identified on Farm B. The data is presented without parity break-down (A) and according to parity (B). Data are shown as mean with the S.E. in brackets below the mean.

	Total Population	High Responders	Low Responders
A.			
No. of pigs	208	62	62
Farrowing rate (%)	88.9	83.3	90.3
Litter size (born alive)	9.2	8.7	9.3
	(0.13)	(0.25)	(0.21)
No. lactational failures	17	7	3
B. Parity 1			
No. of pigs	41	12	12
Farrowing rate (%)	83.3	76.9	90.9
Litter size (born alive)	8.8	7.7	9.2
	(0.37)	(0.72)	(0.66)
No. lactational failures	2	2	2
Parity 2			
No. of pigs	90	27	27
Farrowing rate (%)	87.0	87.0	90.9
Litter size (born alive)	9.1	8.5	9.2
	(0.19)	(0.35)	(0.35)
No. lactational failures	6	1	1
Parity 3			
No. of pigs	77	23	23
Farrowing rate (%)	92.3	90.0	92.5
Litter size (born alive)	9.6	10.0	9.2
	(0.17)	(0.35)	(0.27)
No. lactational failures	9	7	1

CONCLUSIONS : THE PREVENTION OF SEASONAL INFERTILITY

There is no doubt that seasonal infertility is a complex problem and that several forms of infertility are manifested as a common clinical symptom. On the evidence presented so far, it seems reasonable to conclude that seasonal infertility is a result of the pig's adrenal response to a variety of stressors in its environment i.e. a generalized stress response. These stressors may act persistently and in combination on the pig. When the combined level of stressors reaches a threshold, a stress response will occur. The glucocorticoids secreted in this stress response may interfere with various aspects of the reproductive process or the immune system. This may lead to the appearance of seasonal infertility. We strongly believe that it is not just one particular stressor which causes seasonal infertility but that it is a combination of stressors acting at any given time and which are especially critical around the mating period. It is suggested that whenever a chronic stress response occurs, the clinical symptoms of seasonal infertility can be manifested. Therefore, it is suggested that the answer to reducing the incidence of seasonal infertility lies in reducing the magnitude and frequency of the stress response of the individual, particularly in the period from one week before mating until three weeks after mating. Basically there are two ways to reduce the impact of a stress response.

The first way to reduce a stress response is to ensure the pigs are housed and managed in a manner that reduces, in as many ways as possible, the stressful stimuli in the pig's environment. Detailed attention should be given to the physical and social environment, to husbandry and

management practices and to the thermal environment of gilts and sows from the time they enter the boar shed, during mating and until about three weeks after mating. It is worth emphasizing that changing or improving one factor is not the answer to effectively reduce the occurrence of seasonal infertility (and other stress related problems), since one must try to reduce the stressor effect of as many aspects of the environment as possible.

The second way to reduce the impact of a stress response is a new approach that shows considerable promise and that is to select pigs that are genetically and physiologically better suited to their environment. We have previously shown that pigs vary in their adrenal response to stressful stimuli. Pigs that have a low response to stress are more likely to grow faster and use their feed more efficiently. We have shown in this study that low responders have a higher reproductive performance than those pigs which are more responsive to stress. We have also developed a simple test that will allow individual pigs to be classified according to their type of adrenal response. The application of this test to the selection of replacement breeding stock should lead to an overall lower stress response of the herd, and to an improvement in the welfare and the productivity of that herd.

EFFECTS OF HEAT STRESS ON BOAR FERTILITY WITH PARTICULAR REFERENCE TO THE ROLE OF THE BOAR IN SEASONAL INFERTILITY

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Seasonal infertility in pigs has been recognized for many years, and the accompanying reduced reproductive efficiency is more often associated with symptoms in the sow, including anoestrus, an increase in returns to service and small litter size. The effect, and its importance in the boar however, is not as clear as with the sow and therefore the role of the boar in seasonally associated infertility is difficult to elucidate. Many reports are available that suggest hot summer temperatures can produce variation in boar fertility. Also, experimental evidence has shown that the boar, like other males, is susceptible to an increase in testicular temperatures resulting in reduced fertility.

This paper reviews the present knowledge of the effect of heat stress on boar fertility, presents results of work carried out at the University of Queensland related to heat stress in boars, and concludes by discussing the value of further research and its importance to the modern pig industry.

THE EFFECT OF SEASON

A decline in reproductive performance associated with hot summer conditions has been observed in a number of species and the boar is no exception. For example, Burger (1952) reported a deterioration in reproductive performance in boars reared in South Africa when exposed to high but natural summer heat in a temperate climate. Higher conception rates with more pigs weaned were found by Jensen (1964) to result from breeding pigs during the cooler months of the year. Thibault et al. (1966) in Europe observed a decreased farrowing rate after gilts were bred with semen from boars maintained outside and exposed to the high ambient temperatures of summer (35°C) as compared with boars maintained in a building at 22°C.

Seasonal changes in the semen of boars in Oklahoma have been studied by Lawrence et al. (1970), who found an increase in the percentage of abnormal sperm in ejaculates following summer. However, ejaculate volumes and total sperm per ejaculate were highest following summer, and motility was not affected by season. In the study of Lawrence et al. (1970) only a small number of boars were used (8 boars divided into 3 groups) and statistical analysis of the results was not carried out.

A seasonal decrease in the farrowing rate of pigs during the summer months in Southwest France was reported by Corteel et al. (1964). Boars in this study were housed in a piggery with controlled temperature and light, and the impairment in the farrowing rate during summer was considered to be due to a cumulative effect of high environmental temperature and light/dark ratio.

Seasonal reproductive inefficiency, including returns to service, has been observed in several large pig breeding units in the United Kingdom, and it has been suggested that at least part of the cause was reduced fertility in boars subjected to temperatures as high as 32°C during the summer (Stork, 1976, 1979). However, in a study of seasonal infertility in Australia, Love (1978) did not consider boar involvement was important.

In Nigeria, Steinbach (1972) showed that the percentage of boars showing insufficient sexual interest to mount a dummy sow and ejaculate was directly proportional to the environmental temperatures. More boars were affected in the hotter months and the time for ejaculation by those that did mount tended to be longer. Seasonal variation in sperm production rates of boars in the humid tropical environment of Nigeria was studied by Egbunike and Steinbach (1979) who found sperm production per gram of testicular parenchyma increased from January to April and then

decreased by August. Sperm production was correlated with daylength change but not dry bulb temperature or daylength, and they concluded that there was a photoperiod influence on the sperm production rate of boars.

THE EFFECT OF ELEVATED AMBIENT TEMPERATURES ON SPERMATOGENESIS, SEMEN QUALITY AND FERTILITY WITH PARTICULAR REFERENCE TO THE BOAR

Both laboratory and domestic animals have been used extensively to study the effect of high ambient temperatures on spermatogenesis and seminal quality (VanDemark and Free, 1970). Workers have shown that high environmental temperatures can affect spermatogenesis and semen quality in the ram (Gunn et al., 1942) and the bull (De Alba and Riera., 1966). In these studies it was generally found that high ambient temperatures had little effect on semen volume but reduced sperm motility, concentration and total sperm numbers, while at the same time increasing morphologically abnormal sperm.

A number of studies on the effect of controlled but high ambient temperatures on semen production and quality have been carried out on boars. Also, several of these studies have examined the effect on fertility by inseminating sows with semen produced after heat stress. Table 6 summarizes the treatment regimes and results of these studies. From this Table it must be concluded that semen production and semen quality can be adversely affected when boars are exposed to temperatures around 31°C to 35°C for at least 72 hours. Also, fertility studies suggest a clear relationship between seminal changes following heat stress and the fertility of semen.

THE EFFECT OF HEAT APPLIED DIRECTLY TO THE SCROTUM OF BOARS

To help in our understanding of the effect of elevated ambient temperatures or heat stress on male fertility, studies on the effect of heat applied directly to the scrotum provide valuable information. A few such studies have been done using the boar. In particular, the work of Mazzarri et al. (1968) documents the effect of local heat on spermatogenesis in the boar.

Mazzarri et al. (1968) studied the effect of temperatures of 44°C or 48°C applied to the testes of boars to raise the testicular temperatures to 39.5°C and 40.5°C, respectively, for a period of three hours. Semen was collected from some of the boars for two months after treatment and the others were castrated after one, eight or 16 days in order to carry out histological studies of the testicles.

The local heat treatment which produced a testicular temperature of 40.5°C, resulted in a decrease in sperm motility and sperm numbers commencing 15 days after treatment and continuing through to the 58th day, with the most severe effect being seen between the 37th and 51st day after treatment. The histological studies showed that local heating had an immediate effect on spermatogenesis, the principal damage being to the spermatocytes, and in particular, the pachytene spermatocytes in stage 8 of the cycle of the spermatogenic epithelium. The diplotene spermatocytes in stages 2 and 3 and the younger round spermatids were also obviously affected, but to a lesser degree.

By comparing the histological findings and the semen changes seen in all boars, Mazzarri and his colleagues concluded that changes in semen quality due to heat treatment are delayed for a period directly related to the time interval required for young round spermatids to complete their change to spermatozoa and pass through the epididymis. The spermatids were the most advanced stage affected in the spermatogenic cycle but the most severe effect on sperm production was due to the effect of heat on the pachytene spermatocytes.

These findings were confirmed by examination of the conception rates of sows inseminated with the semen of boars subjected to the local heat treatment. The conception rates fell from the level before heat treatment of 47.3% to 14.2% by 22 days and to zero between 37 and 51 days after treatment. They then increased again to 14.2% for inseminations carried out 58 days after heat treatment. Similar findings were given in another report by Mazzarri (1971).

Stone (1981) studied the thermal characteristics of the testis and epididymis of the boar and

Table 6. A summary of controlled experiments carried out to study the effect of high ambient temperatures on semen production and quality in the boar and subsequent boar fertility.

Reference	Treatment regimes used	Effect of treatment on: Ejaculate volume	Sperm motility	Sperm numbers	Sperm morphology	Fertility
Mazzarri el al. (1968)	Exposure to temperatures of 15°C & 35°C for 10 h & 16 h over a 4 & 8 week period. Daylight also varied. Semen used for A.I.	Not effected.	Decreased significantly at 35°C & after long daylength.	Decreased at 35°C	———	Conception rates reduced in boars at 35 °C & exposed to 16 h daylight at 35°C & 15°C.
McNitt and First (1970)	Exposure to temperatures of 33°C & 50% RH for 72 h after control temp. of 21.5°C. Control group at 20°C & 50% RH.	Not affected.	Decreased from day 20 to 28.	Decreased after 28 days. Max. effect 32 days.	Increase in primary abnormalities days 12 to 28. Secondary abnormalities day 24 to 32	Concluded that semen changes were consistent with reduced fertility seen following short periods of heat stress in field.
Christenson et al. (1972)	Exposure to temperatures of 33.3°C for 72 h. Control group at 23.3°C Semen used for AI.	Decreased 30 to 60 days after.	Decreased 155 to 20 days after.	Decreased 30 30 to 60 days after.	Increased abnormalities 15 to 20 days after.	Fertility normal for 15 days after. Conception rates, embryo survival and litter size adversely affected day 16 to 58 after.
Wettemann and Desjardins (1975)	Exposure to 34°C for 8 hours & 31°C for 16 h for 90 days.	———	———	Spermatids significantly reduced.	———	———
Wettemann et al. (1976)	Exposure to 34.5°C for 8 h & 31°C for 16 h. Controls at 23.0°C. Daylight constant. Treated over 90 days.	Volume and gel weight not changed.	Decreased markedly during week 3 to 6.	Decreased significantly during week 2 to 6. Sperm reservoirs reduced.	Increased during 3rd to 6th week incl. acrosome to 48%. abnormalities	Nearly 50% reduction in conception. Embryonic survival reduced from 70%

Table 6. (continued)

Reference	Treatment regimes used	Effect of treatment on Ejaculate volume	Sperm motility	Sperm numbers	Sperm morphology	Fertility
Cameron and Blackshaw (1980)	Exposed to temps. between 33°C & 38°C 6 h daily for 4, 5, & 7 days.	Total volume & gel volume generally not affected.	Decreased in weeks 3, 4 & 5 after heat stress.	Not significantly affected.	Abnormal sperm increased significantly during 2, 3 & 4 weeks after heat stress.	———
Winfield et al. (1981)	Exposed to 40°C for 8 h and 30°C for 16 h each day for 4, 7 or 10 day periods.	Not measured.	Decreased only in some boars.	Decreased in some boars treated for 7 days or more.	Increase, in particular tail morphology, 3 to 5 weeks after.	Considered to reduce fertility for up to 4 weeks commencing 2-3 weeks after treatment.
Stone (1982)	Exposed to increasing ambient temp. from 20°C by 1 C daily to a max. of 40°C and maintained at 40°C for 24 h.	Reduced semen and gel volume.	Decreased to 19% motile sperm at 35°C.	Not significantly affected.	Increased in incidence at 35°C.	———
Larsson and Malmgren (1984)	Exposed to 35°C & 40% RH for 100h. Semen collected before & after stress used for AI. 6 h studied.	No change.	Decreased 2 to 4 weeks after heat in 4 boars.	No change.	Increase in abnormal heads & tails in 4 of the 6 boars 2 to 6 weeks after stress.	Semen from severly affected boars resulted in 50% & 64% fertilization rate 2-3 weeks & 4-6 weeks, respectively.

found the intra-testicular temperatures of conscious boars were in the range of 35.0°C to 36.5°C, while the values for the caput epididymis were 35.5°C to 37.0° C, and for the cauda epididymis 35.3°C to 37.0°C. These temperatures were found to be 2.5, 1.5 and 1.9°C, respectively, below the rectal temperature (38.2°C), and each varied diurnally. Stone showed that as the environmental temperature increased from 23°C to 34°C, testicular and epididymal temperatures increased. The difference between testicular and rectal temperature diminished as the environmental temperature increased.

From the literature it is apparent that seasonal infertility in pigs may be due partly to the effect of temperature on the boar. Both direct heat applied to the testis and exposure to high ambient temperatures, especially during long periods of daylight, have been shown to cause a decline in semen quality and a reduction in conception rate for several weeks after. Most experiments reported have used high temperatures (33.3°C) continuously for up to 72 hours or high temperatures alternated with slightly lower temperatures for periods as long as 90 days. Under normal conditions, at least in Australia, these extremes of heat may be considered excessive. Further, most of the studies cited have used boars bred in a more temperate climate than many pigs produced in Australia.

STUDIES CARRIED OUT TO DETERMINE THE EFFECT OF SEASON AND ELEVATED AMBIENT TEMPERATURES ON SEMEN QUALITY IN THE BOAR

A total of 34 boars were used in a series of studies to determine the effect of season on semen characteristics over a three-year period. The boars were housed in a 50 sow commercial piggery in South East Queensland where temperatures ranged from maximum temperatures in winter of 20°C to maximum summer temperatures of around 30°C. Semen was collected using a dummy sow and evaluated only after repeated collections had been made to stabilize epididymal sperm reserves. The studies included: (i) Comparison of boars' semen characteristics during summer and again during the following winter; (ii)Comparison of boars of the same age collected during the two seasons; and (iii) Boars collected over 3 summers and 3 winters. The results (Table7) showed that season had no adverse effect on any semen characteristics that could be related both to quality and production. In fact, in some cases ejaculate volumes and sperm numbers were greater when collected during summer.

Table 7. Characteristics of semen from 34 boars collected 3 times a week (Monday, Wednesday, Friday) for 6 weeks*.

Semen characteristics	Season	
	Summer	Winter
Total volume (ml)	367**	274
Fluid volume (ml)	202**	182
Gel volume (ml)	163**	92
Sperm concentration (10^6/ml)	144.6	164.1**
Total sperm (10^9)	27.9	27.3
Daily sperm output (10^9)	11.9	11.7

* ; 17 of the boars were collected during the summers and the other 17 during the winters over a 3-year period.

** ; $P < 0.01$

These studies, together with others, suggested that in boars reared in a sub-tropical environment, where seasonal variation in temperatures is relatively slight, semen quality and production are not affected by season to the extent that they can be in a temperate climate. Also, boars bred and reared in the relatively mild sub-tropical environment of Australia and protected by well-ventilated housing are unlikely to show any adverse changes in semen production or quality which can be attributed to season.

The effect of elevated ambient temperature on semen quality and production was studied in 12 boars divided into three treatment groups and heated in a controlled climate room for 6 hours per day using maximum temperatures ranging from 33.4 to 37.7°C (Table 8).

Table 8. Mean (± s.e.) maximum temperatures achieved during the heating of the boars (4/ group).

Temperature (°C)	Group I (24 h)	Group II (32 h)	Group III (43 h)
Ambient	36.2 ± 0.9	35.3 ± 0.6	35.6 ± 0.2
Rectal:			
Maximum	39.8 ± 0.2	39.0 ± 0.2	39 4 ± 0.7
Increase	1.67 ± 0.2	1.17 ± 0.1	1.37 ± 0.2
Scrotal skin:			
Maximum	36.7 ± 0.3	36.3 ± 0.3	37.0 ± 0.2
Increase	4.2 ± 0.3	4.6 ± 0.1	4.3 ± 0.7

As can be seen in Table 9, ejaculate volumes, gel volumes, sperm concentration and daily sperm outputs were not affected significantly in any of the groups, although changes were seen in individual animals. In some boars heat stress early in the treatment period produced an acute rise in body temperature which appeared to have a greater effect on semen quality than did the duration of exposure. Significant increases in the proportion of morphologically abnormal spermatozoa were seen in all groups by the end of week 2 and up to week 5 after treatment. Boars exposed for 7 days were, in general, more severely affected.

Table 9. The semen characteristics (mean ± s.e.) for 12 boars before and after exposure to elevated ambient temperatures.

	Weeks								
	-1	0	1	2	3	4	5	6	7
Fluid vol.(ml)	208.0 ±12.2	208.5 ±16.0	216.8 ±17.6	198.5 ±14.2	200.1 ±14.6	196.3 ±12.6	197.9 ±10.2	199.6 ±12.4	196.1 ±12.5
Gel vol.(ml)	110.1 ±10.5	131.1 ± 9.1	120.7 ±8.6	107.8 ±10.3	106.3 ± 7.7	109.9 ±8.7	93.9 ±6.7	98.5 ±7.8	103.8 ±8.6
Sperm conc. ($x10^9$/ml)	139.1 ±18.6	117.5 ±10.9	102.7 ±8.8	107.3 ±9.9	109.1 ±10.2	99.2 ±9.1	86.8 ±8.4	94.2 ±8.8	106.5 ±8.2
Total sperm no. ($x10^9$/ml)	26.4 ±2.9	21.5 ±1.4	20.8 ±1.7	20.5 ±1.9	21.8 ±2.4	19.9 ±2.2	18.6 ±2.2	18.5 ±2.0	20.9 ±2.0

THE FUTURE DIRECTION OF RESEARCH

A number of areas for further research need to be considered although it would appear that under normal field conditions, especially in our modern pig industry, there is little reason why boars should be exposed to environmental conditions that adversely effect fertility. Nevertheless, intensively used boars, and especially young boars, require maximum efficiency in semen production, maintenance of sperm reserves and semen of high quality to meet the needs of the modern pig breeding unit. All factors influencing these parameters should be the subject for further research.

Now, and in the future, the emphasis will be on the use of artificial insemination (AI), *in vitro* fertilization and embryo transfer. The production and use of good quality semen in all these techniques is paramount and therefore a more detailed understanding of the effect of season, temperature, photoperiod and adaptation to the environment will be required.

This paper has concentrated on the effect of season and temperature on semen production

and quality. However, their effect on libido and male endocrine function have yet to be fully resolved, especially considering that Winfield et al. (1981) found courting behaviour of boars was depressed at temperatures of 40°C. These workers considered boars were likely to be less willing to mate at temperatures above 30°C. In addition, the influence of photoperiod on boar fertility has been the subject of only a few studies and its importance is still not clear. Photoperiod may prove to be as important as temperature is on the onset of puberty and the subsequent production and quality of semen in the modern breeding boar.

Future research should include:

(1) The influence of season, ambient temperatures and photoperiod on the quantity and quality of semen to be harvested, processed and used for AI under Australian conditions.

(2) Studies of the relationship between the semen characteristics traditionally used to evaluate semen quality for artificial insemination and fertility. The variable characteristics of sperm concentration, sperm motility and the presence of morphologically abnormal sperm seen in boar semen at the time of collection may have an important influence on its suitability for use in AI. The influence of semen quality, as affected by season on embryo survival, also requires further research.

(3) Studies of the inter-relationship between frequency of ejaculation/collection, seasonal factors, the environment and semen quality to provide a better understanding of the useage of boars under different environmental and housing conditions.

CONCLUSIONS

There is ample evidence to show that the fertility of boars can be influenced by season and especially high ambient temperatures. The influence of photoperiod is, however, still not clear. Research has shown that the main effect of heat is on spermatogenesis and boars can be adversely affected when exposed to ambient temperatures over 33°C for at least 72 hours. Shorter periods of heat stress may also effect boar fertility.

Environmental stress in boars will continue to be important as AI and embryo transfer becomes more frequently used and where boars are transported over long distances and subjected to a variety of different environmental conditions.

SEASONAL INFERTILITY IN THE PIG: CONCLUSION

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In the Introduction to this symposium the complexity of seasonal infertility was outlined. In brief, the main symptom of seasonal infertility is a seasonal decrease in the farrowing rate of both sows and gilts. These infertile pigs either show a delayed return to oestrus, or a failure to return to oestrus after normal mating. We reviewed the evidence that the main symptom of seasonal infertility is really many different forms of infertility, such as early embryonic death, luteinized ovarian cysts, small ovarian cysts, silent oestrus or an early undetected abortion and each of these may present as a common clinical symptom. Before this symposium, there were two main thoughts as to the cause of seasonal infertility, heat and a generalized stress response.

In the light of the papers presented, it is now possible to fit names to a few more pieces of the jig-saw puzzle called seasonal infertility. It is clear that excessive temperature may have a direct effect on the gametes, embryo survival or uterine function, although the heat load would have to be quite extreme and probably prolonged in duration. The effects of high temperatures on the sow are more likely to be indirect by causing disturbances to the endocrine milieu at sensitive stages, particularly just before and around ovulation. It is also quite clear that the ancestral pigs from which the domestic pigs were derived, were seasonal breeders, and thus the modern sow may still retain some tendency for seasonal reproduction. If the pig does have an underlying seasonality to its reproduction, then it is likely that photoperiod, the pineal gland and melatonin are involved in some way in regulating this seasonality. However, the absence of a predictable, nocturnal rise in melatonin in the domestic sow, as is seen in other species of short day breeders, is rather perplexing. This area of the pig's reproductive physiology is in need of further research. The results of the University of Sydney's project on photoperiod and reproduction in the pig are eagerly awaited.

We have also seen that prolonged high temperatures can directly effect semen quality in the boar, and result in reduced conception rates and reduced litter sizes. It is also possible that temperature may indirectly affect the reproductive performance of the boar, by reducing the boar's sexual behaviour. Finally, a generalized stress response can cause seasonal infertility and individual pigs vary in the way they respond to stress.

Two important questions that we must keep in mind when looking for causes of seasonal infertility are:

1. Why do some herds have the symptoms of seasonal infertility present all year?
2. Why some herds do not experience the problem even over the hottest of summers?

I would like to put forward the following hypothesis, that the occurrence of seasonal infertility is like a jig-saw puzzle. To that puzzle we must add a variety of pieces or factors before the clinical picture of seasonal infertility appears. For example, pieces of the jig-saw may be added for the following:

1. The direct and the indirect effects of heat on either the boar or the sow;
2. An underlying trend for seasonal reproduction in the pig's ancestors;
3. A whole variety of management, husbandry and environmental factors that are acting as stressors;
4. The individual variation between animals, in both the way they respond to those stressors, and their ability to tolerate heat loads.

As we add pieces or factors to this jig-saw puzzle we reach the point where we can see the symptoms of seasonal infertility start to appear. Which factor(s), or whether any individual factor, finally causes the clinical picture of seasonal infertility will depend upon the combination of other factors that are acting on that particular herd at that time. For example, in a herd where the total level of management, husbandry and environmental stressors is quite low then by adding the additional heat load pieces to the puzzle will not cause the clinical picture of seasonal infertility to

appear. On the other hand, if there are other "stressor" factors acting on the herd during the year, then over the hotter months the clinical picture of seasonal infertility will appear. This explanation for seasonal infertility could also be used to explain why some herds have the symptoms of seasonal infertility all year round.

In light of the data presented it is proposed that the most important mechanism contributing to the occurrence of seasonal infertility is the animal's response to stressors in its environment. Therefore, the secret to reducing the incidence of seasonal infertility lies in reducing the magnitude and frequency of the stress response of individual pigs, particularly in the period from one week before mating until about three weeks after mating. Basically there are two strategies that can be taken to reduce the impact of a stress response.

The first way to reduce a stress response is to ensure that the pig is housed and managed in a manner that reduces in as many ways as possible, the stressful stimuli in the pig's environment. Detailed attention should be given to the physical and social environment, to husbandry and management practices, and to the thermal environment of sow and gilts from the time that they enter the boar shed, during mating and until about three weeks after mating. The second way to reduce the impact of a stress response is a new way that shows considerable promise. That is to select replacement breeding stock that are genetically and physiologically better suited to their environment. Techniques to achieve this aim should be available in the near future.

Therefore, future research in the field of seasonal infertility should concentrate on trying to identify which of the many stressors acting on the pig are the important ones in precipitating seasonal infertility, designing production systems that reduce the level of stressors acting on the pig, further developing the tests to allow the detection of "stress tolerant" breeding stock and finally identify the mechanisms that control true "seasonal breeding" in pigs and the possible ways to manipulate that control.

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SHORT TERM ELEVATIONS IN PLASMA CORTISOL LEVELS REDUCE PITUITARY RESPONSIVENESS TO EXOGENOUS GnRH IN PREPUBERTAL GILTS

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Puberty attainment in the gilt is stimulated by exposure to mature boars. Maximum efficacy of boar-induced precocious puberty requires physical contact with the boar (Karlbom, 1982), and this has been shown to induce rapid elevations in plasma cortisol levels in recipient gilts (Pearce and Hughes, 1987). Acute elevations in plasma cortisol have been reported to enhance the release of luteinizing hormone (LH) in response to exogenous gonadotrophin releasing hormone (GnRH) in male pigs (Liptrap and Raeside,1983), and may therefore be involved in the changes in the hypothalamic - pituitary axis thought to occur at puberty attainment in the gilt.

The present experiment was designed to investigate the influence of cortisol on the endogenous and GnRH-induced release of LH in pre-pubertal gilts. A total of 24 gilts were fitted with indwelling jugular vein and carotid artery catheters at 148 days of age and allocated according to litter in groups of six to the following treatments at 160 days: (i) infusion of saline (40mls), (ii) infusion of saline + GnRH injection, (iii) infusion of cortisol (10mg in 40mls of saline), and (iv) infusion of cortisol + GnRH injection. Infusion was carried out via the carotid artery catheter from 1517 to 1617 h. Midway through the infusion half of the gilts received 5μg synthetic GnRH (Gonadorelin Intervet, Australia) via the jugular catheter. Blood samples were taken every 15 mins. from 1400 to 1800 h and hourly thereafter until 2200 h and plasma concentrations of LH and total corticosteroids were measured using the methods of Niswender et al (1970) and Barnett et al. (1981), respectively.

Analysis of variance showed a significantly reduced release of LH in response to GnRH injection during cortisol infusion compared to during saline infusion ($P<0.05$, Figure 1). However the secretion of LH was greater during infusion of cortisol alone than during infusion of saline alone ($P=0.054$). Plasma cortisol levels were significantly elevated above baseline in cortisol infused gilts only ($P<0.01$). Thus acutely elevated plasma levels of cortisol reduced pituitary responsiveness to exogenous GnRH challenge, but marginally increased endogenous LH release in prepubertal gilts.

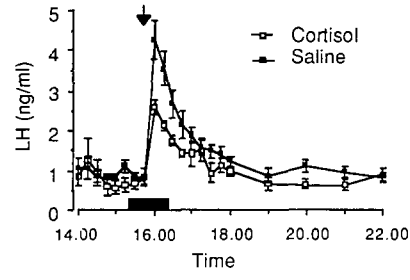

Figure 1.
The effect of 5 μg i.v. GnRH (arrow) on LH secretion in prepubertal gilts infused with cortisol (n=6) or saline (n=5). Each point represents mean ±S.E. Time of infusion is indicated by the shaded bar.

In conclusion, these results indicate that the elevations in plasma cortisol levels in gilts induced by physical contact with boars are unlikely to stimulate puberty attainment by enhancing pituitary responsiveness to GnRH but may be involved in direct stimulation of LH release.

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PUBERTY IN GILTS HANDLED PLEASANTLY OR UNPLEASANTLY

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Repeated unpleasant handling by humans can induce chronic stress in pigs (Hemsworth et al., 1981, 1986). The stressed pigs are fearful of humans, grow slowly and may have impaired reproductive performance. They also have elevated basal cortisol levels and at mating they do not exhibit a normal, acute release of cortisol. An acute release of cortisol is characteristic of prepubertal gilts exposed to mature boars and this release is essential for maximal stimulation of puberty (Pearce and Hughes, 1987). If gilts are chronically stressed they may not be able to respond to boar exposure with an appropriate release of cortisol and the onset of puberty may be disrupted.

In this experiment 70 gilts housed in pen groups of eight or nine were handled pleasantly or unpleasantly for 3 minutes, 3 days per week for 12 weeks from 80 to 164 days of age. Unpleasantly handled gilts were shocked with a battery operated goad if they approached the handler whereas pleasantly handled gilts were patted and stroked. Fear of humans was then assessed in a three minute behaviour test. From 165 days of age the gilts were exposed daily for 30 minutes to a mature boar until they reached puberty or 225 days. The data were analysed with ANOVA and Chi-square procedures. The behaviour test data involving time were transformed ($Y = \ln x + 1$) prior to analysis and the values presented here are the back transformed means.

In the behaviour test pleasantly handled gilts were quicker to approach within 0.5 m of the experimenter (6.1 vs 26.4 seconds, $P< 0.001$), spent more time there (21.9 vs 8.4 seconds, $P< 0.05$) and interacted more quickly (8.6 vs 41.8 seconds, $P< 0.001$) and more frequently (2.7 vs 1.3 interactions, $P < 0.01$) than gilts handled unpleasantly. Growth rate between 80 and 164 days of age was not affected by treatment (645 vs 639 g/day). The mean interval from first boar exposure to puberty was shorter for unpleasant gilts (27.5 vs 20.6 days, $P< 0.05$). Regression analysis showed that the interval from boar exposure to puberty was not significantly correlated with growth rate, body weight or any of the variables measured in the behaviour tests. The overall proportion of gilts reaching puberty within 60 days of boar exposure was not statistically different between pleasant (31/34) and unpleasant gilts (29/36) but more unpleasantly handled gilts reached puberty before day 20 (7/31 vs 17/29, $X^2_1 = 6.68$, $P< 0.01$).

We conclude that the unpleasantly handled gilts were not chronically stressed because their growth rates were not affected. They were highly fearful and this suggests they may have been more prone to acute stress which could explain why they were more, rather than less, responsive to boars. The experiment has wide implications because it shows that pigs housed in groups respond to stressors differently to pigs housed alone and also that fear of humans is not necessarily indicative of chronic stress in pigs.

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DAILY BOAR CONTACT MAXIMIZES THE BOAR EFFECT IN GILTS

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In most studies of the boar effect,boar contact has been applied daily until puberty is reached. Exactly when the signal(s) that initiate final development towards puberty is perceived by the gilt is unknown, as is whether the boar effect is comprised of a single stimulus or repeated stimulation. In this experiment we investigated the effect of limiting the number of days of boar exposure on the onset of puberty. At the practical level this may simplify the application of the boar effect while at the physiological level the period when boar contact initiates the endocrine changes leading to puberty may be identified.

A total of 123 gilts aged 164.6 $\pm$ 0.44 days were divided among four treatments in two herds (Medina and Muresk) in two seasons (winter and summer). The treatments were full boar contact for 20-30 minutes per day for (a) 1 day, (b) 10 consecutive days or (c) daily until puberty and (d) an isolated control. Those not detected in oestrus were slaughtered 80 days (Medina) or 135 days (Muresk) after first boar exposure.

Table 1. Percentage of gilts reaching puberty and mean interval to puberty

	Medina				Muresk			
Treatment	Control	1D	10D	Daily	Control	1D	10D	Daily
Gilts reaching puberty (%)								
Winter	100^{a}	75	100	100	86^{a}	100^{a}	100^{a}	86
Summer	33^{b}	78	67	100	0^{b}	0^{b}	38^{b}	86
Interval (days)	51.4^{x}	41.5^{xy}	28.5^{y}	12.7^{z}	105.8^{e}	95.4^{ef}	84.7^{fg}	71.7^{g}

Comparing winter and summer, percentages with different superscripts are significantly different ($P< 0.05$). Comparing intervals, means with different superscripts are significantly different at $P<0.01$ (except y-z, $P<0.05$).

In the winter, treatment and herd had no effect on the percentage reaching puberty (93% in both herds). In the summer, fewer control gilts reached puberty at Medina than in the winter but the percentage in each group exposed to boars was not affected by season. At Muresk the percentage of control,1 day and 10 day gilts reaching puberty in the summer was lower than in winter but the percentage of daily gilts was the same. More gilts exposed for 1 day in the summer reached puberty at Medina than at Muresk ($P< 0.01$). Season had no significant effect on the mean interval to puberty which decreased with increased boar exposure. In both herds gilts receiving 10 days or daily boar exposure reached puberty earlier than control gilts with 1 day gilts intermediate between the control and 10 day groups. At Medina daily gilts reached puberty significantly earlier than 10 day gilts but this difference just failed to reach statistical significance at Muresk. At each treatment level the Medina gilts reached puberty earlier than the Muresk gilts.

Significant differences between herds and between seasons in both the natural and boar-induced attainment of puberty were observed in this study.When boar exposure was limited to 1 day or 10 days some gilts were stimulated to reach puberty but daily exposure continuing until puberty was necessary for the maximum stimulation of precocious puberty.

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THE BEHAVIOUR AND GROWTH OF MALE PIGS HANDLED PLEASANTLY OR UNPLEASANTLY DURING REARING

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Pigs regularly subjected to unpleasant handling by humans have been reported to exhibit chronically elevated levels of plasma cortisol and reduced growth rates compared to pleasantly handled animals (Hemsworth et al., 1981). The performance of adjunctive behaviours such as chain pulling has been reported to reduce plasma cortisol levels in individually stressed pigs (Dantzer and Mormede, 1983). Thus if unpleasant handling reduces growth rates via chronically elevated cortisol levels (as suggested by Barnett et al., 1983), the provision of facilities to enable the performance of adjunctive activities may alleviate the stress and hence avoid the depression in growth rates.

The present experiment investigated the effects of pleasant (P) and unpleasant (U) handling for 3 minutes 3 times per week in normal (N) and enriched (E) environments on the behaviour and growth rates of 60 male pigs housed in groups of 7 or 8 between 40kg and 90kg bodyweight. Unpleasantly handled pigs were shocked with an electric goad if they approached the handler whereas those pleasantly handled were stroked. The enriched environment pens were fitted with chains, tyres and bars. The behaviour of each pig was recorded using instantaneous scan sampling every minute for 40 minutes once per week around handling at 10am and once per 2 weeks around feeding at 1pm. Number of observations made per treatment (number of 40 minute observation periods x number of pigs observed in each period) varied from 141 to 163 around handling and from 43 to 62 around feeding. Results are expressed as the median occurence of the behaviour per 40 minute observation period.Fear of humans was assessed after 24 handling periods by recording the time taken for each pig to enter an area within 0.5m of the stationary experimenter (area A).

Table 1.The effects of the envirionment and handling on behaviour and growth rates of pigs.

Treatment	Mean time to enter area A (secs)	Behaviour around handling period active	Behaviour around handling period inactive	Active behaviour around feeding	Mean rate (g/day)
UN	126.7^{c}	8.0(1-26)^{e}	5.0(2-23)^{x}	23.0(0-29)	700
UE	52.9^{a}	7.0(1-24)^{e}	5.0(0-13)^{x}	21.0(0-28)	670
PN	23.7^{a}	11.0(0-34)^{f}	2.0(0-34)^{y}	21.5(0-25)	685
PE	8.2^{b}	11.0(0-30)^{f}	1.0(0-16)^{y}	22.0(0-26)	688

a,b,c: back transformed means with different superscripts are significantly different ($P<0.001$) (Analysis of Variance on log transformed data) e vs f, x vs y: median (ranges) with different superscripts are significantly different ($P<0.001$), (Mann-Whitney U test).

In both types of environment U handled pigs were significantly more fearful of humans than P handled pigs ($P<0.001$, Table 1). However, pigs in the E environment were less fearful of humans than those housed in the N environment ($P<0.05$). Significantly less active behaviour (such as moving, nosing the floor, chains etc.) and more inactive standing and sitting was observed around U handling than P handling ($P<0.001$). No significant treatment differences were observed in behaviour around feeding. Mean growth rates were not significantly effected by treatment.

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The results suggest that the pigs subjected to unpleasant handling were acutely stressed by the presence of humans and that the provision of an enriched environment reduced this stress. However the absence of any effect of treatment on growth rates or the occurrence of adjunctive activities would suggest that they were not chronically stressed, in contrast to the findings of previous studies using individually housed pigs. It is possible that the presence of conspecifies in group housing enables social interactions which allow the animals to cope with stressors without recourse to the physiological and behavioural responses observed in individually housed pigs.

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PRE-PARTUM TESTS TO PREDICT STILLBIRTHS IN SOWS

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Losses from stillbirths in Australian piggeries average around 7%. This trial investigated several simple tests on sows with a view to predicting those likely to farrow stillborn piglets. In the week prior to farrowing, blood and urine samples were collected from 404 sows which had farrowed at least three litters. Blood haematocrit (PCV, %) was measured, and the urine was tested for leukocytes, nitrite, pH, protein, haemoglobin and blood using "Nephur-Test + Leuco" test strips (Boehringer Mannheim Australia, North Ryde, NSW 2113), and then cultured using "Urotube 2s" to determine total bacterial counts, which were scored from 1 (1,000 organisms per ml) to 5 (1,000,000 per ml) (Roche Diagnostics, Dee Why, NSW 2099). Backfat (BF, mm) was determined at the P2 level. The data from these tests were analysed to determine any relationships with the subsequent prevalence of stillbirths (SB).

Table 1. Relationship between some tests and the stillbirth rate.

Urine Tests	No.	pH	PCV	BF	Bact.	Alive	Dead	Total	%SB
pH:<7	240	6	34	30	2	11.72	0.80	12.52	6.4
: 7+	164	8	33	30	2	11.07	0.96	12.03	8.0
Nitrite: Neg	314	6	34	30	1	11.42	0.87	12.29	7.
: Pos	90	7	33	29	5	11.56	0.88	12.44	7.1
Protein: <100	385	6	33	30	2	11.48	0.85	12.33	6.9
: 100+	19	7	33	32	3	10.84	1.26	12.10	10.4
Leukocyt: <10	387	6	34	30	2	11.49	0.83	12.32	6.7
: 10+	17	7	32	27	3	10.53	1.76	12.29	14.3
Bacteria: <4	305	6	33	30	1	11.43	0.88	12.31	7.1
: 4+	99	7	33	29	5	11.53	0.85	12.38	6.9
Blood Test									
PCV: 33+	270	6	35	31	2	11.40	0.71	12.11	5.9
: <33	134	7	30	28	2	11.56	1.18	12.74	9.3
Litter Statistics									
All sows	404	6	33	30	2	11.45	0.87	12.32	7.1
No. Alive : <12	193	7	34	30	2	9.32	1.13	10.45	10.8
: 12+	211	6	33	30	2	13.41	0.63	14.04	4.5
No. Dead : <2	316	6	34	30	2	11.71	0.30	12.01	2.5
: 2+	88	7	33	31	2	10.52	2.91	13.43	21.7

Eighty-eight of the 404 sows (22%) produced two or more stillborn piglets, and these made up 73% of all stillborns. The nitrite test detected 85% of sows with 100,000 or more organisms per ml, but this infection had no apparent effect on the stillbirth rate. Although one-third of the sows had more than 10% of their litter stillborn, no significant relationship was found between the proportion of stillborns per litter and any of the factors measured.

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PREDICTING THOSE SOWS LIKELY TO FARROW STILLBORN PIGS

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In commercial herds stillbirths in piglets account for between 4.9 and 7.9% of all pigs born (English and Wilkinson, 1982). Spicer et al (1986) reported that pigs which died during farrowing accounted for 29% of the preweaning deaths. Stillborn pigs were born towards the end of the litter, were lighter than litter mates (1.17 kg vs 1.30 kg) and were born 70 minutes after the previous birth compared to 16 minutes for liveborn litter mates. This paper reports the results of studies to predict those sows likely to farrow stillborn pigs. The data were drawn from the farrowing records of a 6000 sow farm in Victoria and from four farms with 3700 sows in North Carolina, USA.

Frequency of Stillbirths: Most (60. 44%) sows farrowed litters without stillborn pigs. In Victoria 14% of the sows produced 62% of the stillborn pigs and in North Carolina, 18% of the sows produced 70% of the stillborn pigs. These sows farrowed two or more stillborn pigs.

The Effect of Parity on Stillbirths: In both Victoria and North Carolina parity one sows farrowed a higher percentage of stillborn pigs than parity two sows. From parity two, stillbirth rate increased linearly with parity. As the sows aged, those which farrowed stillborn pigs farrowed multiple stillbirths in greater numbers. Thirty percent of the parity 10 sows which farrowed stillbirths produced 3 or more stillbirths. Only 16% of parity one sows farrowing stillbirths farrowed 3 or more.

Repeatability of Stillbirths: Analysis of Victorian data demonstrated that most sows which had multiple stillbirths in their previous litter had an increased chance of farrowing multiple stillborn pigs in their current litter. For sows which farrowed multiple stillbirths in their current litter, 36.5%farrowed multiple stillbirths in their previous litter. The relationship was highly significant (P<.001) for parities 4-7 but not for younger sows (Table1). This effect was not tested with data from North Carolina.

Table 1. The effect of stillbirth rate in the previous litter on stillbirth rate in the current litter. Data from 415 sows, parities 4-7, Victoria 1987.

Number of Stillbirths		% Sows
Current Litter (No. Sows)	Previous Litter	
NONE (205)	NONE	59
	ONE	25
	MULTIPLE	16
ONE (114)	NONE	46
	ONE	33
	MULTIPLE	21
MULTIPLE (96)	NONE	38.5
	ONE	25
	MULTIPLE	36.5

The results demonstrate the importance of parity and previous farrowing history with regard to determining and implementing strategies which reduce stillbirth rates in pig herds.

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PROFILE OF CHANGES IN PIGLETS WHICH DIE PRIOR TO BIRTH

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Approximately 7% of all fully-developed piglets are stillborn, i.e. they are born dead, or found dead behind the sow after birth. Some of these stillborn piglets may have died prior to farrowing, but intra-partum deaths have been estimated at 80% of stillbirth losses (English et al., 1982). Although the majority of stillborn piglets are regarded as having died during parturition, there is little evidence as to the actual time of death. In studies on stillbirth losses it is important to know the time of death, and so an experiment was designed to measure gross, histological, and biochemical changes over time in term-piglets which died from anoxia and were maintained in utero at 38°C. Only changes seen at autopsy, and in blood glucose and lactic acid concentrations are reported in this paper.

Two sows at 114 days of gestation, and one at 115 days, were killed by shooting. The gravid uterus was immediately removed and placed in a saline water bath at 38°C. Piglets were withdrawn from the uterus within 10 min, or at 1, 4, 8 or 24 h after the hysterectomy, and autopsied, using 7, 11, 14, 6, and 5 piglets, respectively.

Table 1. Gross changes in piglets after death "in utero".

Tissue	0 h	1 h	4 h	8 h
cornea	clear	slight opaque	opaque	opaque
skin	wet	meconium-stain	meconium	red blotch
subcut. oedema	no	yes	yes	yes
skel. muscle	normal	firmer	most rigor	rigor
colon	filled	empty segment	empty seg.	empty seg.
stomach	fluid present	meconium	meconium	meconium
abdom./thorax	normal	slight fluid	fluid/fibrin	fluid/fib.
liver	yellow-tan	red edges	all red	all red
heart	flabby	firm	firm	firm
lungs	uniform pink	sl. darker	red blotches	red blotch
intestines	firm tone	some tone	flaccid	flaccid
blood lactic acid	13.1mmol/l	15.0mmol/l	20.1mmol/l	23.9mmol/l
blood glucose	2.9mmol/l	15.0mmol/l	17.0mmol/l	15.9mmol/l

Blood lactic acid concentrations increased gradually with time after death. Blood glucose concentrations also increased, especially between 0 and 1h ($P<0.025$). Field evaluations of the significant changes reported here are in progress.

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THE EFFECTS OF MATING CONDITIONS ON THE SEXUAL BEHAVIOUR OF MALE AND FEMALE PIGS

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There has been only limited research conducted to determine the optimum conditions under which matings should be carried out. The objective of this experiment was to compare the sexual behaviour of pigs mating in the boar's accommodation pen with that of pigs mating in a pen specifically designed for mating.

Sixteen 7 month-old boars and 48 7 month-old ovariectomized gilts were randomly allotted to one of two treatments; the pigs were mated in either the boar's accommodation pen (1.9 x 2.2 m in size with a floor area of 4.2 m^2) (BP treatment) or an octagonal-shaped mating pen (minimum width of 2.8 m with a floor area of 10.5 m^2) (MP treatment). Both pen types had a concrete floor but in the MP treatment there was a light sprinkling of saw dust on the floor. Gilts were injected with 0.8 mg of oestradiol benzoate every two weeks to induce behavioural oestrus. During oestrus gilts were given daily opportunity to mate with a boar from the same treatment in a 5-min. mating test in the appropriate pen. The treatments were imposed for 15 weeks and the sexual behaviour of the pigs was observed.

Similar numbers of mating tests were conducted on each treatment group (3.6 and 3.7 mating tests per week for each boar in the BP and MP treatments, respectively), however the percentage of mating tests which resulted in successful copulations (mating rate) was significantly lower for the pigs in the BP treatment than for those in the MP treatment (76% and 88%, respectively, $X^2_1 = 23.0$, $P<0.01$). The sexual behaviour of the gilts in the two treatments were similar, but there was a trend for the sexual behaviour of the boars in the treatments to differ (Table 1). This suggests that the low mating rate for the BP treatment was mainly mediated through an effect on the sexual behaviour of the boar rather than that of the gilt.

Table 1. Summary of the sexual behaviour of the pigs in the two treatments.

Parameter	Mean (per pig) for Treatment	
	BP	MP
GILTS		
Duration of oestrus (days)	2.4(0.14)*	2.6(0.20)
Interval between boar mounting and gilt displaying the standing response (min).	0.3(0.08)	0.3(0.13)
BOARS		
Total number of copulations	40.5(3.61)	49.6(3.45)
Duration of ejaculation (min)	3.9(0.23)	4.3(0.36)
Time to first mount (min)	0.9(0.24)	0.6(0.07)

* Standard errors

These results indicate that the BP treatment may affect the sexual behaviour of the boar to the extent where mating rate is reduced. This has considerable practical implication since this treatment is very similar to the mating conditions of many commercial pigs. Further research is required to identify the factors in the BP treatment that inhibit or interfere with the sexual behaviour of boars.

THE EFFECTIVENESS OF ALTRENOGEST IN SYNCHRONIZING OESTRUS IN GILTS

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Under normal management conditions extra gilts are kept so that adequate numbers can be mated each week to maintain regular production. A reliable system for regulating the number of gilts available for mating each week would require fewer gilts being held, and result in economic benefits to the producer.

Altrenogest, an oral synthetic progestagen (Roussel-Uclaf, France), has been used successfully to synchronize oestrus in cycling gilts (Webel, 1982). This experiment was run in a commercial piggery to evaluate its effectiveness under Australian conditions. Each week, for 20 weeks, ten 28-week-old gilts were allocated unseen to the treatment, and the remaining 30 or so acted as controls. Altrenogest (20 mg per gilt per day) was mixed in the feed of treated gilts for 18 days. All gilts received 15 minutes of boar exposure each day for 13 days, and were then moved into the boar shed and mated as they exhibited oestrus.

Control gilts were available for mating from the first day in the boar shed, but the first mating of the treated gilts did not occur until the ninth day, i.e. four days after removal of treatment. Two-thirds of matings of the treated gilts occurred on days five and six after altrenogest treatment, demonstrating its synchronizing effect.

Table 1. The effect of altrenogest on reproductive performance.

	Altrenogest		Control		X^2	LSD
number of gilts	200		731			
number mated	175	(87.5%)	619	(84.7%)	NS	
number farrowed	153	(76.5%)	526	(72.0%)	NS	
(% of mated)		(87.4%)		(85.0%)	NS	
Litter: Born alive	9.92		9.48			<0.05
Born dead	0.44		0.40			NS
Total born	10.36		9.88			<0.025
First 14 days in boar shed (9 days off altrenogest):						
Number mated (% total)	163	(81.5%)	453	(62.0%)	<0.001	
(% of all mated)		(93.1%)		(73.2%)	<0.001	
No. farrowed (% mated)	143	(87.7%)	383	(84.5%)	NS	
(% of all farrowed)		(93.5%)		(72.8%)	<0.001	

If gilts were kept in the boar shed for two weeks, instead of the usual six, 93% of the matings of treated gilts would have occurred, compared with only 73% in the controls. Regular use of altrenogest requires less labour, since gilts would not be in oestrus until four or more days after treatment; and less feed and space, since fewer animals would be needed as replacements. Those kept would be mated earlier, and any gilts not mated by nine days after treatment could be culled.

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ENERGY AND PROTEIN METABOLISM IN THE PIG

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INTRODUCTION

Knowledge of the factors affecting the partitioning of energy between maintenance, protein and fat is fundamental to understanding the consequences of change in nutrient intake on growth performance and body composition, determining the animal's requirements for dietary nutrients, how these change as it grows and for the design of biologically and economically efficient diets and feeding strategies.

This review attempts to highlight the more critical aspects of energy and protein metabolism affecting the performance and body composition of pigs from birth to maturity, and to indicate where current information may be either inadequate or misleading. The first section of this paper deals briefly with the effects of dietary protein status on energy and protein metabolism. The remainder of the paper relates only to situations in which protein accretion is not restrained by protein or amino acid intake.

In the final section, some of the more recent information on the effects of exogenous growth hormone administration on pig growth and development is discussed. Advances in biotechnology have enabled this area of research to progress at a much faster rate than could have been anticipated two years ago, and some of the initial research findings indicate that implementation of this new technology may necessitate a complete re-evaluation of current concepts of energy and protein metabolism.

PROTEIN AND ENERGY METABOLISM UNDER CONDITIONS OF DIETARY PROTEIN DEFICIENCY

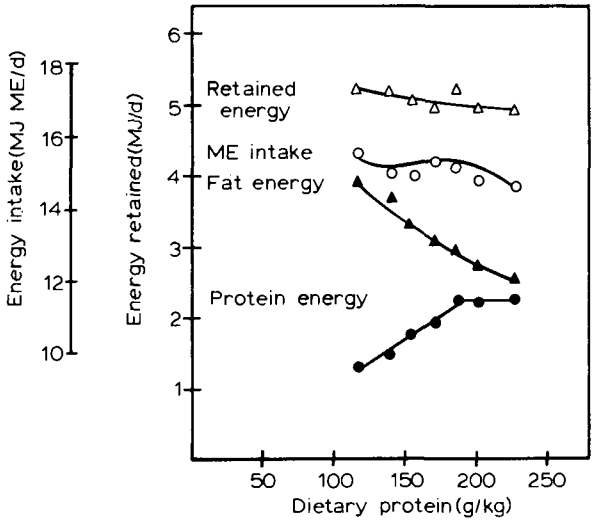
Although there is evidence that potential energy retention and voluntary feed intake may be depressed in pigs given protein-deficient diets (Likuski et al., 1961; Rogerson and Campbell, 1982), there is little definitive information on the effects of increasing protein deficiency on the rate of protein and fat deposition in pigs offered feed *ad libitum*. This is ably demonstrated by the fact that in the simulation model published recently by Black et al. (1986) the authors were forced to use the data of Radcliffe and Webster (1976) for rats to predict the effects of dietary protein status on the voluntary feed intake and growth performance of pigs. Radcliffe and Webster (1976) found that voluntary feed intake of rats began to decline once dietary protein concentration was reduced below requirement and was accompanied by a reduction in energy retained as protein. Fat deposition on the other hand, did not begin to decline until dietary protein concentration had been reduced below 0.7 of requirement.

These responses are not entirely consistent with those which have been observed in growing pigs given diets of varying protein content (Giles et al., 1986; Campbell and Taverner, 1986). This is also indicated by the results of a more recent study involving pigs from 8 to 20 kg liveweight, which are summarized in Figure 1. In contrast to the situation with rats (Radcliffe and Webster, 1976) these results (Figure 1) showed that voluntary energy intake was highest for pigs given the diets of lowest protein concentration and tended to decline when protein intake exceeded the animal's requirement at the tissue level. The results also showed that reducing dietary protein below requirement was associated with a linear decline in protein accretion and a concomitant linear increase in fat accretion. There was however, no evidence of any decline in rate of fat accretion even at the lowest dietary protein concentration tested (0.62 of requirement).

Because the feed intake of pigs to 20 kg is limited by ingestive capacity (Campbell et al., 1975) it is possible that the increased voluntary energy intake indicated in Figure 1 for pigs given

the lowest protein diets may be more pronounced in pigs of heavier body weight and, as such, may result not in reduced but increased fat accretion under conditions of severe protein deficiency. There is evidence that this occurs in broiler chickens (Campbell et al., 1987) when dietary protein concentration is reduced below 0.6 of requirement and these relationships need to be established over a wide range of dietary protein concentrations for pigs up to 100 kg.

Figure 1. Effect of dietary protein content on the metabolizable energy intake, total energy retained and as protein and fat in pigs offered feed *ad libitum* from 8.0 to 20.0 kg (Campbell and Taverner, unpublished results).



PROTEIN AND ENERGY METABOLISM UNDER CONDITIONS OF DIETARY PROTEIN ADEQUACY

Under conditions of protein adequacy the partition of energy between fat and protein is determined by the relationship between energy intake and rate of protein deposition (Black,1974; Williams, 1976; Campbell and Dunkin, 1983a). Knowledge of this relationship is fundamental to the development of diets and feeding strategies and for determining the consequences of change in feed intake on the performance and body composition of pigs growing over specific live weight stages up to commercial slaughter weight.

On the other hand, to determine or predict the pigs potential requirement for dietary nutrients and its body composition and performance capabilities at any stage of development, information is required on the relationship between live weight and maximal protein and fat accretion between birth and maturity.

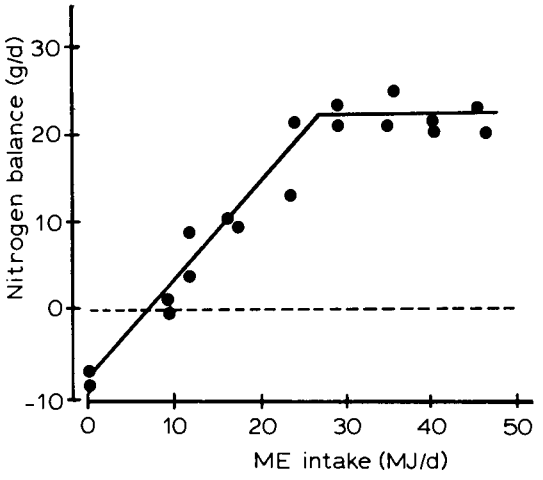
The relationship between protein deposition and energy intake for pigs to 100 kg.

There is limited support in the literature for each of three alternative response relationships, namely, linear, curvilinear and linear/plateau. On the basis of limited information the Agricultural Research Council (1981) concluded that the relation between energy intake and protein accretion was probably linear. It was however, pointed out that most of the data related to young pigs and that the relationship may be affected by factors such as live weight, sex and breed or strain.

In contrast Whittemore and Fawcett (1976) proposed that protein deposition responded linearly to energy intake up to a maximum point at which it plateaued. This linear/plateau relation between energy intake and protein deposition (Figure 2) has since been confirmed experimentally (Tullis, 1982; Campbell et al.,1985; Dunkin et al., 1986) and it now appears certain that there is an

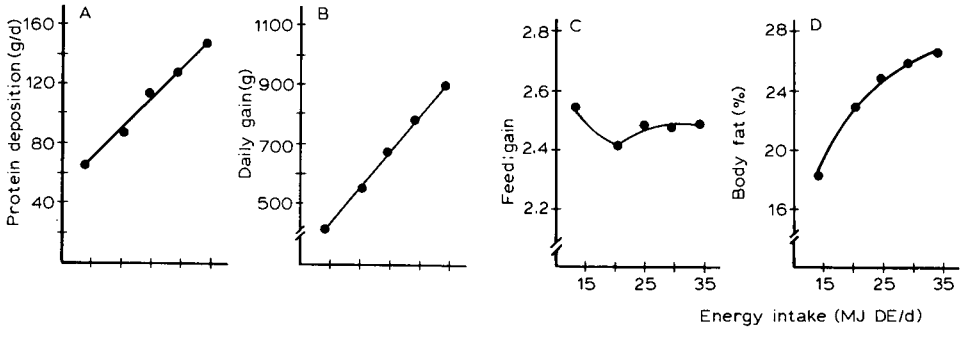
intrinsic limit to protein deposition for pigs at any stage of growth and development (Hodge, 1974). However, because pigs up to 50 kg are generally unable to consume sufficient energy even when offered diets of high energy concentration, to reach the plateau for protein growth, the relation between protein accretion and energy intake during the earlier stages of development is essentially linear (Williams,1976, Close et al; 1979; Campbell and Dunkin, 1983b; Campbell et al., 1983).

Figure 2. The relation between metabolizable energy intake and rate of protein deposition for boars weighing 75 kg live weight (from Dunkin et al., 1986).



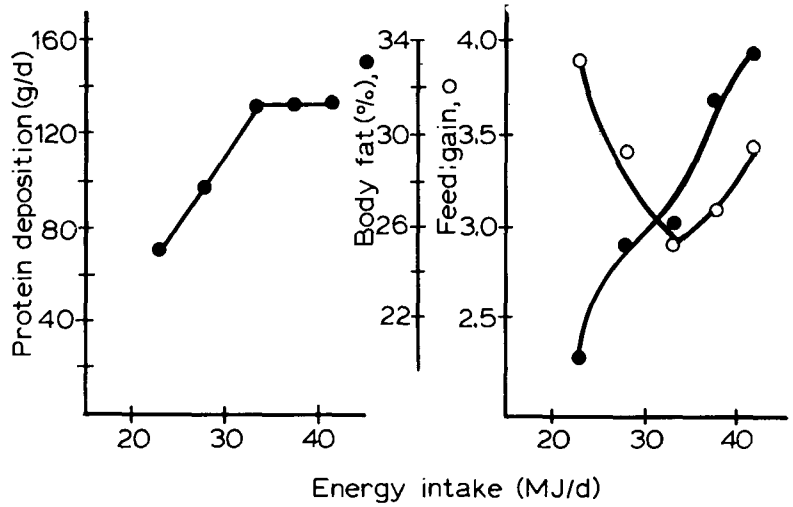
Under these circumstances raising energy intake results in concomitant linear increases in the rates of deposition of water, fat and ash and thus in growth rate. In contrast, feed: gain decreases as energy intake is raised from an initially low level and reaches a minimal value determined by dietary energy concentration and environmental temperature. Body fat content on the other hand, increases in a curvilinear manner and approaches a maximal value determined by environmental temperature, initial body fat content and to a lesser extent by sex and genotype. These responses which have been reported for pigs up to 50 kg by numerous researchers (Burlacu et al., 1973; Close et al., 1979; Close et al., 1983; Campbell and Dunkin, 1983a; Campbell et al., 1983) are illustrated in Figure 3.

Figure 3. The effects of energy intake on A) protein deposition, B) growth rate, C) feed: gain and D) body fat content of entire male pigs grown from 20 to 45 kg (Campbell et al., 1983).



However, when energy intake is sufficient to maximize protein accretion (reach the plateau) any extra energy supplied is deposited as fat resulting in an overall decline in the rate and efficiency of growth and marked increase in body fat content. These responses which are commonly observed in pigs above 50 kg live weight are illustrated in Figure 4. Although the general linear/plateau relation between protein accretion and energy intake appears to be constant for pigs of any age, weight, sex or genotype, there is evidence that each of these factors may affect the height and position of the plateau, and possibly the slope of the linear response phase of the relationship.

Figure 4. The effects of energy intake on protein deposition, feed gain: and body fat content of entire male pigs grown from 48 to 90 kg (from Campbell et al., 1985).



Effects of live weight

It has been suggested by Whittemore and Fawcett (1976) and Whittemore (1986) that for pigs of the same sex and genotype the slope of the linear component of the relationship between energy intake and protein deposition and the plateau (maximal protein accretion) are largely independent of live weight. This proposition however, is inconsistent with the decline in the rate and efficiency of growth and increase in carcass fatness which commonly accompany increase in live weight in pigs given the same level of energy intake, even after differences in maintenance energy requirement have been allowed for.

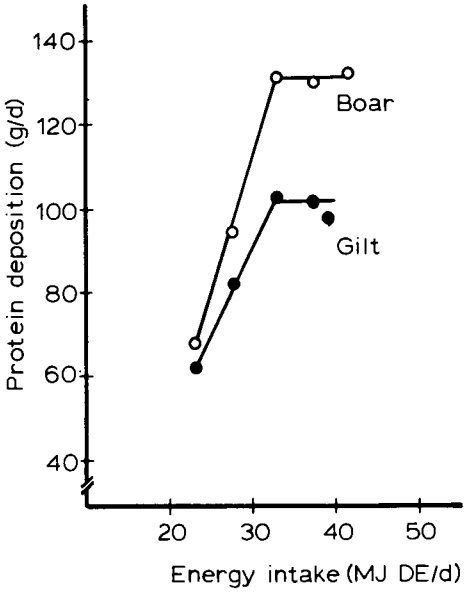
The results of Dunkin et al. (1984) and Dunkin and Black (1985) indicate that the slope of the relationship between energy intake and protein deposition falls with increasing live weight. The same results also indicated that the change in slope was greater as weight increased for lighter pigs than for heavier pigs. The results of Dunkin and Black (1985) which have been incorporated in the simulation model published by Black et al. (1986) further indicate that the ceiling for protein growth and the energy intake at which it is expressed increases with increasing live weight. There is however, little other experimental evidence to support this contention and the true biological effect of live weight on the relation between protein deposition and energy intake probably lies somewhere between the two different models proposed by Whittemore (1986) and Dunkin and Black (1985). Because precise knowledge of this effect is fundamental to the development of economically optimum feeding strategies for pigs at different stages of growth, it is important that this point of contention be resolved as soon as possible.

Effects of sex

There is limited evidence that sex may affect both the linear and plateau response phases of the relation between energy intake and protein accretion without necessarily affecting the energy

intake at which the plateau is reached. For example, in a study involving entire male and female pigs given five levels of energy intake from 48 to 90 kg, Campbell et al. (1985) reported that protein deposition in the two sexes increased linearly with energy intake up to 33 MJDE/d (0.82 *ad libitum* energy intake) and remained constant thereafter. The results which are summarized in Figure 5 showed however, that both the slope of the linear response phase of protein accretion and maximal protein accretion (plateau value) were lower for females than for entire males.

Figure 5. The relationships between energy intake and rate of protein deposition in entire male (boar) and female (gilt) pigs grown from 48 to 90 kg (from Campbell et al., 1985).



Similar but more dramatic differences in the two components of the linear/plateau relation between energy intake and protein accretion have been demonstrated between entire and castrated males of the same genotype (Campbell and Taverner, 1985). However, again the response was found to plateau at approximately the same energy intake (33 MJDE/d or 0.82 and 0.73 *ad libitum* energy intake for entire males and castrates, respectively) for the two sexes. These results and those discussed previously suggest that entire males will exhibit more rapid and leaner growth at any level of feeding than either of the other two 'sexes' but that deterioration of growth performance and carcass quality (increased fat content) in all three pig types will commence at approximately the same level of energy intake.

Because of their greater lean body mass and higher rates of protein accretion and turnover, entire male pigs also have a higher energy requirement for maintenance than females or castrates (Campbell et al., 1985; Campbell and Taverner, 1985). This further reduces energy available for lipogenesis in entire male pigs and enhances between sex differences in body fat content, particularly at low levels of feeding, but at the same time reduces the magnitude of the differences in growth performance which might otherwise be expected from the differences in protein accretion rates between the sexes.

Effects of genotype

Numerous experiments have been conducted to compare the growth performance and body composition of different breeds and strains of growing pigs (Wood et al., 1975; Gregory et al., 1977; Moody et al., 1978; Ellis et al., 1983). However, because the majority of these studies involved a single level of feeding they provide no information on how breed and strain might modify the relations between energy intake and protein and fat accretion.

Siebrits and Kemm (1982) reported differences in maximal protein and fat accretion rates between genetically 'lean' and 'obese' strains of gilts offered feed *ad libitum* from 20 to 110 kg, indicating that the plateau value for the relationship between energy intake and protein deposition may be altered by genotype. However, the same authors reported no difference in maximal protein accretion rates between genetically lean and obese boars. These data also provide no information on the extent to which genotype might affect the linear response phase of protein growth leading to the plateau, and thus the growth performance and body composition of different strains or genotypes fed restrictively.

In a series of studies involving control and selected lines of Large White pigs, Ellis et al. (1983) and Henderson et al. (1983) reported that selected pigs exhibited more rapid and leaner growth under both restricted and *ad libitum* feeding than their control counterparts. These results indicate that the selection of pigs under *ad libitum* feeding may increase maximal protein deposition (plateau) and either raise the position of or increase the slope of the linear/plateau relation between protein deposition and energy intake. In a similar study involving lean and obese strains of broiler chickens selected under *ad libitum* feeding, Leclercq and Saadoun (1982) reported that birds of the leaner strain exhibited more rapid protein deposition at levels of energy intake from 0.5 *ad libitum* to *ad libitum* than their more obese counterparts.

For pigs, the most definitive information available on the response of different genotypes to energy intake is probably that published by Campbell and Taverner (1985). These authors measured protein deposition in two strains of entire male pigs (Large White x Landrace) from 45 to 90 kg. One of the strains (Strain A) used in this experiment was from a 70 sow experimental piggery and representative of genotypes from smaller commercial piggeries in Australia. The other (Strain B) was obtained from a 6000 sow commercial unit where all breeding stock were selected on the basis of growth performance and carcass fat thickness under *ad libitum* feeding between approximately 50 and 100 kg live weight.

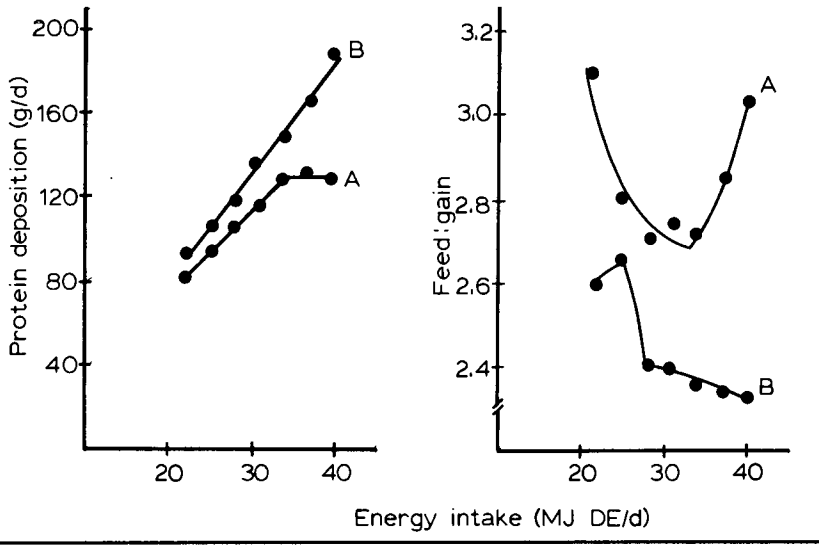
The results which are summarized in Figure 6 showed that for strain A pigs, protein deposition increased linearly with energy intake up to 32 MJDE/d and remained constant at approximately 132 g/d thereafter. For strain B pigs however, there was no evidence of an intrinsic limit to protein deposition which increased linearly with energy intake to almost 190 g/d when feed was offered *ad libitum* (approximately 40 MJDE/d). The results indicate that the intense selection of these animals (strain B) under *ad libitum* feeding had raised their ceiling for protein growth beyond the upper limit of appetite. Consequently the effects of raising energy intake on growth performance and body composition of these pigs (strain B) were qualitatively the same as those previously described (Figure 3) for pigs up to 45 kg live weight.

The results of Campbell and Taverner (1985) also indicate a degree of interdependence between the linear and horizontal (plateau) components of the relation between protein deposition and energy intake since the slope of the relation for strain B pigs was also approximately 20% steeper than that of the linear response phase of the relationship for strain A (Figure 6). This finding is consistent with the results of Henderson et al. (1983). The results of both experiments and others indicate that genetic improvement in growth performance is probably associated with increase in both maximal protein growth (plateau) and, albeit of a lower magnitude, the slope of linear response phase of protein growth. Thus pigs of superior genotype should exhibit faster and leaner growth at any level of feeding than those of lower protein growth potential, though the differences will be most evident at higher levels of energy intake. These contentions are certainly supported by the responses shown in Figure 6.

Nevertheless, it is probable that the relative improvement in the two components of the linear/plateau relation between protein deposition and energy intake will be influenced by the animals nutritional environment during selection. For instance, selection for growth performance under conditions of restricted energy intake should theoretically result in a relatively larger increase in the slope of the linear response phase for protein deposition than in the ceiling for protein growth. Regardless of how improvement in protein growth potential is achieved, it will almost invariably be associated with increase in maintenance energy requirement (Webster et al., 1977, 1978; Campbell and Taverner, 1985; Campbell et al., 1985). The implications of this on growth

performance and body composition have been discussed previously in the section dealing with the effects of sex on energy and protein metabolism.

Figure 6. a) The relationship between energy intake and protein deposition in entire males of slow (A) and fast (B) growing genotype (b) the responses of feed: gain to increasing energy intake (from Campbell and Taverner, 1985).



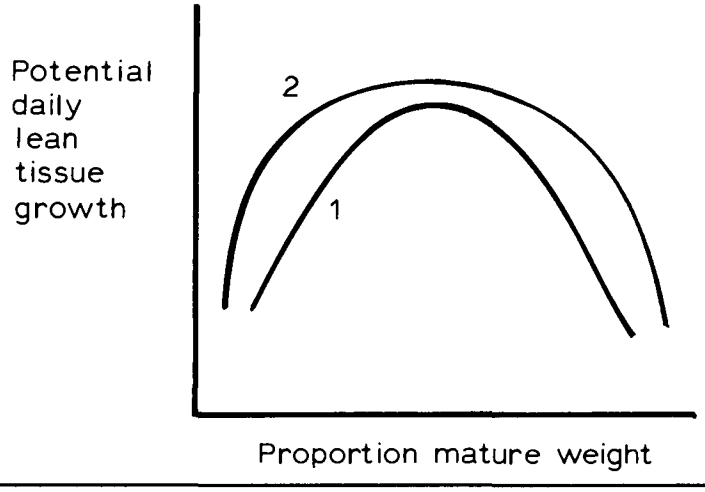
THE RELATIONSHIPS BETWEEN LIVE WEIGHT AND POTENTIAL PROTEIN AND ENERGY RETENTION

Knowledge of the relationships between live weight and maximal protein and energy accretion rates is basic to understanding how the growing pig's nutrient requirements might change as it grows and for determining its body composition and growth capabilities at any stage of development between birth and maturity.

There are currently two schools of thought concerning the form of the relationships between live weight and potential protein and energy accretion. The first presumes that the responses are quadratic in nature with maximum protein deposition occurring at 70 to 90 kg depending on genotype and sex and then gradually falling to zero when mature body size is attained (Thorbek, 1975; Carr et al., 1977; Black et al., 1986). The second also assumes that protein deposition increases from birth but reaches a maximum relatively early in life (20 to 40 kg) and remains constant over a wide range of live weights (up to 120 kg depending on sex and genotype) before falling to zero at maturity (Fuller and Boyne, 1971; Siebrits et al.,1986; Whittemore, 1986). The two different response lines are shown in Figure 7.

Clearly it is important to establish which of the two relationships between live weight and potential protein accretion is correct. There is probably adequate information in the literature to accurately describe the ascending response phases for both protein and energy accretion (Hodge, 1974; Williams, 1976; Siebrits and Kemm, 1982; Campbell et al., 1985; Siebrits et al., 1986). On the other hand, two most critical values needed to describe the descending response phases are mature body size and mature body composition. Because of the long term nature of studies required to obtain these values it is perhaps not surprising that this information is extremely limited. Information is required for the different sexes and for the various genotypes available both in Australia and the rest of the world. The modelling approaches adopted by Whittemore and Fawcett (1976), Black et al. (1986) and others clearly demonstrate the importance of these longer term studies, and the accuracy of these simulation models will continue to improve as the appropriate information becomes available.

Figure 7. Potential protein deposition as a function of weight. Case 1 suggests a maxima at 40 to 50% of mature weight. Case 2 suggests a plateau attainable in early life.



EFFECTS OF EXOGENOUS GROWTH HORMONE ADMINISTRATION ON PROTEIN AND ENERGY METABOLISM

The recent increase in availability of highly purified pituitary porcine growth hormone (pGH) and the almost unlimited supply of a number of forms of recombinant pGH has provided animal scientists with an unparalleled opportunity to probe and define the mechanisms controlling growth and development. The initial experimental results of Etherton et al. (1986) and Boyd et al. (1986, 1987) showed that exogenous pGH therapy for 50 to 70 days from approximately 60 kg reduced the voluntary energy intake of castrated males in a dose related manner by as much as 30%, but improved growth rate 16 - 19%, feed: gain by 18 - 27% and reduced carcass fat content by as much as 45%.

However, while these experiments demonstrate the dramatic effects elicited by pGH they provide little information on the modes of action of the hormone. Also because pGH administration reduces voluntary feed intake, the results do not permit the direct effects of pGH on energy and protein metabolism to be separated from those which may have been associated with the concomitant decline in energy intake.

Some of this information is however, provided by the results of Campbell et al. (1987) who compared the response of excipient and pGH treated castrates (0.0 and 0.1 mg pGH/kg/d) to three levels of feeding from 25 to 55 kg. The data which are summarized in Table 1 showed that pGH administration increased protein deposition at each level of feeding by as much as 50% and depressed fat deposition by 30 - 33% resulting in a marked decline in the fat: protein ratio of energy gain and thus in body fat content at 55 kg (Table 1). These results indicate the endocrine system and endogenous GH in particular is a major factor limiting potential protein growth in the pig. They also suggest that pGH acts either directly or possibly via the somatomedins to stimulate protein accretion in muscle tissue and that this and the associated increase in water deposition is the mechanism by which pGH therapy increases the rate and efficiency of growth. The decline in fat deposition however, appears to result from the concomitant decline in energy available for lipogenesis. The marked effect of these changes in protein and energy metabolism on the partition of energy between fat and protein are shown in Figure 8. The results of Campbell et al. (1987) also showed that because of their greater lean body mass, pGH treated pigs had a higher maintenance energy requirement than excipient pigs (Figure 8). This pGH- induced increase in maintenance energy costs further reduces energy available for lipogenesis and as such accentuates the effect of pGH therapy on body composition.

Table 1. Effects of feeding level and porcine pituitary growth hormone (pGH) on performance and protein and fat accretion rates of pigs from 25 to 55 kg.

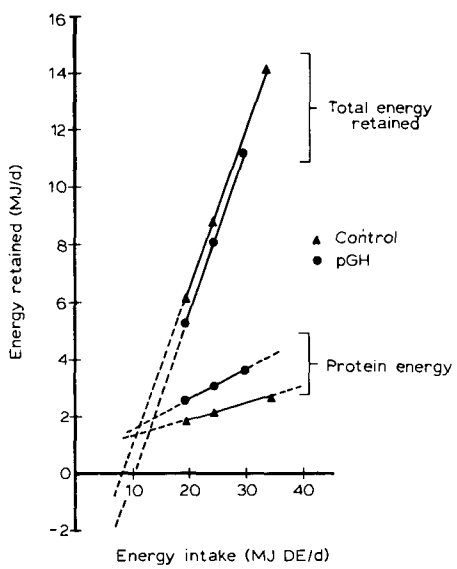
Feeding level (kg/d)	*Ad libitum*		1.64		1.38		
pGH (μg/kg)	0	100	0	100	0	100	AOV*
Item							
Energy intake, MJ DE/day	34.0	29.6	24.4	24.4	19.9	19.9	GH**, EI, GH x EI
Daily weight gain, g	905	1051	670	842	543	681	GH, EI
Food conversion ratio	2.57	1.96	2.45	1.92	2.54	1.92	GH
Body length, cm	64.3	65.3	64.1	66.3	65.0	66.2	GH
Body fat, g/kg	258	188	230	153	196	140	GH, E I
Body protein deposition, g/day	109	151	84	127	77	106	GH, EI, GH x EI
Body fat deposition, g/day	283	192	171	126	107	76	GH, EI
Body ash deposition, g/day	20.5	36.8	15.4	22.0	11.9	17.1	GH, EI, GH x EI

* indicates significance, P<.05, from analysis of variance (AOV).

** GH indicates main effect of growth hormone level; EI indicates main effect of energy intake; GH x EI indicates interaction between growth hormone and energy intake.

The improvements in growth performance and carcass quality (less fat) elicited by exogenous pGH administration appear remarkably consistent at least for castrates (Machlin, 1972; Etherton et al., 1986; Boyd et al., 1986; Campbell et al., 1987). However, castrates have an inherently low capacity for muscle growth compared with gilts or boars and the growing pig's responsiveness to pGH therapy may be modified by its intrinsic potential for protein accretion. This contention is supported by the findings of Campbell and Steele (unpublished data) who compared the responsiveness of boars, gilts and castrates to pGH administration (0.0 and 0.1 mg/kg/d) for 31 days commencing at 60 kg. The results (Table 2) showed that the pGH-induced improvements in growth performance were highest for castrates and lowest for boars with gilts being intermediate. For instance, pGH improved the feed:gain of castrates, gilts and boars by 34, 33 and 19%, respectively. The corresponding decreases in carcass fat content were 35, 37 and 23%, respectively though it should be noted (Table 2) that control boars exhibited faster, more efficient and leaner growth than castrates or gilts.

Figure 8. The partition of energy retained between fat and protein for control and porcine growth hormone (pGH) treated pigs from 25 to 55 kg (from Campbell et al., 1987).



An interesting point about these results was that the effects of sex on voluntary feed intake, growth performance and carcass composition, which were clearly evident between three control groups (0.0 mg pGH/kg/d), were effectively eliminated by pGH. This effect appeared to be associated with the action of pGH on protein growth which increased in castrates, gilts and boars from approximately 128, 133 and 164 g/d in the eviscerated carcass of the control animals to 200 222 and 214 g/d, respectively for pGH treated pigs (Table 2). These results indicate that the growing pig's potential for protein deposition probably lies somewhere between 200, and 250 g/d and that, because of differences in endocrine status, animals such as boars and pigs of superior genotype are operating closer to this ultimate ceiling than others such as castrates and less improved genotypes. They also indicate that the steroids and growth hormone play similar roles in the control of growth and development and that they are probably involved in the same control system.

The fact that a single daily injection of pGH enables almost full expression of the pigs potential for protein growth means that protein and amino acid requirements of pGH treated pigs will be much higher than those of normal pigs. This technology will enable carcass fat content to be reduced to levels not previously thought biologically possible and completely alter current concepts of energy and protein metabolism as they are influenced by nutrition, sex, genotype and possibly even live weight. Appropriate changes will have to be made to simulation models to allow these effects to be predicted. These changes however, will have to await further and more precise information on the actions of pGH on energy and protein metabolism.

Table 2. Effects of sex and exogenous porcine pituitary growth hormone administration (pGH) on the performance and carcass protein accretion rate of pigs (N=36) from 60 to 100 kg (Campbell and Steele, unpublished data)

Sex	Boar		Gilt		Castrate		SEM
pGH (μg/kg/d)	0	100	0	100	0	100	
Voluntary feed intake (kg/d)	3.2	3.0	3.4	2.7	3.7	2.8	0.13
Daily gain (g)	1180	1340	1011	1237	1060	1210	43.2
Feed: gain	2.7	2.2	3.3	2.2	3.5	2.30.	07
Carcass fat (g/kg)	242	186	302	190	328	215	10.3
Carcass protein deposition (g/d)	164	214	133	222	128	200	9.4

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AMINO ACID REQUIREMENTS OF THE GROWING PIG

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INTRODUCTION

The aim of this review is to examine current concepts related to the amino acid requirements of growing pigs. It is not the intention to make an exhaustive review of the experiments which have been undertaken to assess amino acid requirements. That has been done by a number of others (e.g., Rerat and Lougnon, 1968; Agricultural Research Council (ARC),1981; National Research Council, 1987) and we could not add usefully to the sum of their efforts. Nor does it seem particularly valuable to comment in detail on the differences between the various estimates; some possible reasons for these differences have been discussed elsewhere (Lewis, 1984; Fuller, 1986). It seems more useful to take a broad view of the whole question of requirements and to examine some of the underlying problems involved in their estimation and in the application of those estimates. From that discussion some of the underlying reasons for differences in estimates will emerge. First, it is necessary to ask the most basic question: "What is a 'requirement'?"

DEFINITION OF A REQUIREMENT

Definitions of amino acid requirements tend to be tautologous, for example: 'The amount that must be supplied to meet the animal's needs...'; 'Requirement is the amount in the diet to meet the net requirement...'; and so on. We all have some idea of what we mean by a nutrient requirement, but the way we define it depends on our standpoint. The clinician, for example, might think of the amount needed for the prevention of deficiency and for the maintenance of normal well-being, whereas the animal nutritionist is more likely to consider it in terms of maximum productivity. There is clearly no unique definition and even if, for the present purpose, we confine our attention to the growing pig, we can suggest a number of criteria which might be used and we have to say precisely what we mean. In fact, there are three questions which must be answered:

Requirements of what animals?
Requirements for what criteria?
Requirements for amino acids in what form?

What is a pig?

Although our estimates are derived from past experiment with a particular set of animals, we usually want to predict how much of the amino acid we must supply to meet specified criteria in some other population at a future time. There are two problems; one arises from the differences which exist between populations of pigs at any one time, the other from continuous but variable changes with time in pigs in any particular herd, in terms both of their genetic make-up and of environmental factors which may affect their responses to nutrition. The extent to which animals of different sex and genotype can differ in their potential responses to nutrient supply was excellently illustrated by recent studies at the Animal Research Institute, Werribee (Campbell, 1987; Campbell and Taverner, 1985) where maximum rates of protein accretion ranged from 90 to 190 g/d. Obviously we can specify the sex of the animal but the problem of characterizing the genetic differences between animals in a way that allows us to predict how they will respond to nutrient supply has yet to be satisfactorily answered.

In applying results from experiments at one place and time to another set of animals some time later we are, of course, making the assumption that the two populations respond to amino acids in the same way. Ideally we would conduct experiments to estimate requirements on the population of pigs in which we wish to use the estimates and we would repeat these experiments frequently

to take account of secular changes in the population. The closer we come to this ideal, by using recent experimental data and applying it to genetically similar pigs subject to similar environmental constraints, the less will be the error of extrapolation that we introduce.

The extrapolation is particularly important when we consider 'committee' estimates of nutrient requirements made by reviewing a number of experiments done at various times past with a range of animals, usually poorly characterized. Such reviews invariably encounter much unexplained variation and are unavoidably obsolete.

Requirement for what?

The criteria we use to judge the adequacy of the dietary amino acid supply depend on the use we wish to make of the information. In the context of formulating practical diets for growing pigs we generally require data on the effects of amino acid supply on those measurements which are of economic significance. The criteria of response most frequently used to deduce requirements are those related to commercial production, i.e. growth rate, feed:gain ratio, carcass composition and maybe others. If all the chosen measures give similar estimates we have no problem, but it is quite common to find, for example, that a higher amino acid concentration is required to achieve maximum growth rate than is needed for minimum feed: gain ratio. This may lead us to a compromise or, more formally, to an economic index. Assuming that the aim in providing pigs with amino acids is to produce pig meat profitably and that amino acids cost money, a more useful estimate may be the intake that leads to the greatest difference between the cost of the input and the value of the output. That is clearly an economic optimization and not an estimate of the animal's intrinsic requirement.

How should requirements be expressed?

Amino acids are used for a variety of metabolic processes and the rates of those processes determine the rates at which amino acids are required. Thus, amino acid requirements must ultimately be expressed in terms of rates, e.g. g per day, rather than as dietary concentrations, which have relevance only if some rate of food intake is assumed. Thus, although for practical convenience in diet formulation it may be necessary to prescribe amino acid concentrations, we should expect clearer relationships between inputs and responses to emerge if we regard the rates of amino acid and non-protein energy supply as the primary nutritional variables (e.g. Fuller and Crofts, 1979).

Obviously, animals can only utilize, in cellular metabolism, amino acids that have been absorbed. It follows that their intrinsic needs must be expressed in these units. Yet for practical application we need to know how much of a given dietary source of amino acids must be supplied to meet these needs. This depends on the extent to which it is digested and absorbed (its digestibility) and the extent to which its utilization is impaired by the form in which it is supplied (its availability). These matters are considered in another contribution to this conference (the symposium by Batterham and others). Again, we are concerned in practical nutrition less with the animal's intrinsic needs than with how those may be met by an appropriate dietary supply.

Requirements and allowances

In addressing these questions the inherent impossibility of specifying 'the amino acid requirements of the pig' become strikingly evident. We are familiar with the tables of 'requirements' published by various committees round the world and we tend tacitly to assume that these embody some underlying biological truths about the intrinsic needs of 'the pig'. In fact, despite the protestations of their authors to the contrary, the values provided by such tables are not really estimates of requirements at all but recommended dietary allowances. This point is more than semantic; there is a need to distinguish clearly between the specific requirements of the animal to maintain a certain rate of a particular metabolic activity and the amounts we suggest should be given

to pigs in practice. Recommended dietary allowances may be derived from estimates of specific requirements but need not be. They may equally be derived from empirical experiment, traditional practice, accumulated experience and prejudice.

THE BASIS OF AMINO ACID NEEDS

In general, an essential amino acid can be defined as one, needed for the metabolic functioning of the body, which the animal cannot itself synthesize from simpler substances and which must therefore be supplied, perhaps in the short term from body stores, but ultimately in the diet. It may be noted that most amino acids transaminate freely and can thus be replaced by their alpha keto analogues; threonine and lysine are exceptions to this and, in the strictest sense are therefore the only essential amino acids.

For a given rate of a particular metabolic activity a certain supply of each essential amino acid is needed. If the supply is less, the metabolic activity will eventually be impaired giving rise to signs of deficiency and at some greater intake there will be signs of excess. Between these extremes is a range of adequacy. In these terms an animal's intrinsic requirement for a nutrient can be defined as the minimum supply compatible with unimpaired metabolic function. This is the requirement of an individual at any one moment, or in a steady state over a period of time.

A definition of this sort presupposes that an animal has some inherent rate of metabolism which is to be supported by nutrient supply. According to this view a growing pig has a certain genetically determined potential for protein accretion which may in some cases be attained, when the nutrient supply is sufficient and no other constraint supervenes; in other cases, the animal's nutrient intake may be insufficient to allow that potential to be realised. This view gives rise to a model of pig growth in which increasing intakes of limiting nutrients allow linear increments of growth until the animal's potential is reached, when no further response is possible.

The alternative view is that there is no inherent rate of anabolism, but that nutrients act as stimuli or modulators of metabolism and the animal's metabolic activity is a dynamic function of their effects. The dichotomy of these views and their consequences will reappear later.

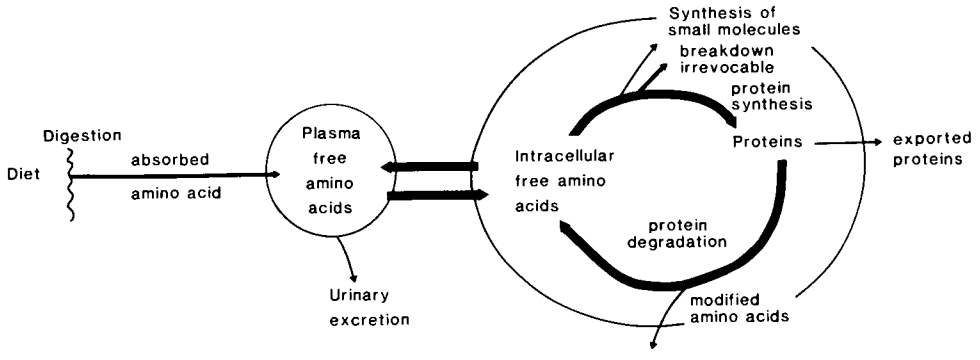
The metabolic fate of absorbed amino acids

Absorbed amino acids can enter a number of metabolic pathways. Many are used as precursors for the synthesis of other essential molecules, such as hormones, neurotransmitters, pigments, etc. Small amounts of amino acids are inevitably lost in the urine as a result of incomplete reabsorption from the renal tubules. These account for a small but continuous disposal from the circulation. Quantitatively, however, by far the most important routes of disposal of essential amino acids are through incorporation into proteins and irrevocable catabolism. A simplified scheme of amino acid metabolism is shown in Figure 1. Amino acids absorbed from the gut are taken up into the extra- and intra-cellular pools from which they are removed for protein synthesis and oxidative metabolism, as well as the other, quantitatively and minor, pathways mentioned. These pools are also replenished by the continuous flux of amino acids liberated during protein degradation. Some proteins are not subject to degradation but are lost intact from the body, especially the keratins of skin and hair and the mucins of the gut. Some amino acids are modified after incorporation into protein (e.g., 3-methyl histidine, hydroxylysine, etc.) and are not reutilized. Again, these routes account for a very small proportion of amino acid flux.

Protein synthesis and degradation proceed continuously throughout life, and greatly exceed in their rates both the intake and the deposition of protein (Reeds et al., 1980). Body protein accretion (or loss) results from inequalities in rate between these two proceses and can be expressed simply as the algebraic sum of the two. Figure 2 shows how the rate of each process changes during the course of growth; viewed in this way, growth is seen to be the relatively small difference between them. Both processes respond to a variety of stimuli, amongst which the most significant in the present context is food. Figure 3 shows the responses of growing pigs to increases in daily food intake. It can be seen that with increases in food intake, the rates of both synthesis and

degradation increased as did the difference between them, giving rise to the observed increase in body protein gain. The rate of body protein accretion was tending towards an asymptote but there is no suggestion in these data that the rates of protein synthesis and degradation were; rather they were tending towards a parallel increase that would signify the attainment of a maximum rate of body protein accretion. The rate of amino acid breakdown also increased steadily through this range of intake. In subsequent experiments (Reeds et al., 1981; Fuller et al.,1987a) we have looked at the effects of individual nutrients on protein synthesis and degradation, giving supplements of carbohydrate, fat, protein and an individual limiting amino acid. These results are summarized in Figure 4. The important point about these results is that increases in body protein gain can be brought about by increases in synthesis, reductions in breakdown or both, and can be accompanied by either acceleration or suppression of amino acid breakdown. From such results it is clear that growth of the protein mass of the body does not involve constant relationships between protein synthesis and breakdown but that each of these processes is modulated independently by nutrients through mechanisms that are separate yet concerted.

Figure 1. A simplified scheme of amino acid metabolism.



The part of the total flux of an essential amino acid which is not incorporated into protein is, by one route or another, irrevocably lost. What we are referring to here is not necessarily complete oxidation since some of the carbon may enter synthetic pathways and certainly the nitrogen may be rather ubiquitously distributed through transamination reactions; the important step, which varies amongst the amino acids, is the first irreversible reaction beyond which the complete amino acid cannot be recovered.

For each amino acid there is an irreducible minimum rate of irrevocable loss which contributes to the maintenance need and, in the absence of a dietary supply of amino acid, is met from the amino acid flux released by body protein degradation. With increases in dietary amino acid supply there is a slight increase (Figure 2), accompanying the increased rate of amino acid flux. This reflects the continuous decline in the efficiency of utilization seen with all proteins, however high their quality (e.g. Forbes et al.,1958). At some greater intake the catabolic flux increases much more steeply with amino acid supply (Kang-Lee and Harper,1977; Bergner et al.,1978) and clearly when amino acid intake is in excess of that needed to achieve the maximum rate of body protein accretion, increments of amino acid intake are quantitatively catabolized.

When a protein of very high biological value is given at a suboptimal intake, virtually all of an increment in protein supply is directed to protein synthesis. The proportions or pattern in which they are thus utilized is dictated primarily by the amino acid composition of the proteins being synthesized which in turn is determined by the nucleotide sequences of the genes. To a first

approximation, therefore, the amino acid requirements of the growing animal are determined by the amino acids of the proteins being synthesized.

Figure 2. Rates of protein (circles) and degredation (triangles) during the course of growth in a) rats and b) chickens. Data of a) Goldspink and Kelly (1984) and (b) of Muramatsu and Okumura (1975) and of Muramatsu et al. (1978 a, b).

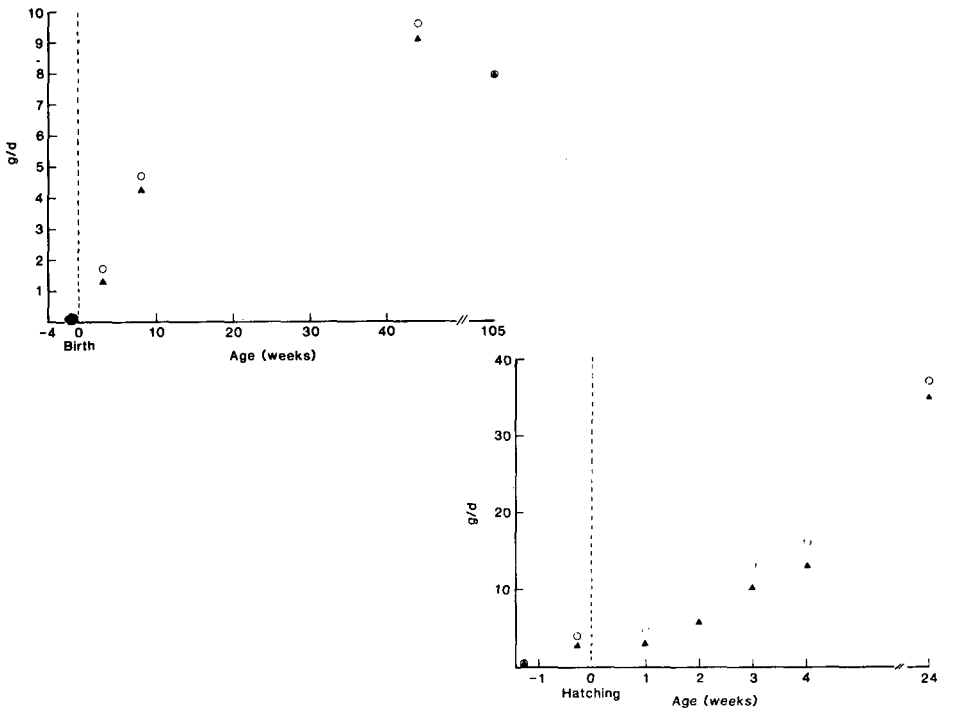


Figure 3. Rates of protein synthesis, degradation and accretion and of total amino acid oxidation in growing pigs given food at three rates, with energy intakes one, two or three-fold their maintenance requirements. Data of Reeds et al. (1981).

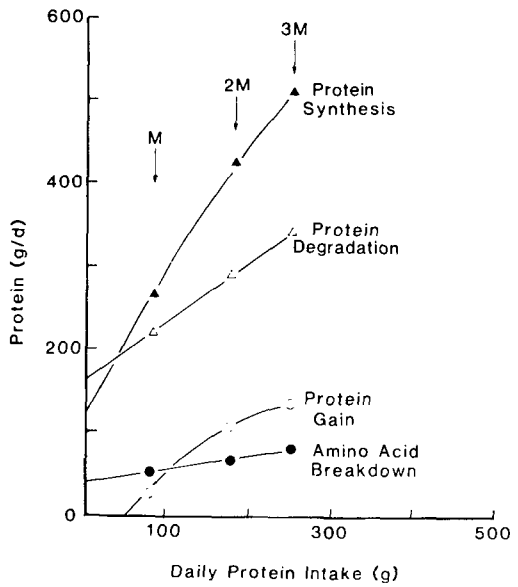
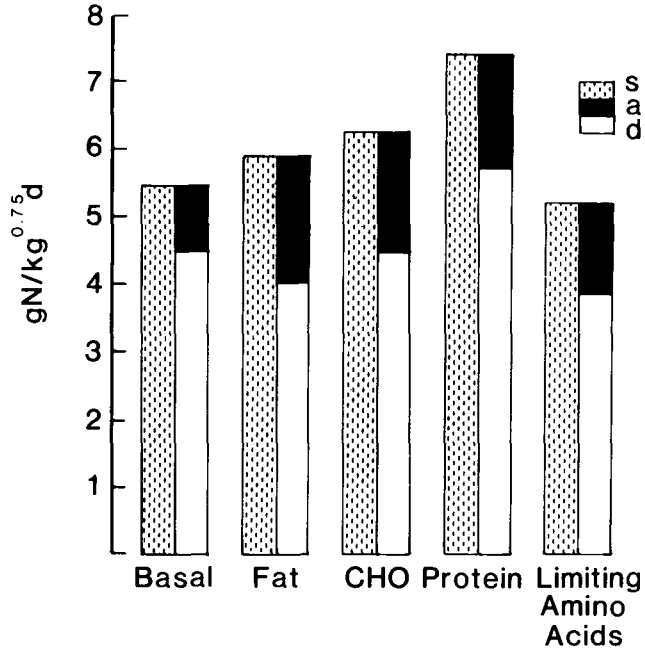


Figure 4. Effects of dietry supplements of single nutrients on protein synthesis (S), accretion (a) and degredation (d). data of Reeds et al. (1981), Fuller et al. (1987b).



This proposition was expounded many years ago, most explicitly by Mitchell (1950) and it was shown that amino acid needs for growth, determined by empirical experiment, were well correlated with the amino acid composition of the animal in question (Fisher and Scott, 1954; Price et al., 1953; Williams et al., 1954).

Ideal protein

An ideal protein can be defined as one which supplies the animal with amino acids in exactly the proportions in which they are required and which is therefore potentially fully utilizable. The concept of an ideal protein has been in the literature on protein and amino acid requirements for at least 40 years. Mitchell (1964) put it this way:

'a yardstick of comparison, represented by an amino acid mixture or by a protein with complete availability in digestion and in metabolism. .....An amino acid mixture of this description would be one identical in composition with the amino acid requirements of the animal for growth and maintenance.'

It is a useful concept in two ways: First, it assists us in coping with variations in amino acid requirements amongst animals by allowing amino acid constraints in diet formulation to be specified in terms of a single entity (either 'ideal protein' itself or any one of the amino acids in the 'ideal' pattern). Second, it provides a reference protein against which other proteins of lesser quality may be compared.

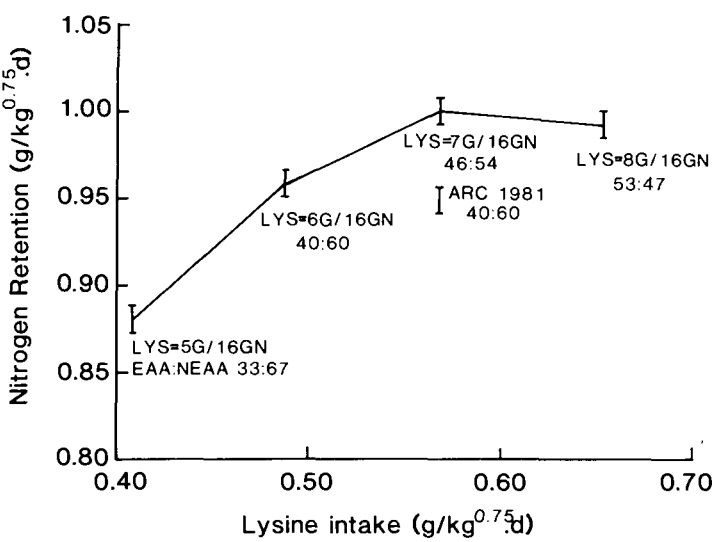
An ideal protein appropriate for the growing pig was proposed by the ARC (1981). The estimates it comprises were collated from a number of disparate sources, augmented by data on the amino acid composition of body tissues, using the arguments advanced above. Because this proposed ideal protein forms the basis of recommendations for use in practice it seemed important that they be validated or reassessed by independent experimentation. We have recently reported the results of three series of studies with this aim.

In the first series of experiments (Wang and Fuller, 1987a) the method of selective amino acid deletion was used to establish the optimum dietary amino acid balance for the growing pig. The method is based on the proposition that in an ideally balanced protein all amino acids are

equally limiting (or none is) and that removal of a certain proportion (we used 20%) of any one amino acid would have the same effect as removing the same proportion of any other. There are three possible outcomes to the selective removal of amino acids: 1. There is no effect, in which case all the quantity removed is assumed to have been in excess relative to the first limiting amino acid; 2. There is a substantial reduction in protein utilization, which is greatest for the amino acid that is limiting in the control diet; 3. There is a response intermediate between these extremes, indicating that some of the 20% removed was in relative excess. This fraction was estimated by interpolation between the control diet and the response to the most limiting amino acid. This principle was used in a sequence of experiments and the results were used to estimate the optimum pattern of essential amino acids.

These experiments addressed the question of the optimum balance of the essential amino acids and it remained to determine the optimum ratio between the sum of the essential and the sum of the non-essential amino acids. This was estimated in a separate experiment which included the ARC (1981) ideal protein for comparison. The results are shown in Figure 5. A minimum ratio of 46:54 (essentials:non-essentials) was required to maximize protein utilisation. The diet with this pattern of amino acids was utilized significantly better than that proposed by ARC (1981), even though the lysine concentrations and the total nitrogen intakes were identical. The resulting optimum pattern of amino acids is given in Table 1, together with the ARC (1981) estimates for comparison.

Figure 5. Effect of nitrogen retention of varying the ratio of essential: non-essential amino acids whilst maintaining total N intake constant. The pattern of essential amino acids was that estimated as optimum by Wang and Fuller (1987a). The pattern proposed by the Agricultural Research Council (1981) is included for comparison. Data of Wang and Fuller (1987a).



There is ample evidence from other species that the pattern of amino acids required for maintenance is different from that needed for body protein accretion (Said and Hegsted, 1970; Hegsted, 1970). As a result, the overall pattern which an animal requires, which is the sum of its requirements for these two functions, should be expected to vary as a function of its rate of body protein accretion; the higher this rate, the less should be the influence of the maintenance component and the nearer the total pattern should approach that for body protein accretion alone.

The only direct estimates of the amino acid requirements for maintenance in the literature appear to be those of Baker et al. (1966a,b,c) and Baker and Allee (1970). The second experiment (Fuller et al., 1987b) was designed to estimate, separately but in the same experiment, the patterns of amino acid requirements for both maintenance and for body protein accretion. Diets similar to those used in the first experiment were used; the lowest N intakes used maintained the pigs below N equilibrium, while the high N intake allowed them to gain close to 1 g N per kg $W^{0.75}$ per day, or about 100 g protein per day at the mean weight of 44 kg. The principle of these experiments was to estimate the gradient of N retention in response to each essential amino acid when it was limiting in the diet. For an amino acid like tryptophan, with a relatively small requirement, the gradient is steep; for lysine, which is required in larger quantities, it is much less. We took the reciprocals of these slopes and calculated the pattern amongst them. The specific requirements for maintenance were estimated from the intercepts on the x axis.

Table 1. Estimates of the amino acid pattern (g/16gN) for growing pigs from experiments using specific partial amino acid deletions (Wang and Fuller,1987a). The estimates of the ARC (1981) are included for comparison.

	Present estimates	ARC (1981)
Lysine	6.5	7.0
Threonine	4.7	4.2
Methionine + cystine	4.1	3.5
Isoleucine	3.9	3.8
Tryptophan	1.2	1.0
Leucine	7.2	7.0
Histidine	n.d.	2.3
Phenylalanine + tyrosine	7.8	6.7
Valine	4.9	4.9
Arginine	n.d.	n.d.

n.d. : denotes not determined.

The results, shown in Table 2, confirm that the optimal pattern of amino acids for maintenance and for body protein accretion are very different; only for the sulphur amino acids are the requirements equal.

Table 2. Estimates for the amino acid patterns (g/16gN) required for the maintenance of N equilibrium, for body protein accretion and for both at a normal rate of protein accretion. Data of Fuller et al. (1987a).

	Maintenance pattern	Tissue accretion	Both
Lysine	2.4	6.8	6.4
Threonine	3.4	4.7	4.6
Methionine + cystine	3.6	3.6	3.6
Methionine	0.8	1.9	1.8
Isoleucine	1.1	4.3	4.0
Tryptophan	0.7	1.2	1.2
Leucine	1.7	7.8	7.2
Histidine	n.d.	n.d.	n.d.
Phenylalanine + tyrosine	3.0	8.4	7.9
Phenylanine	1.5	4.1	3.9
Valine	1.3	5.2	4.8
Arginine	n.d.	n.d.	n.d.

n.d. not determined.

It is interesting to note that the methionine requirement for body protein accretion is estimated to be 1.9 g/16 g N, close to half the total sulphur amino acid requirement of 3.6 g/16 g N, whereas for maintenance, it appears that more than three quarters of the total methionine + cystine requirement can be supplied by cystine. According to these results, tyrosine can supply half the total phenylalanine + tyrosine requirements for both purposes.

The sum of the essential amino acids (excluding histidine) in the 'growth' pattern is 48 g/16 g N (100 g protein) whereas for maintenance it is only 20 g. The reason for the large requirement for 'non-essential' nitrogen at maintenance remains to be elucidated.

There was no response to the removal of histidine in these experiments and no estimate of the requirement could be made. The most likely explanation for the pig's insensitivity to dietary histidine is the increased catabolism of carnosine which is known to be depleted in dietary histidine insufficiency (Chung-Kwang et al., 1976; Quinn and Fisher, 1977). In growing pigs, muscle carnosine concentrations continue to decline over several weeks on histidine deficient diets (M.F. Fuller, C.I. Harris and A. Cadenhead, unpublished data), making it unlikely that reliable estimates of dietary histidine requirement could be estimated in any shorter time.

All these experiments involved relatively short periods of feeding the deficient diets. We were concerned by the possibility that the short term effects of specific amino acid deficiency might not be maintained in the longer term. This was clearly the case for histidine but, when we repeated the deletion experiment a third time, we observed that leucine and valine also gave less than the expected response. To examine the extent of such adaptative effects we have now carried out further experiments in which the specific deficiencies were maintained for periods of 12 or 25 days (Wang and Fuller, 1987b). In general, the results were consistent with those obtained in the short term. There was very little effect of removing histidine and, again, deficiencies of leucine or valine produced less than the expected fall in protein utilization.

These results have led us to reconsider one aspect of the 'ideal protein' concept. First, it is necessary to point out that there can be any number of ideal proteins. It is easy to see that if, with a particular amino acid pattern, a BV of 1.0 is measured then nitrogen utilization is complete, but this does not mean that the utilization of every amino acid is complete. A small excess of an essential amino acid could contribute N for the formation of non-essential amino acids such that the utilization of N as a whole could still be complete. Egg protein, for instance, which can be fully utilized (Forbes et al., 1958; Eggum, 1968) may be of this kind, with 64% of its N in essential amino acids. We have a rather more limited definition of an ideal protein, one which contains the minimum of each essential amino acid necessary to match the animal's needs. This predicates a unique pattern.

In our recent studies on the optimum dietary amino acid pattern we have assumed that in an ideal protein every essential amino acid is equally limiting. This may not necessarily be so. It seems to us quite possible that, although the fractional catabolism of each essential amino acid is low, it may not be equally low for every amino acid. Removal of (say) 20% of the supply would reduce the fractional catabolism to some degree and it seems possible that different amino acids could be conserved to different degrees (Beckett et al., 1987).

We have never achieved, in giving our best balanced amino acid patterns to growing pigs, a BV greater than about 0.93 (Fuller et al., 1979; Wang and Fuller, 1987a). The fact that complete utilization of absorbed N is obtainable with rats given 10% protein diets is, ultimately, something of a coincidence and does not mean that we should expect this to be obtainable with pigs in similar dietary circumstances. A BV of 0.93 does, however, mean that it is possible for some amino acids to be utilized better and others worse compared with the average or with total N.

It should be made clear that in all these experiments we have been using sources of amino acids which are essentially fully digested so as to describe the pig's intrinsic requirements. The synthetic diets were based on mixtures of casein and amino acids, and the faecal N excretion of pigs on these diets was no greater than that of pigs given protein-free diets. Differences in digestibility and availability between amino acid sources must of course be taken into account when comparing these results with the amino acid profiles of practical diets.

METHODS OF ESTIMATING AMINO ACID REQUIREMENTS

Two approaches have been used to estimate the amino acid requirements of pigs; one, the traditional empirical approach, using dose response methods in one form or another, and the other, the factorial approach, summating components of requirement.

We would like to begin by considering the traditional approach, discussing some of the assumptions underlying, first, the experimental method and, second, the way we use the information.

Conventionally, amino acid requirements have been estimated by examining the responses of pigs to graded amounts of the amino acid supplied in a diet considered otherwise adequate. The 'requirement' has been taken to be the least amount of the amino acid which elicits the greatest response from the animal. The answer obtained depends on a number of factors amongst which we would emphasize:

1. What form of response is assumed;
2. What criterion of response is measured.

Form of response to amino acid supply

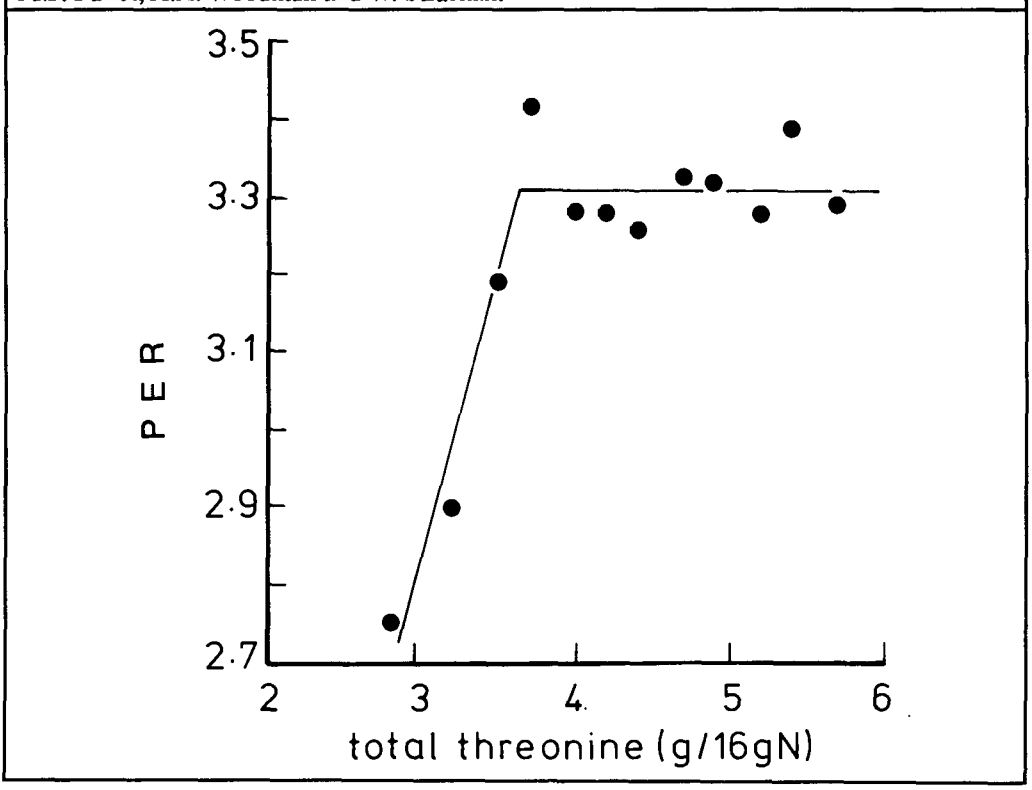
The response of body protein accretion to protein supply is not constant with respect to intake. The efficiency with which protein is used to replace endogenous losses seems to be uniformly high (ARC, 1981; Hegsted, 1970) even with proteins of rather low quality (Said and Hegsted,1969; Inoue et al., 1974), a phenomenon consistent with our observation that (with the exception of the sulphur amino acids) the essential amino acids required for maintenance are low relative to the total N need. With increasing intake, the efficiency (i.e. the marginal response) diminishes and may fall to zero at high intakes. The form of this response curve is of considerable importance in the estimation of requirements. Some assert that it comprises a linear response up to a certain intake and is constant at all higher intakes (the 'linear/plateau' form). Others interpret the response as a continuously diminishing function and use curvilinear expressions to describe the data. The precise form of the response to an individual amino acid undoubtedly depends on the circumstances in which the amino acid supply is varied. We can distinguish three circumstances. First, there is the case in which the intake of an individual amino acid is increased and the intakes of all other nutrients, including all other amino acids, are held constant. This protocol has commonly been employed in estimating individual amino acid requirements. It presupposes an accurate knowledge of the requirement for every other amino acid and the form of the response depends on the correctness of this knowledge. If it is imperfect the response to the amino acid being examined may well be abruptly curtailed by co-limitation of another. An example of this type of response is shown in Figure 6 and no one would argue that this is well described by a rectilinear function. The response may also depend on the alleviation of amino acid imbalances that are inevitable in such protocols.

Second, there is the case where the response to a balanced mixture of amino acids is being examined. If the ideal protein concept is accepted it is reasonable to examine the responses of pigs to amino acid supply in terms of ideal protein or any one amino acid within its invariate pattern. If this is done at a fixed food or energy intake, i.e. examining the response to protein concentration, the proper interpretation is more equivocal. The assumption that the rate of body protein accretion responds linearly up to a point at which energy becomes limiting is easy to handle mathematically and leads to a simple definition of 'requirement'. On the other hand, the data very often seem to show a curvilinear approach to the asymptote. It is very difficult to demonstrate the superiority of one interpretation over the other.

Fisher et al. (1973) have pointed out that, even if the response of an individual is of the linear/plateau form, the combination of a number of those responses to describe the response of a population necessarily generates a curvilinear function. The response of the individual animal has rarely been examined experimentally, probably because it is difficult to obtain multiple measurements of the rate of body protein accretion in the same animal. Recently studies have been carried

out at the Rowett Institute to do this (M.F. Fuller, R. McWilliam and P.J. Garthwaite, unpublished data). Growing pigs were given protein of constant composition at six rates with a constant intake of digestible energy. To account for changes with time in the animals' responses, the six diets were given to each animal in a balanced sequence and the results were used to produce response curves for individual animals. In 12 of the 15 animals the data were more closely described by an exponential equation than by a linear/plateau (splined) function and overall the data provided strong evidence that the better fit provided by the exponential model cannot be attributed to chance. However, the measurement of response was nitrogen retention and, despite the low errors we now obtain with this method in female pigs with bladder catheters, the well known tendency for N retention to over estimate body protein accretion and for the over estimate to increase with intake, cannot be excluded.

Figure 6. Response of growing chicks to additions of a single limiting amino acid. The linear response is curtailed by co-limitation of asecond amino acid. Unpublished data of M.F. Fuller, A.A. Woodham and W. Strachan.



The third circumstance is that in which amino acids and non-protein energy are supplied in increasing quantities in constant proportions, i.e., increasing food intake. Again, we should expect individuals and populations to be best described by different functions.

The mathematical convenience of the rectilinear model cannot be denied but modern methods of data handling have removed the difficulties formerly inherent in fitting complex exponential functions to response data. A few years ago we described (Fuller and Crofts, 1979) the responses of growing pigs to rates of protein and non-protein energy supply by means of a double exponential function which accounted for more than 98% of the variance.

Criteria of response

For the most part, the criteria that have been used to assess requirements in dose response experiments can be classed as measures of productivity;

e.g. rate of body protein accretion,
growth rate,
food:gain ratio,
body composition,
or metabolic responses,

e.g. nitrogen retention,
urinary nitrogen excretion,
urea synthesis or excretion,
plasma urea concentration,
plasma amino acid concentration,
amino acid oxidation.

Considering first the production criteria, if we take it that the rates of body protein and fat accretion are the primary responses of the animal to amino acid and energy supply, and that growth is essentially a function of protein and fat gains, then the other measures are seen to be predictable consequences of those primary variables. Clearly then, responses to amino acids cannot be specified without reference to the concurrent energy supply.

Although the interpretation of the metabolic indices is more complex and each involves different considerations, the inter- relationships between them are basically simple, and relate to the disposal of amino acids into protein synthesis and catabolism, as shown in Figure 2.

N retention is often used as a measure of the rate of body protein accretion on the assumption that the mixed body proteins contain a constant 16 g N/100 g (Franke and Weniger, 1958). However, accreted protein lost from the skin is not measured in conventional N balance work and these estimates should not be expected to correspond exactly with the accumulation of protein in the body. Difficulties may also arise in short term N balance studies from the assumption that the amino acid composition of accreted proteins remains constant. This difficulty is particularly acute at N equilibrium, when the formation of certain proteins, especially those of the integument, continues whilst others are depleted. Nitrogen equilibrium is not synonymous with the equilibrium of every essential amino acid.

If the diet is kept constant in every respect but its amino acid composition (as in our experiments with casein and amino acid mixtures) urinary N excretion alone can be assumed to reflect total protein utilization. The major end product of amino acid catabolism in mammals is urea, the production of which reflects amino acid catabolism more closely than total urinary N excretion, which includes nitrogenous compounds unrelated to amino acid catabolism. In our experiments with growing pigs, 95% of urinary N excreted above N equilibrium is typically in this form. Changes in urea excretion would therefore be expected to reflect closely the changes in total N elimination. Measurements of urea synthesis made in growing pigs with ^{14}C-urea (Fuller et al., 1987b) showed that, as in man (Walser, 1983), urea synthesis exceeded urea excretion by some 20%. Most of the N of the urea hydrolysed in the gut returns to the liver. Labelled urea N may appear by transamination in any amino acid except lysine and threonine, but this does not mean that urea N can contribute to the net amino acid supply or N retention (Walser, 1983). A variable proportion of this N can also be incorporated in microbial protein in the gut and excreted in the faeces; the division of N excretion between urine and faeces depends to some extent on the amount and nature of the digesta reaching the large intestine.

Plasma urea concentration is a function of the rates of synthesis and clearance of urea and of the urea space. In steady state conditions plasma urea concentration is predictably related to production, but in normal meal feeding both synthesis and clearance change rapidly; nevertheless, given careful control of sampling times, plasma urea concentration is inversely related to the utilization of dietary protein, reflecting the extent of amino acid breakdown (Eggum, 1970). The response of plasma urea concentration (measured under standardized conditions) to amino acid supply therefore reflects total amino acid catabolism and can be used to estimate amino acid requirements (Yen et al., 1986).

The plasma concentration of an amino acid is also a function of its entry and clearance from the plasma and its distribution space. If an amino acid is deficient in the diet of a growing animal

its plasma concentration is much lower than when it is not limiting. With increases in dietary intake the combination of greater exogenous supply and smaller incremental responses of protein synthesis result in elevations in plasma concentration (Young et al., 1983) and decreases in the plasma concentrations of other amino acids, notably the second limiting one (Chavez and Bayley, 1976). When the intake exceeds that at which maximum growth rate is achieved, the plasma concentration rises markedly and this point has been used to estimate the requirement (e.g. Keith et al.,1972). However, this point may not be coincident with that corresponding to minimum amino acid oxidation (Kang-Lee and Harper, 1978).

The oxidation of a limiting amino acid in response to increasing dietary supply can be studied with isotopically labelled amino acids. Increases in essential amino acid intake above those required for the maximum rate of body protein accretion are quantitatively catabolized (the possible exception is histidine). Plasma amino acid concentration is an important factor in the regulation of amino acid catabolism (Walser, 1983) but with increasing dietary amino acid supply, plasma amino acid concentration may rise at a lower intake than that at which amino acid oxidation starts to increase (Kang-Lee and Harper, 1978). The 'breakpoint' in amino acid oxidation has been used by a number of workers to estimate the requirements of essential amino acids, in various species including the pig (Chavez and Bayley, 1976). As the supply of a limiting amino acid is increased the oxidation of non-limiting amino acids falls as a greater proportion of their supply can be utilized for protein accretion. This allows the oxidation of a non-limiting 'indicator' amino acid to be used as a measure of the intake at whioh a deficient amino acid ceases to be limiting (Kim et al., 1983).

The factorial approach to estimating amino acid requirements

Growing pigs require amino acids both to meet their obligatory losses and for tissue protein accretion. According to the factorial approach their total requirements are the sum of these two elements and, the greater their rate of protein accretion, the greater their requirements for amino acids. This method thus allows us, in principle at least, to accommodate differences in animal performance and to derive estimates of the amounts of amino acids required to sustain specified rates of protein gain.

The method requires that we solve an equation of the form;

$$R = aE + bcG$$

where E is the obligatory endogenous loss

G is the protein gained

c is the proportion of the particular amino acid in the protein gained

and a and b are coefficients describing the efficiency with which the amino acid is utilized for each of these specific functions.

The efficiency of utilization for maintenance can be taken to be close to1.0 and, provided of course that other nutrients are supplied in adequate amounts, there is little decline in this efficiency with intake up to daily intakes equivalent to 10-12 g of ideal protein per kg $W^{0.75}$. As intake is increased further the efficiency declines and, when the data are seen as a whole, they appear to show a continuous decline in efficiency (Forbes et al., 1958; Fuller and Crofts, 1979; ARC, 1981). The factorial method requires a precise estimate of this efficiency for the specified rate of body protein accretion (or other response). There is no theoretical basis from which this can be established and this means that the factorial method depends ultimately on descriptions of the relationships between amino acid supply and animal response that are as empirical as those generated by dose response studies.

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A SYMPOSIUM: COMPARISON OF METHODOLOGIES TO ESTIMATE AMIMO ACID AVAILABILITIES FOR PIGS

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INTRODUCTION

The determination of amino acid availability is a complex problem that has evoked considerable research efforts. Despite these inputs there is still much uncertainty both in the understanding of the problem and in the acceptance of techniques to measure availability.

The availability problem arises because, although the determination of total amino acids using acid hydrolysis measures the total amount of an amino acid in a sample, this may not reflect that available to an animal. The acids used in the hydrolysis of amino acids liberate bonds that may not be released by the more specific and less powerful digestive enzymes secreted by an animal. Thus, the total amino acid analyses may over-estimate that available to an animal.

Availability is defined as the amount or proportion of an amino acid that is in a form suitable for digestion, absorption and utilization by an animal. It follows from this definition that availability can only be measured by determining the utilization of a test amino acid fed to an animal in a deficient state.

The availability of amino acids is normally determined using slope-ratio assays. With this assay, the animal's response to graded levels of the test amino acid is compared to the animal's response to graded levels of standard amino acid. There are a number of statistical safeguards that attempt to ensure that the responses recorded are due to the test amino acid and are not influenced by other dietary factors (Finney, 1964). Various parameters of response have been used by different workers including liveweight gain, food conversion efficiency, carcass gain, food conversion efficiency on a carcass basis, nitrogen retention and protein deposition in the carcass.

The main limitations of slope-ratio assays are that they are time consuming and expensive, only one amino acid can be determined at a time, dietary formulations are complex and, as with any experiment based on growth response, the standard errors of the estimates are high. Despite these limitations, slope-ratio assays are at present the only way to determine the availability of an amino acid, i.e. its utilization by an animal when it is limiting in a diet.

With pigs, lysine is normally the first and major limiting amino acid in cereal-based diets and most interest has centred on developing techniques for estimating lysine availability. Lysine, being dibasic, has a free epsilon amino group when it forms part of a peptide chain. This free epsilon amino group is thought to react with reducing sugars when heat is applied during processing to form Maillard reactions. These reactions render the lysine molecule unavailable to an animal. A measure of the free epsilon amino group of lysine is the basis of most chemical tests for estimating lysine availability. However, subsequent research has indicated little or no relationship between chemical estimates and lysine availability determined by slope-ratio assays for growing pigs (Batterham, 1986).

In recent years, interest has centred on the use of ileal digestibility assays to estimate amino acid availability. With this assay, a cannula is inserted at the terminal ileum and digestion of amino acids prior to the terminal ileum determined. This avoids the interference of microbial degradation or synthesis of amino acids in the hind gut which limits the use of faecal digestibility in amino acid studies. Some workers record apparent amino acid digestibility at the ileum whilst others make corrections for endogenous secretions and express the results as true digestibility.

The main advantage of the ileal digestibility assay is that all amino acids can be measured at the one time. Its limitations are that the assay is fairly complex and is not suitable for routine estimations, and it is a measure of indigestibility rather than a test of utilization. The assumption

is made that those amino acids not measured at the terminal ileum have been absorbed in a form suitable for utilization. This assumption is not strictly correct as amino acids can be absorbed in forms that are not efficiently utilized (eg. fructo-lysine) and metabolism of amino acids may occur in the lumen of the gut wall. Thus ileal digestibility can over-estimate availability.

This conclusion was also reached by Black and Davies (Unpublished data) when attempting to predict with the AUSPIG[1] computer model the responses of pigs fed lysine limiting diets. In all cases, estimates of lysine availability based on ileal digestibility assays had to be reduced by up to 0.25 before predictions were similar to observed responses.

Despite the intense debate on the relative advantages and disadvantages of the two techniques, there have been very few studies comparing ileal digestibilities with slope-ratio assays for growing pigs. What studies have been conducted have given conflicting results. With growing pigs, lysine availability in soyabean meal (mean 0.88, range 0.80-0.98; Batterham et al., 1979; Batterham et al., 1984; Batterham et al., 1986b) was similar to the ileal digestibility of lysine (0.89; Taverner, 1982). However, with the same sample of lupin-seed meal, the ileal digestibility of lysine (0.86; Taverner, personal communication) was considerably higher than lysine availability (0.37; Batterham et al., 1984). With weaner pigs, Leibholz (1985, 1986) found similar values for both the ileal digestibility of lysine (0.61) and lysine availability in cottonseed meals (0.61-0.80). These values are considerably higher for cottonseed meal than lysine availabilities of 0.43 and 0.39 reported by Batterham et al. (1979) and Batterham et al. (1984). It is not known if these differences with cottonseed meals reflect differences in methodology or feed samples.

In order to provide more definitive information on the problem, a collaborative study was undertaken using the same feed samples. Four protein concentrates were used:- soyabean meal No. 1, a pre-press solvent extracted meal, and three cottonseed meals. Cottonseed meals Nos. 1 and 2 were pre-press solvent extracted and cottonseed meal No. 3 was expeller extracted. The ileal digestibility of lysine in cottonseed meal No. 1 for grower finisher pigs and lysine availability for weaner pigs was assessed by Dr J. Leibholz. The ileal digestibility of the amino acids in the four meals for finisher pigs was determined by Dr M. Taverner, and lysine availability in the four meals for grower pigs by the author.

The papers in this symposium summarize the main results of the collaborative study and some previous studies in these areas. In addition, estimates of amino acid availabilities in feeds using computer simulation studies are presented by Dr J. Black and Mr G. Davies in the fourth section.

[1] AUSPIG *computer simulation model developed by CSIRO, Australia*

A COMPARISON OF ILEAL DIGESTIBILITY MEASUREMENTS WITH GROWER-FINISHER PIGS AND SLOPE-RATIO ASSAYS IN WEANER PIGS FOR ESTIMATING THE AVAILABILITY OF LYSINE

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INTRODUCTION

The availability of lysine from cottonseed meal has been found to be low (0.43-0.39) in growing pigs (Batterham et al., 1979; Batterham et al., 1984). On the other hand, slope-ratio assays with weaner pigs have shown the availability of lysine in cottonseed meal to be higher (0.61-0.80, Leibholz, 1986). The apparent digestibility of lysine in cottonseed meal to the ileum was 0.61 (Leibholz, 1985) which compares well with the slope-ratio assay estimates and with ileal digestibility estimates with grower pigs of 0.62- 0.68 (Tanksley and Knabe, 1984).

The experiments reported here form part of a collaborative study with Drs Taverner and Batterham to evaluate the ileal digestibility (Experiment 1) and availability of lysine (Experiment 2) in cottonseed meal No. 1. In a subsequent growth study (Experiment 3) values from three other proteins, also tested in Experiments 1 and 2, were evaluated.

MATERIALS AND METHODS

Experiment 1

Eight diets were prepared from corn starch and one of seven protein sources: soyabean meal No. 2; cottonseed meal No. 1 (supplied by Dr Batterham); cottonseed meal No. 4; meat meal; sunflower meal; dried skim milk; and wheat gluten. All diets contained 8 g lysine/kg of dry matter. The diets were sprayed with ytterbium nitrate as an indigestible marker.

Six pigs (25 kg initial weight) were fitted with T-shaped cannulae in the terminal ileum and fed 1.3 kg/day from belt feeders. Each pig was offered each diet for 14 days. Ileal digesta, faeces and urine were collected for four days at the end of each 14-day feeding period.

Experiment 2

A basal diet containing wheat, cottonseed meal, wheat gluten and corn starch was prepared similar to that of Leibholz (1986). It contained 7.3 g lysine/kg (air dry). The corn starch was replaced by increasing amounts of L-lysine, cottonseed meal No. 1, cottonseed meal No.4, meat meal, sunflower meal or dried skim milk to supply 7.3-11.0 g lysine/kg of diet in five increments.

The diets were given *ad libitum* to 84 pigs from 21-49 days of age. The pigs were weighed weekly and faeces and urine were collected between days 22 and 28 of the experiment (43 and 49 days of age).

The results for each lysine source were analysed by linear regression with lysine intake as the independent variable and liveweight gain as the dependent variable (Leibholz, 1986).

Experiment 3

Eight diets were prepared to contain 10 and 12 g lysine/kg from soyabean meal No. 2; 10, 12.5 and 14.5 g lysine/kg from cottonseed meal No. 4; and 10, 11.2 and 12.8 g lysine/kg from sunflower meal.

The diets were given *ad libitum* to 72 pigs from 21-49 days of age. Pigs were weighed weekly and faeces and urine were collected between days 22 and 28 of the experiment.

Experiment 3 was designed so that the total lysine content of diets 1, 3 and 6 were the same, while the available lysine contents of diets 1, 4 and 7 and diets 2, 5 and 8 were the same as calculated from the data from Experiments 1 and 2.

RESULTS AND DISCUSSIONS

In Experiment 1, the lysine and dry matter intake was constant for all pigs and this resulted in nitrogen intakes of 23-73 g/d. However, the requirements of all amino acids, except lysine, were met by the protein sources and synthetic amino acids (National Research Council, 1987).

Table 1. The apparent digestibility of nitrogen and lysine for seven protein sources in Experiment 1.

	Apparent digestibility			
	Nitrogen		Lysine	N retention
	Ileum	Faeces	Ileum	: N intake
Soyabean meal No.2	0.826	0.859	0.891	0.559
Cottonseed meal No.1	0.739	0.764	0.886	0.423
Cottonseed meal No.4	0.772	0.778	0.732	0.383
Meat meal	0.801	0.796	0.873	0.468
Sunflower meal	0.857	0.869	0.892	0.469
Dried skim milk	0.905	0.903	0.966	0.654
Wheat gluten	0.967	0.969	0.962	0.352
S.E.M.	0.0117	0.0088	0.0162	0.0031

Table 1 shows that the apparent digestibility of nitrogen to the ileum and in the whole tract depended on the source of protein. Some digestion of nitrogen occurred in the hindgut for the vegetable proteins but not with dried skim milk or meat meal. These were the diets of lowest fibre content.

The apparent digestibility of lysine to the ileum varied from 0.97 for dried skim milk to 0.69 for cottonseed meal No.1. The value for soyabean meal No.2 was 0.89. These values are similar to those reported by Alimon and Farrell (1980) and Tanksley and Knabe (1984). The apparent digestibility of lysine to the ileum was less than that of nitrogen for both cottonseed meals.

Table 2. Utilization of lysine (relative to free lysine) in Experiment 2.

	Weight gain		FCR		N balance	
	Mean	SD	Mean	SD	Mean	S.D.
Cottonseed meal No. 1	0.689	0.069	0.676	0.111	0.685	0.130
Cottonseed meal No.4	0.758	0.081	0.765	0.120	0.716	0.105
Meat meal	0.903	0.095	0.797	0.135	0.855	0.131
Sunflower meal	0.894	0.099	0.901	0.137	0.790	0.150
Dried skim milk	0.998	0.105	1.000	0.151	1.000	0.143

In Table 2, data are presented from a slope-ratio assay with young pigs using the same sources of protein as used in Experiment 1. It is clear that the availability and digestibility of lysine are similar for these protein sources (Tables 1 and 2). For example, the apparent digestibility of lysine to the ileum for cottonseed meal No. 1 was 0.69, which is the same as the value estimated by the slope-ratio assay. Experiments by Batterham et al. (1984) gave a lower availability of lysine in cottonseed meal (0.39) as estimated by a slope-ratio assay with growing pigs, or 0.27 in the paper by Batterham in this symposium.

The lysine content of cottonseed meal No. 1 was 4.9 g/kg of crude protein as estimated in our laboratory. Analysis of 3.9 g lysine/kg of crude protein of cottonseed meal No.1 was made by Batterham (see paper in this symposium). If this latter value is used in our calculations then the value for the utilisation of the lysine in cottonseed meal No. 1 relative to free lysine increases from 0.69 to 0.80.

A comment should also be made on the variation in availability estimates. In Table 1, the SEM for the apparent digestibility of lysine was 0.016. On the other hand, the standard deviation of the regressions in Table 2 were 0.069-0.151, while the standard deviations reported by Batterham

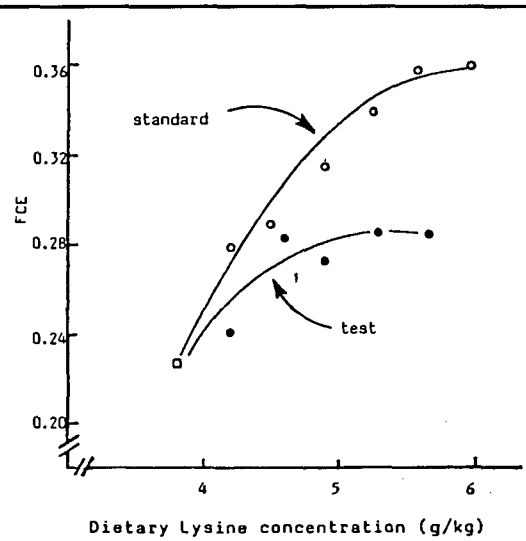
et al. (1979) were 0.082-0.107. It must be concluded that there is considerable variation in availability values measured by slope-ratio assays. In fact, two poor pigs were omitted from calculations of the data for cottonseed meal No. 1. The inclusion of these pigs would have reduced the value for the utilization of lysine of the cottonseed meal No.1 from 0.69 to 0.58. This indicates the sensitivity of the estimates to variation in individual pig performance.

Table 3. Performance of pigs given three sources of lysine in Experiment 3.

Diet No.	Protein source	Lysine (g/kg of diet)	Gain (g/d)	FCR	Apparent digestibility of dry matter	Digestible dry matter intake: gain
1.	Soyabean meal No. 2	10.0	323	1.61	0.806	1.30
2.	Soyabean meal No. 2	12.0	396	1.43	0.811	1.16
3.	Cottonseed meal No. 4	10.0	136	2.46	0.673	1.86
4.	Cottonseed meal No. 4	12.5	270	1.82	0.691	1.26
5.	Cottonseed meal No. 4	14.5	374	1.69	0.682	1.16
6.	Sunflower meal	10.0	199	2.04	0.728	1.45
7.	Sunflower meal	11.2	227	1.84	0.736	1.35
8.	Sunflower meal	12.8	358	1.82	0.727	1.17
	S.E.M.		22.0	0.063	0.0090	0.045

Unfortunately, the digestible energy contents of the diets in Experiment 3 were not the same. To overcome this problem, the last column in Table 3 gives the ratio of digestible dry matter intake to weight gain. This value is the same for diets 1, 4 and 7 and for diets 2, 5 and 8. This shows that the values measured for the availability of lysine from cottonseed meal and sunflower meal from Experiments 1 and 2 are in full agreement with the performance of pigs. Hence, it must be concluded that the values reported here, both the apparent digestibility of lysine to the ileum and from feeding trials measuring the utilization of lysine relative to free lysine, are similar and correct. The measurement of apparent digestibility is preferred, as fewer pigs and less time is involved in these experiments.

Figure 1. The relationship between dietary lysine and FCE (Kadir, 1986).



One final point which needs consideration is the differences in the results obtained for the availability of lysine in cottonseed meal by Batterham et al. (1984) and in the present Experiment 2. The explanation may be found in some elegant work by Kadir (1986). Isonitrogenous and isoenergetic diets were prepared with increasing amounts of lysine from synthetic lysine or

cottonseed meal No. 5 (also supplied by Dr. Batterham). These were given to rats and the results are shown in Figure 1. It is evident that lysine alone was not the limiting factor for optimum FCE. In fact, over the initial range of the experiment, the availability of lysine from cottonseed meal No. 5 was close to 1.0 and then it plateaued to zero.

In the present Experiment 2, the weight gains of the weaner pigs ranged from 43-535 g/d while in the experiments of Batterham et al. (1984) weight gains were 478-643 g/d. If the curves in Figure 1 were applied to this data, it is possible that the data from Experiment 2 falls in the earlier part of the curve, while the data of Batterham et al. (1984) falls in the latter part of the curve. This would give an explanation for the observed results.

In conclusion, it appears that the apparent digestibility of lysine to the ileum agrees well with the results of feeding trials, and a rapid method of measurement of ileal digestibility is required for testing feedstuffs.

ILEAL DIGESTIBILITY ASSAYS FOR FINISHER PIGS

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There are now many studies of amino acid digestibility in pigs using ileal digestibility assays. These have been reviewed and much of their data have been collated in work such as that of Low (1982), Tanksley and Knabe (1984) and Sauer and Ozimek (1986). Such reviews generally have also compared techniques of measuring amino acid digestibility and have concluded that the ileal digestibility assays are the most sensitive and accurate measure of amino acid absorption in pigs; they are more accurate, for example, than faecal digestibility assays.

This paper will describe an assay of ileal amino acid digestibility of the four oilseed meals (cottonseed meals Nos. 1, 2 and 3 and soyabean meal No. 1) previously described in the Introduction and used in the collaborative studies for amino acid availability. These data will serve a threefold purpose. Firstly, they will be used to demonstrate important features of the assay. Secondly, they will demonstrate the variability of amino acid digestibility both within and between Australian oilseed meals and, finally, these data will provide a real comparison between digestibility and growth assays as indices of protein quality for these meals.

MATERIALS AND METHODS

There were five diets used in this experiment. The basal diet contained (g/kg) wheat (976), dicalcium phosphate (15), salt (2) and a vitamin/mineral supplement (5). In the four test diets, 300 g/kg of the wheat was displaced by either soyabean meal or one of the three cottonseed meal samples. Chromic oxide (2 g/kg) was added to all diets for the estimation of ileal and faecal digestibility values.

Four male pigs were used with a mean live weight of approximately 65 kg. The pigs were prepared with T-shaped cannulae and each pig was given each diet in a completely randomized design. The diets were offered for 7 days and samples of faeces were collected on the sixth day. On the sixth and seventh days digesta samples of 70-100 g were collected from the cannulae at periods of approximately 2, 4, 6 and 8 h after feeding.

The treatment of samples and the analytical methods used were the same as those described by Taverner et al. (1983).

True digestibility (TD) values were calculated by subtracting a calculated amount of endogenous lysine from the total amount of ileal lysine and expressing the residual or undigested dietary lysine as a proportion of total lysine intake. The value of endogenous lysine output used in this calculation was 320 mg/kg dry matter intake (Taverner et al., 1981).

RESULTS AND DISCUSSION

The digestibilities of amino acids and nitrogen (N) in the three cottonseed meals were less ($P<0.05$) than those in soyabean meal (Table 4). Furthermore, there were significant differences in amino acid digestibility among the three cottonseed meals. When cottonseed meal No. 1 was added to the basal diet the apparent ileal digestibility of lysine (mean $\pm$ S.D.) decreased from 0.81 (0.023) in the basal diet to 0.69 (0.019) in the test diet. Thus, it was calculated that only 0.56 (0.042) of the lysine in cottonseed meal No. 1 was absorbed from the pig's small intestine.

Significantly more ($P<0.05$) lysine was absorbed from cottonseed meal Nos. 2 (0.67) and 3 (0.71) than from No. 1. However, the average and the range in values of apparent digestibility of lysine in the three cottonseed meals in this experiment (mean 0.64, range 0.56-0.71) was similar to that reported by Sauer and Ozimek (1986) in a collation of published values of ileal lysine digestibility in cottonseed meals (mean 0.62, range 0.53-0.70).

Table 4. Digestibility for pigs of nutrients in soyabean meal and three cottonseed meals.

	Cottonseed meal			Soyabean	S.E.M.
	No. 1	No. 2	No. 3	meal No. 1	
Faecal digestibility					
N - apparent	0.77[a1]	0.78[a]	0.83[b]	0.89[c]	0.032
Ileal digestibility					
N - apparent	0.70[a]	0.73[a]	0.78[b]	0.83[c]	0.024
Average of amino acids[2] - apparent	0.66[a]	0.76[b]	0.82[c]	0.86[d]	0.030
Lysine - apparent	0.56[a]	0.67[b]	0.71[b]	0.88[c]	0.042
- true	0.58[a]	0.68[b]	0.72[b]	0.89[c]	0.042

[1] Within each row, meals followed by different superscripts are significantly different ($P<0.05$).

[2] Average of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine.

Similarly, ileal N and lysine digestibility values averaged from published data on five soyabean meals by Sauer and Ozimek (1986) (0.82 and 0.87, respectively) are close to those values in the present experiment (0.83 and 0.88, respectively). Indeed there is remarkably little variation among published values of apparent digestibility of lysine in soyabean meal determined at the end of the small intestine using a variety of methods. For example, the previous values were determined using pigs with simple T cannulae and a variety of collection, marker and sampling procedures but the values are similar to those determined earlier by Holmes et al. (1974) (0.91) who used re-entrant ileal cannulae. This supports other studies such as Taverner et al. (1983) who found no differences between simple and re-entrant ileal cannulae in the measurement of amino acid digestibility with a range of different feeds. This work and other reports on the techniques of the ileal assay such as Haydon et al. (1984) suggest that the assay is reasonably robust. Refinement of the technique is continuing with new techniques such as ileorectal anastomosis (Fuller and Livingstone, 1982) and the ileocolic post-valvular procedure (Darcy et al., 1980).

There was a consistent but small difference between the true and apparent digestibility values of lysine in this experiment. Previous work (Taverner, 1979) has found that the differences between true and apparent amino acid digestibility diminish as the protein content of the feed increases. Clearly, the relative contribution of endogenous lysine to the total ileal lysine pool is greater with diets containing low levels of lysine than those with higher lysine levels. Taverner (unpublished data) found that the difference between true and apparent ileal lysine digestibility decreased from 0.08 for feeds containing 120 g/kg protein to approximately 0.04 for feeds with 200 g/kg protein. Thus, while there are reasons for using TD values for comparing amino acid digestibility among individual feedstuffs such as cereal grains and protein concentrates, the greatest difficulty lies in determining levels of endogenous amino acid excretion.

The endogenous lysine values used in this paper were those presented by Taverner et al. (1981) and determined by extrapolation to zero intake of linear regressions of ileal lysine on dietary lysine intake. This value was adjusted for the dry matter intake of each diet but it is possible that there are other factors influencing endogenous amino acid output from pigs given diets varying in their levels of fibre and containing possible anti-nutritional factors (Sauer and Ozimek, 1986).

Thus, if TD values are to be more widely used, methods such as that using isotope markers (15N) as proposed by Souffrant et al. (1982) need to be developed to distinguish more directly and accurately between endogenous and dietary amino acids in ileal digesta.

In both this work and other (Tanksley et al., 1981), lysine was considerably less digestible than the average of the other indispensable amino acids in cottonseed meal. The situation is reversed, however, in soyabean meal where lysine is more digestible than most other indispensible amino acids. It is known that, because of its free epsilon group, lysine is primarily affected when heat is applied to cottonseed protein in the presence of gossypol (Baliga and Lyman, 1957).

Furthermore, Craig and Broderick (1981) suggested that, in addition to the effect of gossypol, reactions of lysine residues with reducing sugars and oxidized fats also reduce lysine availability in cottonseed meal. They found that, with increasing heat damage to cottonseed meal, the TD of protein was decreased but the decrease was not as severe as that in availability assessed by the FDNB available lysine assay. Craig and Broderick (1981) concluded from their work that with excessive heating the TD of protein is not a good indicator of the changes in lysine availability in cottonseed meal. The present work supports this conclusion for protein digestibility but demonstrates that ileal lysine digestibility can be considerably different from N digestibility and may well be a better indicator of protein quality in cottonseed meal.

The large variation in the digestibility of lysine and other amino acids among cottonseed meals is of concern. Clearly there will be inaccuracies in using an average value of lysine digestibility for cottonseed meal. Such a value for lysine digestibility is not likely to be the same for other amino acids or conversely, an average amino acid digestibility value or a N digestibility value is unlikely to truly reflect lysine digestibility in cottonseed meals.

AVAILABILITY OF LYSINE FOR GROWER PIGS

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INTRODUCTION

The slope-ratio assay has been used to determine the availability of lysine in the major protein concentrates for growing pigs. Availability has been shown to vary from approximately 0.90 in fish meal, soyabean meal and field peas to 0.40 in cottonseed meals (Batterham et al., 1979, 1984). Meals of intermediate values include sunflower meal (0.60), lupin-seed meal (0.55) and meat and bone meals (0.40-0.96; Batterham et al., 1981, 1986b, 1986c, 1986d).

There has been little comparative work determining to what extent reduced digestibility at the terminal ileum is responsible for the reduced availability in the above meals. Direct comparisons between different samples of the one type of protein concentrate are always limited in the extent that variation between samples may affect the results. In comparative work with the one sample of lupin-seed meal, the ileal digestibility of lysine was high (0.86; M. R. Taverner, personal communication) whilst the availability determined by slope-ratio assay was low (0.37; Batterham et al., 1984).

EXPERIMENT 1. SLOPE-RATIO ANALYSIS FOR LYSINE

In order to provide comparative data on the same feed samples, the availability of lysine in the four oilseed meals (cottonseed meals Nos. 1, 2 and 3 and soyabean meal No. 1) was determined. The technique of Batterham et al. (1979) was used. Five points were used to determine the response to free lysine and four points for each of the four protein concentrates. Response was assessed in terms of feed conversion efficiency on a carcass basis. The results are given in Table 5.

Table 5. Availability of lysine in the four protein concentrates for growing pigs.

	Soya 1	Cot 1	Cot 2	Cot 3	S.E.M.
Lysine availability (proportion of total)	0.90	0.27	0.30	0.29	0.09

The result for soyabean meal No. 1 is similar to previous determinations (Batterham et al., 1984) whilst the values for the three cottonseed meals are slightly lower than those recorded previously (0.43-0.39; Batterham et al., 1979, 1984).

EXPERIMENT 2. TESTING THE SLOPE-RATIO VALUES

In order to test these values, a second trial was conducted using soyabean meal No. 1 and cottonseed meal No. 2. The aims of the trial were, (a) to compare the performance of pigs fed diets formulated to the same available lysine content from soyabean meal No. 1 and cottonseed meal No. 2 and, (b) to determine whether the availability of lysine could be used as a correction factor for the other essential amino acids in cottonseed meal.

Four diets were formulated (Table 6). Diets 1 and 4 contained either wheat and cottonseed meal No.2 (diet 1) or wheat and soyabean meal No.1 (diet 4) and were formulated to contain the same total lysine content (8 g/kg). Diet 2 contained cottonseed meal No.2 plus free lysine added to equalize the available lysine content with that of the soyabean meal diet (diet 4). Diet 3 was as for diet 2 plus a supplement of methionine, threonine and tryptophan to ensure adequacy of these essential amino acids on an available amino acid basis according to the recommended balance of amino acids of the Agricultural Research Council (1981). The diets were fed restrictively to pigs over the 20-45 kg growth phase.

Table 6. Response of grower pigs to diets formulated on either a total or available lysine basis.

	Diet 1 Cot 2	Diet 2 Cot 2 + lysine	Diet 3 Cot 2 + lysine + other aa	Diet 4 Soya 1	S.E.M.
Total lysine content (g /kg)	8	10	10	8	
Available lysine content (g /kg)	5	7	7	7	
Pig response					
Carcass gain (g/d)	410	470	480	480	6.3
FCR (carcass basis)	3.1	2.8	2.7	2.7	0.04
Carcass results					
Estimated lysine retention					
total lysine intake	0.48	0.48	0.48	0.63	0.010
available lysine intake	0.78	0.66	0.70	0.72	0.015

The results in Table 6 indicate that the growth and FCR responses are in agreement with the available lysine values. The higher retention of total lysine of pigs fed the soyabean meal diet reflects a higher availability than for the cottonseed meal. However, once the diets were formulated to an available lysine basis, and the other essential amino acid added, lysine retention of pigs fed the cottonseed meal diet was similar (0.70 v 0.72). These results also indicate that the availability of lysine can be used as correction factor for the availability of some of the other essential amino acids in cottonseed meal.

EXPERIMENTS 3, 4 AND 5. EVALUATING SLOPE-RATIO VALUES IN LUPIN-SEED MEALS

Previous work has indicated little agreement between the ileal digestibility of lysine and lysine availability in the same samples of lupin-seed meal for growing pigs (Taverner, personal communication; Batterham et al., 1984). The reason for this difference is unclear. The lower availability of lysine in lupin-seed meal could indicate that the lysine is absorbed in a form that is not fully utilized. Alternatively, it could be due to some factor that interfered in a linear manner with the growth response of pigs fed diets containing increasing levels of lupin-seed meal in the slope-ratio assays.

Table 7. Response of grower pigs to diets containing lupin-seed meal or soyabean meal and formulated to the same total lysine and digestible energy contents (Batterham et al., 1986a, 1986b).

	Diet 1 Lupins	Diet 2 Soya[1]	S.E.M.
Experiment 3			
Carcass gain (g/d)	422	453	6.9
FCR (carcass basis)	3.04	2.83	0.052
Experiment 4			
Carcass gain (g/d)	445	523	9.4
FCR (carcass basis)	2.93	2.48	0.053
Experiment 5			
Carcass gain (g/d)	440	508	6.1
FCE (carcass basis)	0.34	0.38	0.005

Whilst no direct comparisons of diets formulated on a lysine digestibility or availability basis have been conducted, there have been a number of experiments comparing diets containing lupin-seed meal and soyabean meal formulated on a total lysine and equal digestible energy basis (Batterham et al., 1986a, 1986b) (see Table 7).

These experiments confirm that growth responses of pigs fed the diets containing lupin-seed meal are inferior to those given diets containing soyabean meal. Whilst not direct evidence, these experiments do show that the lower lysine availability values have greater application in dietary formulations for lupin-seed meals than the ileal digestibility values, which are similar to those of soyabean meal.

CONCLUSIONS

The results indicate that while the availability of lysine in soyabean meal is high (ca 0.90), that in cottonseed meal is low (ca 0.30). Furthermore, lysine availability was consistently low in the different samples of cottonseed meal that were produced by either expeller or pre-press solvent extraction processes. The results of Experiment 2 confirmed that these values were applicable in dietary formulations. Because of the large differences in availability in local protein concentrates, there is clearly a need to formulate on an available rather than a total lysine basis.

ESTIMATION OF AMINO ACID AVAILABILITY USING THE AUSPIG COMPUTER SIMULATION MODEL

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The AUSPIG computer model, that simulates amino acid and energy utilization in pigs and predicts animal performance, provides an additional way of assessing the availability for metabolism of amino acids in feeds. The model predicts, in relation to the weight, strain and sex of the animal, and environmental conditions, its energy intake, the maximum rate and amino acid composition of body protein deposition and the quantity of amino acids inevitably lost through catabolism and in the faeces. It calculates whether sufficient amino acids are available from the feed to allow the maximum rate of protein deposition for the defined situation and determines the order in which the essential amino acids would be limiting. When the supply of an amino acid is limiting protein deposition, the model assesses the effect of the deficiency on protein gain, fat deposition and live weight change. Hence, the AUSPIG model, when used to simulate experiments investigating the effects of increasing the intake of either total protein or individual amino acids, can provide an assessment of the availability of the most limiting amino acid. In addition, the model demonstrates quantitatively the probable effect of a change in amino acid availability on animal performance and dietary amino acid requirements.

CALCULATION PROCEDURES AND ACCURACY OF THE METHOD

Details of the simulation model are described by Black et al. (1986). The pattern of amino acids deposited in body protein was assumed to be that of protein in the whole pig which, along with the patterns for inevitable catabolism and metabolic faecal losses, have been calculated by Black and Davies (unpublished data). These patterns were assumed to be unaffected by the weight, strain or sex of pig, and variation in the overall amino acid pattern required by an animal resulted only from changes in the relative rates of amino acid use for each function. Because inevitable losses represent only a small proportion of total amino acid requirements in most situations with growing pigs, the strain and sex of the pig or its energy intake were predicted to have little effect on the rate of protein deposition when it was limited by amino acid supply. However, accuracy of using the AUSPIG simulation model to assess the availability of a limiting amino acid depends on the amino acid patterns selected for each body function because these affect the rate of protein deposition and live weight gain coincident with a given availability of the most limiting amino acid. The accuracy of the procedure is also greater when experimental results are given in terms of protein deposition rather than live weight gain because the latter is often affected by largely unrelated factors such as the efficiency of energy utilization, activity of the animal and adverse climatic conditions. Unfortunately, reports of many experiments fail to adequately describe housing conditions of pigs.

The AUSPIG model has been used to assess the availability of amino acids in the diets of several experiments described in the literature. Initially, the availability of amino acids in each ingredient was assumed to be the mean of many measurments using the ileal digestibility technique. Values published before May 1984 were collated by P.A. Moughan and W.C. Smith (unpublished), and the mean, range and co-efficient of variation for amino acid availability in several ingredients have been calculated by Black and Davies (unpublished data). Following comparison of predictions from the model with experimental results, availabilities of the limiting amino acids were adjusted until prediction and observations were similar.

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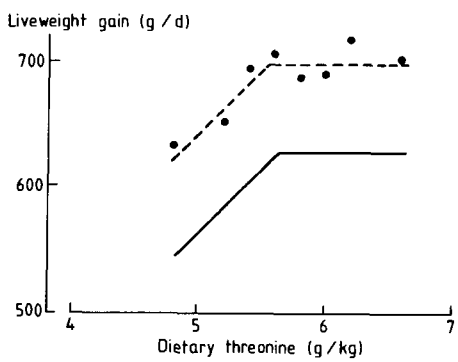
RESULTS

Example 1: Predicted and observed results for the experiment of Taylor et al. (1982) that was designed to determine the dietary threonine requirements of growing pigs are presented in Figure 2. The basal diet contained (g/kg) barley (899), soyabean meal (40) and white fish meal (20) and seven increments of DL- threonine were added to provide eight dietary treatments that were offered at a restricted level, once daily to pigs growing from 25 to 55 kg live weight. The reported dietary amino acid pattern was used in the simulation and the availability of each amino acid was calculated from the mean of ileal digestibility estimates for each ingredient. The resulting availabilities for threonine, histidine and valine were 0.74, 0.81 and 0.77, respectively, in the basal diet. The availability of DL-threonine was assumed to be 0.33 on the basis that D-threonine is not utilized and L-threonine would be used with an efficiency of only 0.66 because of the restricted once-daily feeding regime (Batterham and O'Neill, 1978). When these experimental conditions were simulated, predicted growth rate was considerably less than observed. However, the increases in growth with increments of threonine were initially similar to the observations, indicating that added DL-threonine was used with an efficiency of about 0.33. By the fourth increment of threonine in the simulation, histidine and valine were predicted to be the first and second limiting amino acids and there was no further increase in growth with additional threonine. These predictions indicate that the availability of threonine, histidine and valine, calculated from the mean of ileal digestibility values reported in various studies, is less than occurred in the experiment. The broken line in Figure 2 shows the results of simulations when the availability of three amino acids was raised by 0.15 to 0.85, 0.93 and 0.88, respectively, for threonine, histidine and valine. Similarity between these results and the observations suggests that the availability of amino acids in the experiment of Taylor et al. (1982) was probably considerably higher than suggested from the mean of reported ileal digestibility determinations.

Figure 2. Response in live weight gain of pigs fed increments of DL- threonine

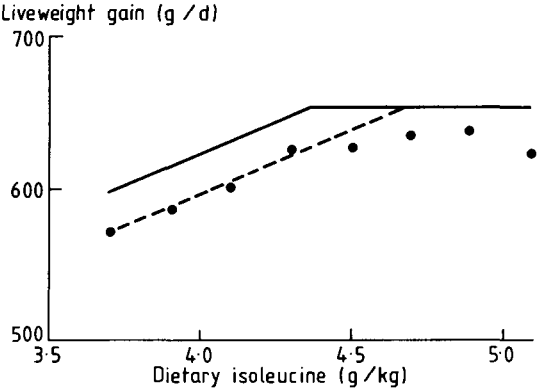


• observed results (Taylor et al., 1982); —— predictions from AUSPIG using published values for the ileal digestibility of amino acids; - - - predictions from AUSPIG when amino acid availability was increased by 0.15

Example 2: Predicted and observed results for the experiment of Taylor et al. (1985) that was designed to determine the dietary isoleucine requirements of growing pigs are presented in Figure 3. The basal diet contained (g/kg) barley (400), maize (400), tapioca (71), toprina (35) and blood meal (25) and seven increments of L-isoleucine were added to provide eight dietary treatments that were offered at a restricted level to pigs growing from 25 to 55 kg live weight. The reported dietary amino acid pattern was used in the simulation and availabilities were calculated from ileal digestibility studies such that the availability of isoleucine in the basal diet was estimated to be 0.82. Added L-isoleucine was assumed to have a utilization 0.66 because of the restricted, once-daily feeding regime (Batterham and O'Neill, 1978). Although the pattern of response was similar, predicted growth rates were higher than observed. When the availability of isoleucine was

decreased by 0.10 to 0.74, observed and predicted growth rates were similar, except when an amino acid deficiency was not limiting performance. This latter disparity indicates a difference in the genetic potential for growth between the animal simulated by the model and those used in the experiment. In addition, reducing the availability of isoleucine by 0.10 resulted in a predicted change in dietary requirement of isoleucine from 4.37 to 4.77 g/kg diet.

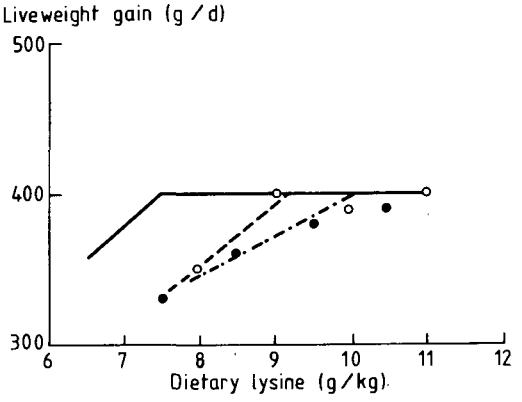
Figure 3. Response in live weight gain of pigs fed increments of L- isoleucine



•observed results (Taylor et al., 1985); — predictions from AUSPIG using published values for the ileal digestibility of amino acids; - - - predictions from AUSPIG when amino acid availability was reduced by 0.10.

Example 3: Predicted and observed results for two experiments of Thaler et al. (1986) that investigated the effect of dietary lysine on the performance of pigs growing from 8 to 20 kg are presented in Figure 4. The basal diets contained maize, dehulled oats, soyabean meal and 100 g/kg dried whey. In each experiment, three increments of lysine were added to the basal diets to provide four dietary treatments which were offered to pigs *ad libitum*. Because of the similarity between the two experiments, predictions for Experiment 1 only are shown in Figure 4.

Figure 4. Response in liveweight gain of pigs fed increments of L-lysine monohydrochloride



Observed results, •Experiment 1, o Experiment 2, (Thaler et al., 1986); — predictions from AUSPIG using published values for ileal digestibility of amino acids and free L-lysine utilization of 1.0; - - - predictions from AUSPIG when availability of dietary lysine was adjusted to 0.65 and free lysine utilization to 1.0; - - - - predictions from AUSPIG when the availability of dietary lysine was adjusted to 0.65 and free lysine utilization to 0.50.

The availability of lysine in the basal diet of this experiment was estimated from ileal digestibility studies to be 0.85 and lysine in the added L-lysine monohydrochloride was initially assumed to have a utilization of 1.0. Simulations using these availabilities indicated that lysine was not limiting the performance of animals given the basal diet containing 7.5g/kg, but growth rate would be depressed if lysine was only 6.5g/kg diet. Lysine availability in the basal diet needed to be reduced by almost 0.25 to 0.65 before the predicted performance of animals given the basal diet was similar to observations. The utilization of added lysine also had to be reduced to about 0.50 before the predicted growth response was similar to observations for all treatments. This analysis suggests that the availability of lysine in the experiments of Thaler et al. (1986) was considerably below that estimated from the mean of values obtained for ingredients using ileal digestibility studies.

DISCUSSION

Only a few of the comparisons between predictions from the AUSPIG model and experimental observations that have been made are presented in this paper. In all comparisons, except when lysine was the limiting amino acid, adjustments in availability of amino acids either up or down of about 0.15 or less, resulted in predictions similar to observations. However, with all the experiments in which lysine was limiting performance availability had to be reduced by up to 0.25 before predictions were similar to observations (Table 8).

Table 8. Reductions in lysine availability required for predictions from AUSPIG to be similar to experimental observations

Reference	Proportional decrease in lysine availability
Campbell (1978)	0.25
Batterham et al. (1979)	0.22
Lewis et al. (1981)	0.16
Thaler et al. (1986)	0.23

Although the technique described cannot be regarded as a highly precise method of estimating the availability of dietary amino acids, the consistent discrepancy between predictions and observations for experiments in which lysine was the first limiting amino acid indicates that its availability may commonly be lower than is determined from measurements of its disappearance from the small intestine. A comparison of available lysine requirements for growing pigs estimated from theoretical considerations with that for dietary lysine requirements recommended by the Agricultural Research Council (1981) also suggests that the availability of lysine in the experiments used to establish the dietary recommendations was low, with likely values of between 0.55 and 0.70 (Black and Davies, unpublished data).

Absorption of an amino acid from the intestine does not necessarily mean that it is totally available for metabolism. Lysine is particularly susceptible to becoming unavailable because of its highly reactive epsilon amino group that remains free when lysine is incorporated into peptide chains. These free amino groups react with reducing sugars under relatively dry and mild conditions to form the deoxyketosyl-lysine compounds of the early Maillard reaction. These compounds are biologically unavailable to mammals, although they can be absorbed from the intestine and excreted quantitatively in the urine (Finot, 1973). Polysaccharides, such as starch and cellulose, bind less readily than sugars to the epsilon amino group of lysine and mainly depend on hydrolysis to provide glucose for the reaction. Nevertheless, lysine availability to rats is reduced when proteins are heated with these polysaccharides (Knipfel et al., 1975).

In addition to the particular problem of estimating the availability of lysine, there are large differences between batches of the same ingredient in the availability of many amino acids. For example, Stephenson et al. (1970) estimated that the availability for chicks of threonine and

histidine ranged from 0.47 to 0.92 and from 0.42 to 0.90, respectively, in sorghum grain from different hybrids grown and harvested under similar conditions. Substantial variation also occurs in estimates of the ileal digestibility of ingredients used in pig diets (Black and Davies, unpublished data). For example, the range in estimated lysine absorption from maize given to pigs is 0.48 to 0.83.

The AUSPIG model can readily demonstrate the effect on animal performance and dietary amino acid requirements of variations in amino acid availability. These effects can be substantial. Because the AUSPIG model provides a simple way of precisely estimating the requirements of available amino acid for pigs of different strain and sex in relation to the particular feeding and climatic environment, it is essential that rapid and accurate methods are developed for determining amino acid composition and availability in feedstuffs. Current methods are slow and tedious. Black and Batterham (1987) have proposed an alternative method for determining amino acid availability that requires few animals for a short time, is independent of the strain, sex or weight of animal and can be used for all essential amino acids.

COMPARISON OF ASSAY RESULTS AND CONCLUSIONS

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The results of the collaborative studies with the three samples of cottonseed meal and soyabean meal indicate considerable differences in the estimates of the ileal digestibility of lysine and lysine availability as determined by the slope-ratio assay with weaner and grower pigs. For comparative purposes, the main results are summarized in Table 9.

Table 9. Summary of the ileal digestibility and lysine availability estimates for the three cottonseed meals and soyabean meal.

	Cot 1	Cot 2	Cot 3	Soya 1	S.E.M.
Ileal digestibility (grower-finisher pigs)					
Apparent:					
Leibholz	0.69	——	——	——	0.016
Taverner	0.56	0.67	0.71	0.88	0.042
True:					
Taverner	0.58	0.68	0.72	0.89	0.042
Lysine availability					
Weaner pigs:					
Leibholz	0.69	——	——	——	0.130
Grower pigs:					
Batterham	0.27	0.30	0.29	0.90	0.090

These results indicate that for cottonseed meal there were (a) only fair agreement in the determination of ileal digestibility between two research centres for cottonseed No. 1, (b) substantial differences in the determinations of lysine availability between weaner and grower pigs for the two research centres and (c) good agreement between the ileal digestibility and lysine availabilities for weaner pigs, but for grower pigs, ileal digestibility was considerably higher than lysine availability. On the other hand, for soyabean meal, there was close agreement for all estimates recorded.

COMPARISON OF SLOPE-RATIO VALUES BETWEEN WEANER AND GROWER PIGS

The main discrepancy in the results is the lack of agreement between the availability estimates for weaner and grower pigs. The results for cottonseed meal No. 1 indicate much higher lysine availability for weaner pigs (0.69) compared to grower pigs (0.27). It is unlikely that weaner pigs could utilize the lysine in cottonseed meal more effectively than grower pigs. If anything, one would expect digestibility and therefore utilization to improve with age. The differences seem more related to differences in methodology. These include the parameters of response used to assess availability, statistical analyses, area of response curve and feeding regimen.

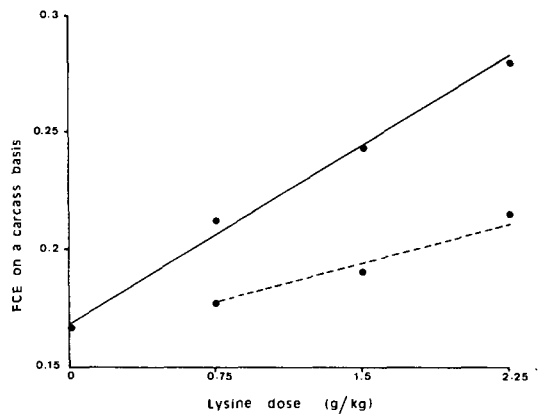
Most of the differences may be due to the different parameters of response adopted in the two studies.The work of Leibholz used live weight gain, FCR and nitrogen balance as the responses, whereas Batterham used FCE on a carcass basis. Previous work had indicated that, with grower pigs, responses based on live weight over-estimated availability in the order of 0.26-0.28 units of availability, presumably due to differences in gut fill (Batterham et al., 1979, 1984).

Part of the differences may also be due to the different statistical analyses conducted. The results for grower pigs were analysed by the slope-ratio analysis of Finney (1964) whereas in the assay of Leihbolz, the results were analysed by linear regression with lysine intake as the

independent variable and live weight gain as the dependent variable (Leibholz, 1986). This method of analysis differs from the slope-ratio analysis in two aspects. Firstly, in the analysis using lysine intake as the independent variable, there is the need to make assumptions regarding the availability of lysine in the basal ingredients in the test diets in order to partition the response due to the lysine in the test protein from that due to the basal ingredients. Secondly, the assay used did not contain the statistical safeguard that the slopes of the responses of the test protein and standard lysine should pass through a common origin. The removal of this safeguard leaves the assay more susceptible to influences of other dietary factors. Both these factors could contribute to the differences in estimates recorded.

The results of Kadir (1986) reported by Leibholz indicate that, in that particular assay, the responses to both the free and test lysine may have been slightly curvilinear rather than linear responses. This may happen in slope-ratio assays and depends on the area of the response curve being utilized relative to maximum response. For this reason it is difficult to apply the results from one assay to another. For example, in a rat assay of the same cottonseed meal (No. 5), there were no indications of curvilinearity of response and the availability of lysine (0.35) was similar to that determined with pigs on the same meal (0.39; Batterham et al., 1984). This is illustrated in Figure 5. Thus it is doubtful that the differences in estimates between weaner and grower pigs are due to differences in the area of response being studied.

Figure 5. The response of growing rats to increasing levels of lysine supplied as either free lysine (—) or as lysine from cottonseed meal (----)



It is possible that differences in feeding regimens may influence the results of availability assays. The assay for availability with weaner pigs was conducted with *ad libitum* feeding whilst that with grower pigs was with restricted feeding. With controlled feeding, all animals receive similar quantities of test proteins (on a live weight basis). However, with full feeding, differences in intake develop and this makes it more difficult to relate the responses to the test amino acid only. This problem is partially overcome by analyzing the results with lysine intake as the independent variable and then necessitates assumptions regarding the availability of the lysine in the basal ingredients.

COMPARISON OF ILEAL DIGESTIBILITY AND AVAILABILITY

The results indicate that, for weaner pigs, reduced digestibility at the terminal ileum is the major reason for reduced availability. However, with grower pigs, reduced ileal digestibility appears to be the main reason for reduced availability in meals of high availability (soyabean meal) but for meals of low availability (cottonseed meal), reduced ileal digestibility only accounts for about 0.3 to 0.5 of the decline in availability. This is in agreement with reports in the literature which indicate that, with heat damage, the decline in protein quality is generally much greater than the reduction in digestibility.

INFLUENCE OF VARIATION IN TOTAL AMINO ACID ANALYSIS ON AVAILABILITY ESTIMATES

The variation in lysine availability in the three cottonseed meals for grower pigs was small (0.27-0.30) and much lower than anticipated considering the high SEM (0.09) of the estimates. However, it is possible that a substantial proportion of the variation in the values for lysine availability in the literature could be related to variation in total amino acid analyses. In the current studies, the total lysine content (g/16gN) of cottonseed meal No. 1 was determined as 3.9, 4.1 and 4.9 at Wollongbar, Werribee and the University of Sydney, respectively. Whilst this variation does not affect the content of available lysine in the cottonseed meal (as assessed against free lysine), it does have a considerable effect on the values when availability is expressed as a proportion of total lysine. The ileal digestibility assay is not similarly affected, as the results are expressed as a proportion of intake. However, if the results are expressed as content of digestible lysine in the meal, then the values will also be affected in a similar manner. The problem of methodology, as applied to total amino acids, is more fully discussed by Davies (1987).

COMPARISON OF THE ILEAL DIGESTIBILITY AND AVAILABILITY OF LYSINE WITH COMPUTER SIMULATED VALUES

The results with computer simulated studies indicate that the availability of lysine is only 0.5-0.7 in diets of reputed good quality proteins. This is much lower than the estimated ileal digestibility of lysine in these diets. It is also lower than the estimated availability of lysine using slope-ratio assay values, as there is generally good agreement between these two techniques in meals of high quality. The computer studies are supported by the data in Table 6 which indicate a retention of only 0.63 of the lysine in a diet of wheat and soyabean meal. In this case, both the ileal digestibility and availability of lysine in the soyabean meal was high (0.89 and 0.90) and, assuming a similar value for the wheat base, a considerable portion of the lysine is unaccounted for. This suggests that either the availability of lysine is lower than estimated or that the metabolic uses and costs of lysine utilisation are not fully understood.

Overall, the results presented in this symposium indicate that there is still much uncertainty regarding the availability of lysine in feeds for pigs and in techniques for estimating availability. There is a need for further information on the uptake and uses of lysine (and other amino acids) by the pig. There is also the need for the differences in methodology to be resolved. To this end, collaborative studies between different research centres and scientists utilizing different techniques is essential if progress is to be achieved. In the long term, there is still a need for simple rapid techniques for assessing availability. However, progress to this end will be slow as long as the information on the causes of reduced amino acid availability are not fully understood.

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COMPOSITION OF THE WEIGHT CHANGE IN SOWS AFTER WEANING

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Sows lose a considerable amount of weight after their piglets have been weaned. The results of Zoiopoulos et al (1983) indicate that in addition to gut fill and water loss, depletion of body tissue also occurs. Furthermore Brooks (1982) suggested that the changes that occur in body composition after weaning may influence subsequent reproductive efficiency. The aim of this study was to apportion the weight loss in sows after weaning into gut fill loss, water loss and body tissue loss.

Twelve first-litter sows were fed either 2.5 or 5.0 kg/day during a lactation period of 24-34 days. The average live weight and backfat thickness (P1) of sows at farrowing was 154.1 (S.E. 3.1) kg and 22.9 (S.E. 0.8)mm, respectively. On the day of weaning and each day thereafter all sows were given 2.0 kg/day. Sows were weighed at weaning both after feeding and after a 24-hour fast and seven days after weaning, again both after feeding and after a 24-hour fast. The amount of water in the body of the sows was determined at the end of each 24-hour fast from the dilution of deuterium oxide injected intramuscularly.

Sows which were given 5.0kg/day during lactation lost more weight after weaning when the measurements were made before fasting (see Table1). Gut fill accounted for most of this loss. The body composition of sows receiving low intakes during lactation remained relatively constant over the week following weaning. In contrast, sows given the higher feeding level lost over 5kg water in the week following weaning. Zoiopoulos et al. (1983) also reported appreciable water loss in newly-weaned sows which had been fed generously during lactation. Furthermore, sows which received high feed intakes appeared to deposit considerable amounts of body tissue in the week following weaning.

Table 1. Composition of weight change in sows in the week following weaning.

Feeding level during lactation (kg)		2.5	5.0	LSD (P=0.05)
Weight change in the week after weaning (kg)				
As measured	: before fasting (WLB)	-0.3	-5.5	4.8
	: after fasting (WLA)	+0.3	-0.6	3.1
	: water	-0.3	-5.3	3.9
As calculated	: gut fill (WLB-WLA)	-0.5	-4.9	5.4
	: tissue (WLB-gut fill-water)	+0.1	+4.7	4.5

The results of this study indicate that sows which were given low feed intakes during lactation failed to deposit any body tissue (dry matter basis) during the week following weaning whereas water loss and accretion of body tissue occurred in newly-weaned sows following high intakes during lactation.

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ESTIMATION OF MILK INTAKE BY PIGS USING DEUTRIUM OXIDE DILUTION

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Sow milk production is an important factor which may limit piglet growth to weaning. Estimates of milk production or intake, based on the weighing of offspring before and after suckling may be inaccurate (Dove and Freer, 1979; Klaver et al., 1981) and the measurement of intake by isotope dilution has been proposed in several species, including pigs (Dove and Freer, 1979; Yang et al., 1980). The purpose of this study was to assess the validity of the deuterium oxide (D_2O) dilution method for estimating milk intake in piglets.

Sixteen piglets were removed from their dams at 24-36 hours after birth and trained to drink a semi-synthetic liquid milk diet. After a pre-experimental period of 7 days, they were fed at one of 3 different levels for a further 3 weeks. Individual milk intakes were recorded daily. Over measurement periods of 1, 3, 5 and 7 days in each of weeks 1 and 3, milk intakes of individual piglets were estimated from their total water turnover, determined by dilution of injected D_2O. Body water was extracted from blood samples by vacuum sublimation and assayed for D_2O by infrared spectroscopy. Total water turnovers were converted to estimates of milk intake using the known milk water content and an estimate of the metabolic water contribution of milk solids.

Milk intake estimated by D_2O dilution was highly correlated with actual intake for all periods (r=0.96-0.99). Furthermore the estimates of milk intake using D_2O dilution did not differ significantly from actual milk consumption (P>0.05).

Table 1. The difference between actual milk intake and milk intake estimated from water turnover measured by D_2O dilution.

Measurement period (days)	Number of measurements	Difference between actual and estimated (D_2O) milk consumption (%) Mean	SE	Range
1	30	- 0.1	1.02	- 15.7 to 11.7
3	30	+ 0.5	1.25	- 13.3 to 15.6
5	30	+ 0.5	1.18	- 15.3 to 16.0
7	45	- 2.1	0.84	- 15.7 to 7.2

The results of this study show that the D_2O dilution technique is an accurate method for estimating the milk consumption of piglets over periods of up to seven days. However, the longer measurement periods require correction for changes in body water pool size (Dove and Freer, 1979).

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A PRELIMINARY EVALUATION OF THE ADEQUACY OF PROTEIN-FREE ENDOGENOUS ILEAL AMINO ACID VALUES USING THE GROWING RAT

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Traditionally, endogenous ileal amino acid loss has been determined following protein-free alimentation. This approach can be questioned, however, owing to the unphysiological nature of the protein-free state. A major criticism is that a protein-free dietary regime will lead to a decreased rate of body protein synthesis which will affect the amount of protein entering the gut. This preliminary study describes a method for the determination of endogenous amino acid excretion at the terminal ileum which overcomes this criticism of protein-free feeding.

Eight male laboratory rats of approximately 200 g live weight were fed a diet in which the sole source of dietary nitrogen was a mixture of free synthetic amino acids devoid of aspartic acid and serine. Six comparable rats received a protein-free diet. Rats were fed these diets for 16 days and were slaughtered three hours after feeding. Digesta from the terminal 20 cm of ileum was flushed out, freeze dried, ground and along with samples of the diet analysed for aspartic acid, serine and chromium (indigestible marker) contents.

Endogenous ileal amino acid flows of aspartic acid and serine, determined using the diet devoid of these amino acids, were not significantly ($P>0.05$) different from values determined on protein-free feeding (Table 1).

Table 1. Comparison of mean (±S.E.) endogenous ileal amino acid flows in rats determined with a diet devoid of key amino acids or a protein-free diet.

	Endogenous flow (µg/g dry matter intake)		
Amino acid	Diet devoid of key amino acids	Protein-free diet	Significance of difference
Aspartic acid	704 ±61	585 ±44	N.S.
Serine	282 ±24	290 ±28	N.S.

Accepting that the method outlined here provides suitable baseline data on endogenous amino acid loss from the terminal ileum of rats, it would appear, at least for aspartic acid and serine, that protein-free feeding provides accurate estimates of endogenous ileal excretion. It remains possible, however, that endogenous amino acid excretion will be greater when intact dietary protein and peptides are present in the gut, although there is evidence (Fauconneau and Michel, 1970) that undigested enzymes contribute only a small proportion of endogenous excretion at the terminal ileum. Further studies involving rats fed synthetic diets devoid of key amino acids are currently in progress to examine whether protein-free feeding provides accurate estimates of endogenous ileal excretion of those amino acids.

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THE EFFECT OF FAT AND FIBRE ON THE VOLUNTARY INTAKE AND GROWTH OF GROWER PIGS

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Grower pigs are able to regulate their energy intake by adjusting feed intake in accordance with changes in dietary energy concentration. However, this ability is limited by liveweight and, therefore, gut capacity (Campbell et al.,1975). It is unclear whether pigs respond only to variations in energy concentration *per se*, or whether the response is also influenced by the level of dietary fat or fibre. For example, Campbell and Taverner (1986) found that the response of grower pigs to dietary energy content was modified by fibre level. The present experiment was undertaken to investigate the response of grower pigs to increasing fat and fibre levels.

Two sets of diets containing either a moderate fibre level, MF (~50 g/kg crude fibre), or a high fibre level, HF(~90 g/kg crude fibre) were formulated. Oat hulls provided the major source of fibre.Within each set, digestible energy (DE) concentration was varied by including tallow at six levels between 0 and 125 g/kg. Lysine and other essential amino acids were kept at a constant ratio to DE. Six individually housed pigs (three males; three females) were allocated to each of the 12 treatment combinations and fed *ad libitum*. The DE contents of the four extreme diets were determined in a digestibilty experiment, and the DE contents of intermediate diets were calculated from these values.

Table 1:Effect of dietary fat and fibre on average daily gain(ADG), voluntary feed intake (VFI), and voluntary energy intake (VEI) (n=6).

Tallow (g/kg)		0	25	50	75	100	125	S.E.M.	Significance	
DE(MJ/kg)	MF	12.9	13.3	13.6	14.0	14.3	14.7		Fibre level	Fat level
	HF	11.5	12.0	12.5	12.9	13.4	13.9			
ADG (g/d)	MF	943	880	900	915	902	944			
	HF	832	842	855	879	907	919	28.3	*	N.S.
VFI (kg/d)	MF	2.17	2.00	2.00	1.97	1.93	1.85			
	HF	2.17	2.04	2.01	2.06	2.02	2.01	0.054	*	**
VEI (MJ/d)	MF	28.0	26.7	27.2	27.5	27.6	27.2			
	HF	24.9	24.5	25.0	26.6	27.1	27.9	0.71	**	*

* P<0.05; ** P<0.01

There were no significant fat x fibre interactions. On average, VFI was only slightly greater (P<0.05) on HF diets, and was insufficient to prevent a decrease in VEI, especially at low dietary DE concentrations. Intake at both levels of fibre decreased (P<0.01) with increasing dietary fat and DE content, although this was more pronounced on the MF diets. For HF diets, VEI and ADG increased linearly with DE concentration (r^2=0.84 and 0.96 respectively); where as for MF diets there was no trend, partly because of the high VEI and ADG of the animals fed zero tallow. The maximum level of DE intake in this experiment (27 to 28 MJ/d) was lower than that reported by Campbell and Taverner (1986), of 30 to 31 MJ/d, for animals of comparable weight which were fed diets of moderate fibre content.

The results indicated that, except for pigs fed the MF diet without fat, growth performance improved with an increase in dietary DE concentration up to 13.5-14.0 MJ/kg. At a given dietary DE concentration, difference in fibre level had little effect on performance.

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NUTRITIVE VALUE OF SMUT-AFFECTED BARLEY FOR PIGS

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Some barley crops in southern Queensland during 1985 were affected with smut due to the fungus *Ustilago hordei*. Growth assay and digestibility studies were used to compare the nutritive value of both clean (CB) and smut-affected (SB) Corvette barleys. SB had a recoverable smut content of 5 g/kg. Neither of the barley lines (Table 1) contained the mycotoxins: aflatoxin B1, B2, G1 and G2; ochratoxin A; sterigmatocystin; zearalenone; 4-deoxynivalenol; nor T-2 toxin.

Table 1. The bulk density (BD, kg/HL) and as fed content (g/kg) of dry matter (DM), crude protein (CP), crude fibre (CF), lysine (LYS), methionine plus cystine (M+C) and threonine (THR) and of gross energy (GE, MJ/kg) for clean (CB) and smut-affected (SB) barley.

Grain	BD	DM	CP	CF	LYS	M+C	THR	GE
CB	68.3	905	94	40	3.51	3.74	3.20	16.53
SB	68.4	895	91	38	3.28	4.06	3.03	16.23

In the growth assay, 32 male pigs of initial liveweight (±SD) of 19.2 ±0.58 kg were used to evaluate eight diets prepared by serially substituting SB for CB in 110 g/kg increments from 0 to 770 g/kg. All diets contained (g/kg): soybean meal, 120; tuna meal, 70; soy oil, 20; lysine HCl, 2.5; and a mineral/vitamin premix, 17.5. Chemical content of all diets was similar (g/kg): Ca, 7.2; total P, 6.5; total Lys, 10.0; M+C, 5.9; and Thr, 6.0. Individually housed pigs were fed *ad libitum*, weighed weekly and slaughtered at 82 kg. Differences between diets were not significant (P>0.05). Means (±S.E.M.) were: daily feed intake, 2.21±0.09 kg; daily growth rate, 914 ±30.3 g; feed conversion, 2.41 ±0.070 g:g; dressing %, 76.5±1.14; and P2 fat, 18.0±1:88 mm.

The apparent digestibility of five diets (Table 2) was determined in a 5x5 latin square experiment using total collection with pigs in metabolism cages.

Table 2. The composition (g/kg) and determined digestibilities of the diets.

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM
Composition						
Clean-barley	950	—	770	330	—	
Smut-barley	—	950	—	440	770	
Additives	50*	50*	230**	230**	230**	
Apparent digestibility						
Dry matter (%)	79.6^{c}	80.4^{bc}	80.6^{bc}	81.2^{ab}	82.3^{a}	0.50
Crude Protein (%)	69.2^{b}	68.9^{b}	78.6^{a}	80.1^{a}	79.9^{a}	0.93
Gross energy (%)	78.0^{c}	78.6^{bc}	79.3^{abc}	79.9^{ab}	81.0^{a}	0.60
Energy (MJ/kg)	12.67^{b}	12.56^{b}	13.46^{a}	13.46^{a}	13.55^{a}	0.100

^{a},^{b},^{c} Means not containing a common letter differ (P<0.05).
*Supplied: soy oil, 8.5; free amino acids, 9; and mineral and vitamins, 32.5.
** Provided feedstuffs in amounts identical to those fed in the growth assay.

The results of both the growth assay and the digestibility study clearly show that the smut had no adverse effect on the nutritive value of the barley.

MUNGBEAN (PHASEOLUS AUREUS): AN ALTERNATIVE PROTEIN SOURCE FOR PIGS

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The traditional protein concentrate meals; fish, meat and soybean, are often in short supply. Mungbeans (MB), grown for the culinary bean sprout trade, are becoming increasingly available as stockfeed. While dry beans of the Phaseolus genus, such as Navy bean, are noted for the presence of heat labile antinutritional factors (Tobin and Carpenter,1978), experiments with rats comparing autoclaved and raw MB have shown MB to be free of these factors (Takken, unpublished data). Two experiments were done with pigs to evaluate the nutritive value of raw MB, of chemical composition in g/kg; dry matter 889, crude protein 245, lysine 15.89, threonine 8.37, methionine plus cystine 5.45 and gross energy 16.47 MJ/kg.

In the first experiment the digestibility of MB was determined by the substitution method with eight pigs in a replicated 4 x 4 latin square design. The determined relationships between apparent digestibility and level of MB (X%) in the diet are given by the following equations;

		(±SEb)
Dry matter digestibility	= 87.23 + 0.0706X	(0.0074)
Nitrogen digestibility	= 87.72 + 0.0555X	(0.0069)
Energy digestibility	= 87.00 + 0.0724X	(0.0071)

In Experiment 2, 48 pigs (equal sexes) were allocated to eight diets which were approximately equal in energy (DE) (13.93 - 13.78 MJ/kg) and lysine (8.3- 8.0 g/kg) contents (Table 1). Pigs were fed restrictively from 25 to 85 kg live weight.

Table 1. The average daily gains (ADG), feed conversion ratios (FCR) and carcass performance of pigs in Experiment 2.

Mungbean (g/kg)	0	40	80	120	160	200	240	280	S.E.M.
Soybean (g/kg)	140	120	100	80	60	40	20	0	
ADG (g/d)	621	608	631	607	590	590	577	594	15.2
FCR (g/g)	2.64	2.69	2.59	2.72	2.78	2.78	2.81	2.77	0.06
Dressing (%)	78.5	78.8	79.0	79.7	80.1	79.7	77.7	80.5	0.82
P2 Backfat (mm)	17.7	20.3	16.3	18.2	17.5	16.6	15.7	16.5	1.39

As the dietary inclusion level of MB (X%) increased, ADG declined ($P<0.05$) and FCR deteriorated ($P<0.01$) linearly according to the relationships:

ADG = 622.5 - 1.44X (±SEb, 0.588)
FCR = 2.63 + 0.0654X (±SEb, 0.0022)

Under the conditions of restricted feeding used here, despite its high digestibility and similarity of nutrient contents of all diets, MB did not perform equally to soybean. The downturn in pig performance accompanying increasing MB may have been due to the effect of antimetabolites in MB that were not evident in the rat studies.

Because the downturn in performance is so slight, feeding levels of 10-15% could be used, but high rates of inclusion might benefit from some heat treatment

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INFLUENCE OF HIGH TEMPERATURE AND SKIN WETNESS ON VOLUNTARY ENERGY INTAKE AND PERFORMANCE OF PIGS FROM 50 TO 80 KG LIVE WEIGHT

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Survey data from Australian piggeries indicate that growth performance is adversely affected during summer (Vajrabukka et al., 1983). The effect of high temperature on voluntary energy intake of growing pigs is unclear because few studies have assessed both the climatic environment and pig performance. The aim of this study was to examine the response of pigs to high temperature under controlled conditions.

The influence of skin wetness and high temperature on digestible energy (DE) intake, growth performance and P2, backfat thickness was examined with 36 pigs, housed in individual pens and fed *ad libitum* a protein-adequate diet, containing 13.6 (± 0.04) MJ DE/kg, air-dried basis, from 50 to 80 kg live weight. Three temperature/skin wetness treatments were applied, each containing 12 pigs. One group was held at 22°C, 70% relative humidity and an air speed of 0.2 m/sec. The remaining pigs were held at 32°C at an air speed of 0.4 m/sec, with one group kept as dry as possible at 70% humidity, whilst the other group was allowed access to water sprays at 15 min intervals, which elevated the humidity to 85%. The mean daily DE intake, growth performance and P2 backfat of each group of pigs are presented in Table 1.

Table 1. Mean daily digestible energy (DE) intake, growth performance and P2 backfat thickness of 36 pigs fed *ad libitum* from 50-80 kg live weight.

Temperature °C	Skin wetness	DE intake (MJ/day)	Daily gain (g)	Food conversion ratio	P2 backfat (mm)
22	dry	34.0[a]	922[a]	2.8	20.4[ab]
32	dry	25.8[b]	729[b]	2.6	17.8[b]
32	wet	34.0[a]	901[a]	2.9	23.1[a]
SEM		0.76**	26.0*	0.10	1.14*

Significance *P<0.05, **P<0.01.

High temperature depressed daily DE intake, daily gain and P2 backfat of finisher pigs housed in a dry environment. Wetting of the skin surface at high temperature overcame these effects but there was a non-significant trend to increase P2 backfat compared to the 22°C treatment. This may indicate a depression in protein retention under high temperature.

The increase in air speed from 0.2 m/sec. at 22°C, to 0.4 m/sec. at 32°C would assist heat loss from pigs in both the dry and wet treatments. However, the evidence of Verstegen and van der Hel (1976) suggested that heat loss would be small.

There was close agreement between the observed performance in Table 1 and the predicted performance from the pig growth simulation model, AUSPIG, described by Black et al. (1986) when the 32°C/dry pigs were assumed to have 15% of their skin surface wet.

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COMPOSITION DIFFERENCES IN PIG CARCASS MIDDLES

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Several studies have shown that P2 fat depth is the best single indicator of lean yield in pig carcasses (Kempster and Evans, 1979; Diestre and Kempster, 1985). However, many processors have expressed concern that in some carcasses with satisfactory P2 measurements, excessive amounts of fat occur between the eye muscle and the streak. It has been suggested this is assocated with poor genotypes and severe feed restriction.

This experiment included Large White pigs which had been either selected for growth rate and back fat depth or selected randomly (controls), and were separated by eight generations from a common genetic base. Forty eight each of selected and control pigs were divided into two equal groups which were fed either *ad libitum* or restricted to 2.2 times maintenance from 40 kg until slaughter at 85 kg live weight. The carcasses were split and broken into three primal portions. The middles were further divided into eye, wedge and belly portions by two craniocaudal cuts, the first at the lateral edge of the eye muscle and the second 8.0 cm ventral to the first. The amounts of muscle, fat and bone in each portion were determined by scalpel dissection. Analyses of variance were performed using carcass weight and P2 as covariates.

Although total middle weights were similar at the same carcass weight and P2, there were significant differences in tissue weights due to genotype and feed level (Table 1). Bone was unaffected apart from an increase in the belly of selected pigs (0.61 vs 0.50 kg, $P<0.05$).

Table 1. The tissue weights of middles and portions at the same P2.

	Muscle (kg)				Fat (kg)			
	Total	Eye	Wedge	Belly	Total	Eye	Wedge	Belly
Genotype-selected	10.34[a*]	5.24	1.66	3.44[a]	9.16[a]	3.40[a]	2.22[a]	3.54
control	9.92[b]	5.18	1.64	3.10[b]	9.66[b]	3.84[b]	2.36[b]	3.46
Feeding-restricted	10.44[a]	5.40[a]	1.72[a]	3.32	9.00[a]	3.46[a]	2.18[a]	3.36[a]
ad libitum	9.80[b]	5.00[b]	1 58[b]	3.22	9.84[b]	3.80[b]	2.38[b]	3.66[b]

* In each column within genotype and feeding regime, means with different superscripts are different ($P<0.05$).

Of the total muscle in the middle, selected pigs had proportionately more in the belly (334 vs 312 g/kg, $P<0.01$) and less in the wedge (160 vs166 g/kg, $P<0.05$) and eye (506 vs 522 g/kg, $P<0.01$) portions. Of the total fat in the middle, selected pigs had proportionately more in the belly (388 vs 358 g/kg, $P<0.01$) and less in the eye (370 vs 398 g/kg, $P<0.01$). Genotype affected neither the proportion of fat in the wedge nor the dorsoventral distribution of bone. Feed level had no effect on tissue distribution apart from a small decrease of muscle in the belly (318 vs 328 g/kg, $P<0.05$) with restricted feeding.

Based on these data we conclude that, at the same P2 measurement, the middles of unimproved pigs can be inferior to those of selected animals. This is more likely to be due to differences in muscle of the belly portion rather than fat in the wedge. Restricting feed intake to the level used in this experiment can improve the quality of carcass middles such that they would be undervalued relative to pigs fed *ad libitum*.

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SIMULATION OF THE DAILY PARTITIONING OF AMINO ACIDS IN THE 50 KG LIVE WEIGHT PIG

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A computerised model simulating the fate of absorbed amino acids in the 50 kg live weight pig has been developed and can be used to determine the daily amino acid requirements of pigs growing at different rates. The inputs required to drive the model programme are: the sex of the animal; genotype; dietary energy density and dry matter content; daily whole body protein and lipid retention rates. Daily food dry-matter intake is calculated on the basis of the digestible energy required to drive body maintenance processes and protein and lipid deposition. There is an upper-limit to digestible energy intake for each sex. Lipid deposition rate is constrained to be $\geq$ protein deposition rate, such that the preferential breakdown of amino acids for energy supply will be minimal. The model calculates the amount (Ri) of an absorbed amino acid required to support a specified level of production: $Ri = \frac{Si + Ci + Gi + Di}{ei}$,where Si denotes the loss of the amino acid via deamination and excretion in the urine, resultant upon inefficiency in whole-body protein turnover; Ci is the loss of the amino acid through the continual shedding of skin and hair; Gi represents loss of the body amino acid from the gut; Di is the amount of the amino acid deposited during growth; and ei describes the efficiency of utilization of the i^{th} absorbed amino acid in meeting the combined needs of maintenance and growth. It is assumed that: (1) Body protein turnover rate is a linear function of the level of protein deposition; (2) Integumental amino acid losses are related to metabolic body weight ($W^{0.75}$); (3) Gut amino acid losses are a function of food dry-matter intake; (4) The efficiency of utilization of an absorbed amino acid is a curvilinear function of body protein deposition rate. Results from the model agree with empirically-derived estimates of requirements given in the literature. The predicted daily requirements for lysine and methionine + cystine for the 50 kg live weight pig, at varying levels of body protein deposition (Pd) but a set ratio of lipid deposition (Ld = 1.5 Pd) and the relative importance of the various underlying physiological processes, are shown in Table 1.

Table 1. Predicted lysine and methionine + cystine requirements related to various physiological processes in the 50 kg live weight pig.

	Requirement (g absorbed amino acid/day)						
	Turnover Loss	Gut Loss	Integument Loss	Pd	Total Net	Utilization†[1]	Total
Lysine							
Pd = 0 g/d	0.46	0.15	0.08	0	0.69	0	0.69
73	0.63	0.56	0.08	4.81	6.08	0.64	6.72
145†[2]	0.80	1.00	0.08	9.61	11.49	6.79	18.28
Methionine+cystine							
Pd = 0 g/d	0.32	0.25	0.06	0	0.63	0	0.63
73	0.43	0.54	0.06	2.34	3.37	0.59	3.96
145	0.54	0.80	0.06	4.68	6.08	5.42	11.50

†[1] Requirement due to inefficiency of utilization of the absorbed amino acid for protein synthesis
†[2] Lipid deposition (Ld) = 1.5 Protein deposition (Pd) except for Pd = 145 g/d, where Ld = 1.3 Pd

The amount of an amino acid required to meet the losses from maintenance processes appears to be a considerable proportion of the total net requirement, even at high levels of body protein deposition, thereby challenging the classical assumption of regarding whole-body amino acid composition as "ideal".

UTILIZATION OF RAW SUGAR BY GROWING/FINISHING PIGS

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Raw sugar is used efficiently as an energy source for growing/finishing pigs. In fact, feed conversion efficiency is often improved when sugar-based diets are compared with cereal-based diets (Schumacher et al.,1986). It is not clear whether this is a response to inclusion of sugar, change in digestible energy (DE) content or the improvement in amino acid balance. The aim of this trial was to determine the response of pigs to inclusion of sugar in iso-energetic diets.

Four diets were formulated using sugar to gradually replace wheat as the energy source in the diet. The diets contained 0, 200, 400 or 600 g/kg sugar,15 MJ DE/kg and 0.64 estimated available lysine/MJ DE. Soybean meal was the only protein source used. Lysine content was decreased (0.51 g/MJ DE) at 50 kg live weight in line with decreased requirements. Twenty pigs (10 male, 10 female) were fed each diet *ad libitum*, from 20-80 kg live weight. Nitrogen and energy retention for each pig was determined by comparative slaughter.

Since sugar inclusion increased dressing percentage (Table 1), daily gain and feed conversion ratio were compared on a carcass basis. There was no adverse response to inclusion of sugar and live weight gains were high (c. 950 g/d). In fact, as well as an improvement in dressing percentage, feed intake and efficiency of energy and fat retention increased with sugar inclusion.

Table 1. Effect of sugar inclusion on growth and nutrient utilization

			Carcass basis			Retention		
Sugar in diet (g/kg)	Soybean meal (g/kg)	Feed intake (g/d)	Dressing percent	Gain (g/d)	FCR	Protein (g/MJ DE) intake	Energy (MJ/MJ DE) intake	Fat (g/MJ DE)
0	268	2.07	81.8	791	2.63	4.1	0.425	8
200	295	2.24	82.6	834	2.69	3.6	0.442	9
400	321	2.26	83.4	845	2.69	3.9	0.451	9
600	348	2.23	83.8	833	2.69	3.8	0.449	9
S.E.M.		.052	.028	16.1	.038	0.13	0.0091	0.2
Linear resp. to sugar		*	**	ns	ns	ns	*	*

* and **: P< 0.05 and P <0.01, respectively.

The response to sugar inclusion may be due to differences in the net energy value of the diets. Sugar-based diets contain less fibre and less excess crude protein, and net energy content may be increased. However, much of the response may simply reflect increased feed intake at all levels of sugar inclusion. Nutrient retention values reflect the efficiency of depositing increased energy intake as fat. A comparison of diets fed restrictively would separate these effects.

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ERRORS IN AMINO ACID ANALYSIS

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INTRODUCTION

Collaborative analytical studies on common samples, e.g. Sarwar et al. (1983), have shown inter-laboratory differences to be the most important source of error in amino acid analysis. There have been no similar Australian studies. This paper examines five pairwise comparisons between four Australian and one other laboratory against Sarwar's data, and discusses likely source of bias.

RESULTS AND DISCUSSION

Data sets showing the smallest and largest differences among five pairwise comparisons are shown in Table 1. In Sarwar's study two thirds of inter-laboratory differences lay between about 0.92 and 1.08. On this criterion some Australian differences appear unacceptably high.

Table 1. Ratio of amino acid levels (g/kg) in two of five data sets (smallest and largest differences in pairwise comparisons).

Comparison	Amino acid								
(sample)	lys	met	thr	ile	arg	his	leu	phe	val
A (casein)	1.02	0.96	0.99	1.04	1.03	1.01	1.01	1.01	1.04
B (lupin)	0.34	1.18	1.35	1.15	1.19	1.01	1.02	1.10	0.46
B (meat meal)	0.84	1.13	1.26	0.41	1.11	0.79	0.99	1.03	0.90

Likely sources of bias of the reference analytical method are discussed under the following headings:

1. sampling error and preparative effects;
2. chromatography (calibration standards and chromatography of other amino acids);
3. non-linear responses;
4. effect of changing resolution;
5. calculation and results (internal standard correction, hydrolysis losses and options for result presentation).

CONCLUSIONS

1. Limited evidence strongly suggests a need to reduce inter-laboratory differences in amino acid analysis by improving accuracy.
2. It is equally important that clients of amino acid services take a collaborative and interpretive approach to these analyses.

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A PROPOSED METHOD FOR DETERMINING AMINO ACID AVAILABILITY IN PIG DIETS

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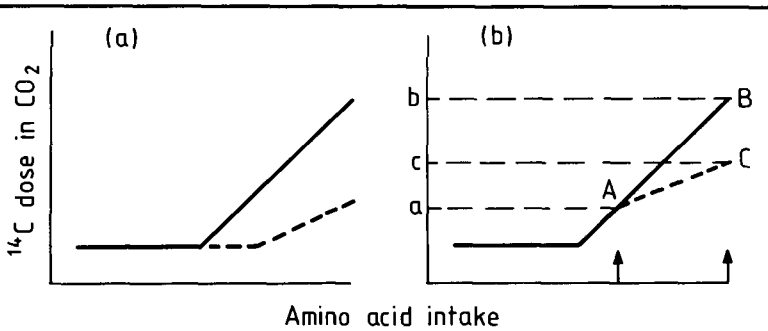
The accuracy with which diets can be formulated to meet specified requirements for available amino acids is severely limited by inadequate knowledge of amino acid availability which varies widely both between and within ingredients. Amino acid availability is currently assessed by chemical or microbiological assays, measurement of amino acid disappearance from the small intestine or growth studies, but all methods have limitations. A procedure for determining the availability of all essential amino acids that is accurate, rapid, uses few animals and that is independent of animal type or environment is required urgently. The following is proposed for evaluation.

Dietary amino acids that are absorbed from the intestine and are not complexed with other compounds such as deoxyketosyl-lysine enter the free amino acid pool of the body and are available for metabolism. When a tracer dose of ^{14}C labelled amino acid is given in the diet, it mixes with the free amino acid pool and may be assumed to enter the various metabolic pathways in the same proportion as the unlabelled amino acid entering that pool. When the supply of an amino acid for protein synthesis changes from a deficit to an excess, there is a major increase in its oxidation and thus in the proportion of the labelled dose appearing as CO_2 (Fig. a). The shape of the relationship between $^{14}CO_2$ excretion and amino acid intake will vary depending on the availability of amino acid, with the increase in $^{14}CO_2$ excretion occurring at a higher intake and the slope of the relationship being less for dietary sources with low amino acid availability (Fig. a). If free amino acid added to a diet is assumed to be totally available, an estimate of the availability of an amino acid in a test protein can be obtained by calculating (c-a)/(b-a) in Fig. b. That is, an increase in $^{14}CO_2$ excretion (c-a) following an incremental increase above requirement in the intake of the amino acid from the protein expressed as a proportion of the increase (b-a) resulting from the same increment of free amino acid. The following conditions must be satisfied, (i) the amino acid is supplied in excess of requirement, (ii) the concentration of the amino acid in the free pool and the proportion of amino acid following each metabolic pathway do not change substantially with increases in amino acid intake, and (iii) the feeding regime is such that the free amino acid is used with complete efficiency. Offering a basal diet (A) and the same amount of the amino acid given either in the free form (B) or as a protein (C) in meals every 0.5 to 1 hr should approximate these requirements (Fig. b). Three groups of animals could receive the three diets in a latin square design over 4 to 7 day intervals.

The major advantages of this proposal are that results are independent of either animal type or environmental conditions because measurements are made when the amino acid is in excess of requirement and few animals are used for a short time.



Relationship between ^{14}C dose excreted in CO_2 and amino acid intake.

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PRELIMINARY REPORT OF A RAPID ASSAY FOR THE MEASUREMENT OF PROTEIN DIGESTION TO THE ILEUM IN PIGS

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There are many studies of the digestibility of amino acids to the ileum. However determination of amino acid availability by ileal digestibility requires ileal cannulation and is time consuming.

Sauer et al. (1983) developed a rapid method for the estimation of digestible energy and protein in feeds using a mobile nylon bag. The bags were inserted in the duodenum and collected in the faeces. The present experiment reports on a nylon bag technique as a rapid assay for the digestibility of protein to the ileum.

Four pigs of approximately 25 kg were fitted with T-shaped cannulae in both the duodenum and ileum and two pigs were fitted with cannulae in the stomach. The pigs were given 1.0 kg of commercial feed daily from a continuous belt feeder.

Nylon bags (pore size 44 μ) were made from pouches of material 70 mm in diameter. The bags were filled with 0.5 g of the test material and tied with surgical silk leaving the ties 100 mm in length.

The bags were incubated in the stomach for 6 hours, removed and then placed in the duodenum of another pig. The bags were collected at the ileum as they appeared, usually in 3 to 4 hours. The bags were withdrawn by pulling on the silk. During collection the ileal cannulas were fitted with removable slides to divert the flow of digesta to the exterior. Other bags were allowed to flow through the tract and collected in the faeces, usually in 36 to 48 hours. Between 4 and 6 bags were placed in the duodenum of each pig on any one day. The bags were inserted at 5 minute intervals.

Table 1. Apparent digestibility of protein supplements in nylon bags.

Source	Meat meal	Milk	Cottonseed meal	S.E.M.
In stomach	0.12	0.22	0.05	0.020
To ileum	0.81	0.98	0.60	0.020
Whole tract	0.88	1.00	0.69	0.018

Preliminary results from 8 bags of each protein source at each site are given in Table 1. These results are in general agreement with reported values for the digestion of protein in meat meal, cottonseed meal and milk to the ileum (Alimon and Farrell, 1980; Sauer and Ozjmek, 1986).

The results of this study will be validated by comparing the results from the nylon bag technique with results of the ileal digestibility of the same protein sources.

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THE INFLUENCE OF HIGH TEMPERATURE AND DIETARY FAT ON THE GROWTH PERFORMANCE OF YOUNG PIGS

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There is little information on the effect of high environmental temperature on the feed intake and growth performance of early-weaned pigs. Increasing the nutrient density of the diet by including fat may reduce the depressive effect of high temperature on appetite (Stahly and Cromwell, 1979). The inclusion of fat has improved growth performance in some studies (Campbell et al., 1975) but not in others (Lawrence and Maxwell, 1983). The aim of the present experiment was to assess the influences of high environmental temperature and dietary fat level on the performance of pigs growing from approximately 8 to 22 kg.

A total of 78 pigs, weaned at 25-27 days of age, were allocated either to an initial slaughter group (6 pigs) or among eight factorially arranged treatments comprising two environmental temperatures, 22 and 32 °C, and four dietary levels of tallow, 0, 30, 60 and 90 g/kg. Room temperatures were maintained within ±1°C ; air speed varied between 0.15 and 0.30 m/sec. All diets were formulated to contain 0.7 g available lysine per MJ digestible energy (DE). Animals were penned individually in two rooms maintained at the prescribed temperatures and feed was provided *ad libitum*. Three pigs from each treatment were slaughtered on reaching 22 kg live weight to provide information on body composition.

Table 1. Effect of environmental temperature and dietary fat level on rate and efficiency of weight gain.

		Growth rate (g/d)	Feed intake (g/d)	DE intake (MJ/d)	Feed:gain	DE intake:gain (MJ/kg)
Temperature						
22°C		539	982[a]	14.61	1.83[a]	27.13
32°C		510	899[b]	13.65	1.77[b]	26.80
Level of significance		n.s.	*	n.s.	*	n.s.
Fat level (g/kg)	DE (MJ/kg)					
0	13.9	511[bc]	1005[a]	13.95	1.96[a]	27.28
30	14.6	502[c]	935[bc]	13.67	1.86[b]	27.22
60	15.7	546[a]	953[ab]	14.77	1.75[c]	27.06
90	16.3	538[ab]	870[c]	14.14	1.62[d]	26.29
Level of significance		**	**	n.s.	***	n.s.

Within columns, means with same superscript did not differ significantly (P>0.05).

The higher temperature resulted in a reduction in feed intake (8%) and an improved feed:gain ratio; growth rate tended to decline (P<0.10). Feed intake decreased with increase in fat level such that DE intake remained relatively constant across treatments. Inclusion of 60 g fat/kg resulted in 7-9% faster growth than lower fat levels. Feed:gain ratio improved approximately linearly with increased fat level; DE intake:gain showed a similar trend but treatment differences were not significant. All interactions between temperature and fat level were non-significant. Chemical composition of empty body at 22 kg was not affected by temperature or fat level.

For pigs between 8 and 22 kg live weight, a temperature of 32°C exerted only a mild adverse effect on growth rate performance. Inclusion of 60 and 90 g tallow /kg, which raised dietary DE

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content to 15.7 and 16.3 MJ/kg, respectively were beneficial.

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VOLUNTARY FEED INTAKE IN THE YOUNG GROWING PIG (10 TO 20 KG LIVE WEIGHT)

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Published estimates of voluntary feed intake of young growing pigs given continuous access to the feed are sparse. Yet such basic information is needed to enable modelling of growth and the interpretation of measurements of tissue deposition in the young growing pig. There is evidence (Campbell et al., 1975) that voluntary feed intake in the young growing pig is controlled by gut capacity up to a dietary energy density of around 15.0 MJ DE/kg and thereafter by the digestible energy concentration of the diet, as in the older animal. This investigation aimed, therefore, to determine the relationship between daily dry matter intake and live weight over the range 10 to 20 kg.

Twelve entire male and twelve female Landrace x (Landrace x Large White) pigs of around 8 kg live weight and four weeks of age were used in each of two separate studies. The pigs, selected at random from a weaner pool, were individually penned in a controlled temperature room (24 $\pm$ 1 °C) and fed a diet containing 13.74 MJ DE and 11.6 g total lysine/kg *ad libitum* with free access to water. Following a seven-day acclimatization period live weight and feed intake were recorded every four days until live weight approximated 20 kg. The pigs appeared healthy throughout and grew, on average, at 561 g and 585 g/day in the first and second studies, respectively. Sex of pig did not influence appetite or growth rate in either study.

Linear regression equations relating daily dry matter intake (DMI) to liveweight (LW) for all pigs in each study were:

Study 1 $DMI_{(kg)} = 0.048LW + 0.183$ (R.S.D. = 0.139; $R^2 = 0.59$)

Study 2 $DMI_{(kg)} = 0.072LW + 0.064$ (R.S.D. = 0 .204; $R^2 = 0.55$)

The regression lines differed significantly in slope ($P < 0.01$). Fitting of quadratic and exponential equations to the data did not improve the accuracy of prediction. Correlation coefficients between daily dry matter intake and liveweight, determined for individual pigs in each study, showed considerable inter-animal variability. This finding, combined with the significant difference between the two studies in the rate of increase in voluntary dry matter intake with increasing liveweight, highlights the inherent difficulties in predicting voluntary food intake in the pig postweaning. Further work is in progress to evaluate the robustness of the present equations and to determine whether or not the relationship is influenced by the energy density of the diet.

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CITRIC ACID AS A DIETARY ADDITIVE FOR YOUNG PIGS

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Citric and other organic acids have been shown to reduce piglet diarrhoea and to improve postweaning piglet performance when used at rates of 15-30 g/kg diet (Falkowski and Aherne 1984; Henry et al., 1985). An experiment was conducted to compare diet acceptance, scouring incidence and growth performance of suckling and weaned pigs given diets without or with citric acid at 25 g/kg.

The basal diet contained (g/kg): wheat, 557; barley, 195; tuna meal, 42; meat meal, 83; soybean meal, 83; soy oil, 30; lysine HCl, 2.5; and a mineral/vitamin premix, 7.5. The chemical composition of the diet was estimated to contain (g/kg): Ca, 11; total P, 9; crude protein, 200; total lysine, 11.2; methionine plus cystine, 5.8; threonine, 6.6; and digestible energy (MJ/kg),14.3. The citric acid diet was prepared by adding the citric acid at the expense of the grains. The diets were offered *ad libitum* to the piglets from seven days of age through to four weeks post-weaning. The treatments were each applied to a total of 15 litters. The severity of scouring was judged on a four point scale (0=no scouring; 3=severe scouring with more than half of the litter affected) and assessed daily. Results are shown in Table 1.

Table 1. Performance of piglets given diets without (B) or with (C) citric acid.

Attribute	B diet	C diet	S.E.M.
		Suckling	
No. born/litter	10.7	10.2	0.50
No. weaned/litter	9.2	8.9	0.39
Weaning age (d)	37.2	37.2	0.51
Total feed consumed/pig (kg)	0.72[b]	1.58[a]	0.203
Growth rate to weaning (g/d)	262	243	11.9
Scouring score*	0.2	0.2	0.04
Sow weight change in lactation (kg)	-4	3	4.7
		Postweaning	
Daily feed consumed/pig (kg)	0.78	0.81	0.024
Growth rate (g/d)	378	394	11.7
Feed conversion (g:g)	2.07	2.06	0.046
Scouring score*	0.2	0.2	0.06

[a],[b] Means are significantly different ($P<0.05$).
*Four-point scale from 0 (no scouring) to 3 (severe scouring).

The addition of citric acid to the diet at 25 g/kg neither had an undesirable effect on diet palatability nor did it support any improvement in piglet growth rate. Suckling pigs ate more of the acidified diet than the basal diet. In the absence of any associated improvement in growth rate, this higher intake of the acidified diet may have been to compensate for a lower sow milk production. Differences in sow weight change during lactation between treatment groups are consistent with this view.

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CHOICE FEEDING OF PIGS

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Poultry have the ability to balance their protein and energy needs when offered the choice between protein and energy supplements (Cumming, R.B. personal communication). However, the responses depend on factors such as position of the feed trough, energy concentration of the supplements and mineral and vitamin supplements. Choice feeding could have application to pigs as it would simplify the problem of supplying the different requirements of males and females and declining protein to energy needs with increasing live weight (Batterham et al., 1985).

Two diets were formulated from wheat, soyabean meal and minerals and vitamins to either an estimated 0.64 or 0.57 g available lysine/MJ digestible energy for males and females, respectively. Pigs were offered these diets complete or with the cereal base and soyabean meal given in separate troughs. There were 10 pigs per treatment and the results were assessed over the 20 to 45 kg growth phase.

Most pigs offered the soyabean meal as a separate supplement learnt to adjust their intakes of the cereal base and protein supplement within the first week. However, whilst there were no differences in total feed intakes, the choice-fed pigs consumed more cereal and less soyabean meal than the pigs given the complete diets ($P< 0.01$). As a consequence, performance of the choice-fed pigs was inferior to that of those fed the complete diets ($P<0.01$).

Table 1. Response of growing pigs offered a complete diet or a choice between the cereal base and the protein concentrate

	Complete diets		Choice feeding		S.E.M.
	Males	Females	Males	Females	
Gain (g/d)	885	860	685	650	40.0
Feed intake (g/d)					
Total	1.72	1.86	1.75	1.76	0.059
Cereal base	1.17	11.36	1.50	1.54	0.056
Soyabean meal	0.55	0.50	0.25	0.22	0.034
Feed conversion ratio	2.0	2.2	2.7	2.9	0.16
P2 fat depth (mm)	13	15	17	17	0.8

One interesting aspect of the experiment was the variability between pigs in their acceptance of the soyabean meal as a separate supplement. Most pigs readily consumed it. However, three male and three female pigs were reluctant to eat the soyabean meal and over the whole experiment their growth performance was markedly inferior to those pigs fed the complete diets. However, even when the results of these pigs were deleted from the statistical analyses, the performance of pigs choice fed was inferior ($P<0.01$) to those given the complete diets (746 and 756 gain (g/d) for the male and female choice-fed pigs, respectively).

The results indicate that feeding the cereal base and soyabean meal as separate supplements is not as efficient as feeding the nutrients as complete diets for grower pigs.

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THE EFFECT OF FIBRE AND FAT ON SOME PHYSICAL CHARACTERISTICS OF THE GUT OF GROWER PIGS

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High levels of fibre in the pig diet affect the carcass and gastrointestinal tract (GIT) in various ways. For example, when compared to pigs on lower fibre diets, dressing percentage may fall because of increased gut fill and heavier GIT (Kass et al., 1980). During the course of a recent experiment, the effects of fibre and fat on some gut characteristics were studied.

Grower pigs (20-60 kg) were fed two sets of diets, formulated to contain either a moderate level of fibre,MF (~50 g/kg crude fibre,) or a high level, HF (~90 g/kg crude fibre). Within each level of fibre six levels of fat (0-125 g/kg tallow) were included. There were six pigs per treatment. The major source of fibre was oat hulls, added to a barley-based diet.

Upon reaching 60 kg, the pigs were fasted for 12 to 15 hours and slaughtered at a commercial abattoir. The following measurements were recorded: full GIT weight; empty weights of the stomach, small intestine, and caecum; and dressing proportion.

Table 1. Effect of dietary fat and fibre level on mean GIT weights(g) and dressing proportion (n=6).

	Fat level(g/kg)						Fibre level(CF)		S.E.M.	Signif.	
	0	25	50	75	100	125	50g/kg	90g/kg		fat	fibre
Stomach	452	425	447	434	460	439	419	466	18.8	NS	*
Caecum	104	107	102	97	97	91	99	100	5.7	NS	NS
GIT	3046	3126	3106	3001	3150	3198	3031	3184	121.2	NS	*
GIT Contents	2999	2782	2921	2362	2355	2355	2544	2714	190.1	**	NS
Dressing Prop.(g/kg)	732	732	735	743	741	735	740	732	71	NS	*

* $P<0.05$;** $P<0.01$

There was no significant effect of either fat or fibre on the weight of the caecum or small intestine. The higher fibre diets resulted proportionally in a 0.08 increase in empty GIT weight($P<0.05$), partly because of 0.11 increase in empty stomach weight($P<0.01$). Stanogias and Pearce (1985) also found a positive relationship between dietary fibre content and stomach weight. There was no difference in gut content weight between fibre levels, but HF diets led to a reduction of 0.8 units in dressing proportion, with approximately one third of this difference due to the heavier GIT. As fat level rose, gut content decreased significantly ($P<0.01$). This was associated with a progressive decrease in feed intake as dietary fat content increased.

It is concluded that fibre level altered the weights of the stomach and total gut, and therefore dressing proportion.

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THE VALUE OF DIETARY LUPIN KERNEL MEAL FOR PIGS

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Lupin kernel meal (LKM) results from the mechanical removal and separation of the testa of sweet lupin seed (*Lupinus angustifolius*). This process increases the crude protein content from 300 g/kg in lupin seed to 380 g/kg in LKM, crude fibre is reduced from 159 to 37 g/kg and the digestible energy (DE) concentration is increased from 14.4 MJ/kg to 16.4 MJ/kg. Two experiments were conducted with growing pigs to compare LKM with soyabean meal (SBM) as main ingredients of diets based on wheat and barley.

In the first experiment 60 pigs were fed diets containing LKM, SBM or a mix of both, either *ad libitum* or at 3.2 times maintenance, from 30 to 88 kg live weight. By calculation, the three diets contained 14.2 MJ DE/kg throughout the experiment, and 0.75 and 0.60 g of total lysine/MJ DE during the growth phases 30-53 and 53-88 kg live weight, respectively. For the LKM, LKM + SBM and SBM dietary treatments, the mean growth rates, daily feed intakes, feed:gain ratios, carcass dressing and percentages and P2 fat depth measurements were 782, 792 and 798 g/day (S.E.M. = 21); 2.24, 2.24 and 2.25 kg (S.E.M. = 0.03); 2.89, 2.83 and 2.85 (S.E.M. = 0.08); 68.7, 69.7 and 69.4 (S.E.M. = 0.5); and 16.1, 15.8 and 16.5 mm (S.E.M. = 0.8) respectively. None of the differences between treatments were significant for any of these parameters.

In the second experiment, 12 dietary treatments consisted of supplementing isocaloric diets containing 262 g/kg of LKM (0.80 g total lysine/kg) or 165 g/kg of SBM (0.81 g total lysine/kg) with nil or five equal increments of L-lysine HCl to a total lysine concentration of 0.75 g/MJ DE at the highest level of supplementation. The concentrations of all other essential amino acids were calculated to be adequate relative to DE. Each diet was fed *ad libitum* to seven entire male pigs during the live weight interval 20-52 kg. Pigs fed the LKM diets grew slower than those fed the SBM diets (738 vs 799 g/d, $P<0.01$). Growth rate increased with increasing lysine levels to a maximum at 0.68 and 0.65 g total lysine/MJ DE for the LKM and SBM diets, respectively. Further lysine additions led to growth depresions with both diets.

Although the LKM and SBM diets were both formulated to contain 13.77 MJ DE/kg, DE values were subsequently measured using pigs in metabolism crates and found to be 13.87 and 14.32 MJ/kg, respectively. Further, the mean daily feed intake for pigs fed the LKM diets was 1.70 kg compared to 1.81 kg for those fed the SBM diets. The superior performance of pigs fed the SBM diets can therefore be largely explained in terms of their higher energy intake. When lysine concentration is calculated as a proportion of the determined DE values, it is evident that maximum growth rates were achieved at total lysine levels of 0.63 and 0.68 g/MJ DE for SBM and LKM diets, respectively. These values are less than those reported by Campbell et al. (1985) who demonstrated a growth response plateau at 0.78 g total lysine/MJ DE.

On the assumption that, with both LKM and SBM diets, growth rates would plateau at the same concentration of available lysine relative to DE, our results indicate that lysine availability in LKM exceeds 0.7 (available/total lysine). Batterham et al. (1984) found lysine availability in lupin seed meal to be 0.53 which suggests the seed testa may have an adverse effect in this respect. The results also suggest there could be genotype differences in the lysine requirements of growing pigs.

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DETERMINATION OF PROTEIN AND ENERGY IN PIG CARCASSES USING NEAR INFRA-RED REFLECTANCE SPRECTROPHOTOMETAY

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The protein and energy content of pig carcasses are used to more accurately describe the response of growing pigs to nutritional treatments. However, these analyses by conventional methods are time consuming and expensive. Near infra-red (NIR) analysis could have application as a rapid and inexpensive method of analyzing for these parameters.

Pigs from a series of nutrition experiments were slaughtered and samples of carcass, blood and viscera (with empty gastro-intestinal tract) were collected. These samples were then frozen, minced, mixed, sampled and freeze-dried. The freeze-dried sample was then ground so that particle size between samples was uniform. It was difficult to grind samples from larger fattier pigs to uniform particle size and there was some variation in composition of carcass samples from 80 kg pigs. One hundred and thirty five pigs (20-80 kg live weight) were used to prepare carcass samples with hair (nitrogen (N)=35-71 g/kg, gross energy (GE)=24.5-33.4 MJ/kg) and blood/viscera samples (N=56-113 g/kg, GE=22.1-30.2 MJ/kg). Fifty pigs (20-50 kg liveweight) were used to prepare carcass samples (hair removed) (N=55-112 g/kg, GE=24.4-30.4 MJ/kg) and viscera samples (N=77-99 g/kg, GE=23.7-27.9 MJ/kg). All samples were analyzed for nitrogen by macro kjeldahl technique and gross energy with a Parr adiabatic bomb calorimeter.

The samples were then used to determine prediction equations for nitrogen and gross energy with a Technicon 400 Infraalyzer. Results of the calibrations were as follows.

Table 1. Comparison between laboratory analyses and predicted values using NIR spectrophotometry for nitrogen and energy content of pig carcasses.

Sample	No. samples	Residual standard deviation	Correlation co-efficient	F-ratio	Standard error
Nitrogen					
Carcass	50	0.072	0.996	349.1	0.083
Viscera	50	0.116	0.982	179.9	0.132
Carcass with hair	135	0.173	0.985	582.6	0.177
Blood/viscera	135	0.195	0.991	858.5	0.201
Energy					
Carcass	50	0.318	0.971	61.2	0.375
Viscera	50	0.128	0.994	198.9	0.151
Carcass with hair	135	0.426	0.975	254.9	0.441
Blood/viscera	135	0.298	0.987	747.3	0.305

The results show that there was good correlation between laboratory analyses and predicted values using the infraalyzer. The correlation co-efficients and F-ratios were high and residual standard deviations and standard errors were low. The physical nature of samples did not appear to interfere with Infraalyzer readings.

NIR analysis has potential as a rapid and inexpensive method for analysing carcass samples. The validity of the NIR technique should be confirmed using an additional population of carcass samples.

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THE IMPLICATIONS OF IMMUNOLOGICAL TECHNIQUES FOR THE IMPROVEMENT OF PRODUCTION EFFICIENCY IN PIGS

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INTRODUCTION

Immunology, one of the more recent specialist disciplines within the science of physiology, has been responsible for spectacular improvements in herd health over the past few decades. It is probably not an overstatement to claim that without these advances in immunology, our modern systems of intensive animal husbandry would not be possible. The initial benefits of immunology were principally in the area of herd health through the availability of vaccines for the prevention of infectious diseases of bacterial and viral origin. Over the past 10 years, through the results of continuing research, we have come to recognize that applications arising from immunology have far wider ramifications for animal production than the prevention of infectious disease (Scaramuzzi and Hoskinson, 1984; Quirke, 1985).

The immune system, the body's main defence against infectious disease, has the capacity to recognize and inactivate a seemingly infinite number of molecules. These molecules, usually proteins or large complex carbohydrates, must also be recognized as foreign to the host organism, that is as non-self, and must be of sufficiently large size to evoke an immune response. Compounds of low molecular weight and/or endogenous compounds normally do not activate the immune system. Many of the steroid and peptide hormones required for reproduction, growth, lactation and the maintenance of homeostasis are of low molecular weight and not species specific; they therefore are not immunologically active. However, immunochemical techniques have been developed over the past 50 years which now allow us to overcome these normal limitations and low molecular weight, endogenous substances can now be made immunologically active (Landsteiner, 1936; Lindner et al., 1972). The immune system can now be stimulated to recognize these molecules as foreign and to produce specific antibodies against them. By using these techniques, we now are able to induce auto-immunity against a range of hormones and produce a variety of physiological responses which can be commercially exploited in animal production.

Antibodies to hormones have the capacity to bind circulating endogenous hormone and as a consequence produce a number of general effects. The ability of the hormone to interact with specific receptor binding sites on target cells may be reduced, or in some cases completely prevented, thereby producing symptoms of a hormonal deficiency (Scaramuzzi, 1975). The metabolism of the hormone may be altered leading to a marked reduction in the metabolic clearance rate (Nieschlag and Wickings, 1978) and hence to elevated blood concentrations of the hormone (Scaramuzzi and Hoskinson, 1984). Depending on the relative binding affinities of the hormone to its receptor and to the circulating antibody, immunization may even lead to prolonged hormonal stimulation (Caldwell et al., 1971; Hillier and Cameron, 1976). Which, if any, of these effects is observed will depend on the properties of the hormone, the type of immunoadjuvant used, the binding affinity of the hormonal antigen to its target cell receptors and the specificity and affinity of the immune response.

Even more promising developments are emerging from research laboratories. The recent description of anti-idiotype antibodies to insulin (Sege and Peterson, 1978) and to the ß-adrenergic antagonist alprenolol (Schrieber et al., 1980) raises the prospect of using these classes of antibodies to modify animal production. The potential applications of anti-idiotype antibodies has been recently reviewed (Farid and Lo, 1985). The ready availability of monoclonal antibodies may lead

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to the use of passive immunization as a practical and cost-competitive alternative to active immunization with the additional benefit of a more precise control of circulating antibody levels in the immune animal. The first requirement is to obtain myeloma cell lines derived from the various domestic species of interest. The fusion of rodent myeloma cells with lymphocytes derived from domestic animals to produce a cross-species hybridoma cell is possible, but the clones established from cross-species fusions tend to be unstable and unreliable. The immunoglobulins produced by a hybridoma cell line will be homologous only for the species from which lymphocytes, used for hybridoma formation, were derived. Monoclonal antibodies from rodent hybridoma clones will therefore be heterologous in farm animals and will most likely produce anaphylatic reactions, especially with repeated dosage, in treated animals.

Antibodies to hormones have been used in a number of ways over the past few years: To increase twinning rate (Scaramuzzi and Hoskinson, 1984); to block the effects of toxins (Cox and Braden, 1974); as a contraceptive (Talwar, 1986); to improve growth (Spencer et al.,1983); to alter body composition (Schanbacher, 1984); and to control libido and aggression (Robertson et al., 1982). While all of these actions have been demonstrated under experimental conditions, most of them have not yet been developed into practical technologies, and none have been specifically developed for the pig industry.

This review will briefly cover aspects of animal production which may be enhanced by immunophysiological methods, with special reference to the pig industry and its problems.

INCREASING LITTER SIZE

Litter size in the various species of domestic animals can be increased by increasing ovulation rate (the number of ova released at ovulation) or by reducing embryonic wastage. Immunity to a number of ovarian steroid hormones will increase ovulation rate in sheep (Scaramuzzi, 1976; Scaramuzzi et al., 1977), cattle (Wise and Schanbacher, 1983; Hoskinson et al., 1986b) and rabbits (Armstrong et al., 1978). The increase in ovulation rate in sheep is usually followed by a somewhat smaller increase in lambing rate (Figure 1) because of the increased level of embryonic mortality associated with increased ovulation rate (Hanrahan and Quirke, 1985). Recently a commercial product, Fecundin (Glaxo Animal Health, Harefield, Middlesex, U.K.), comprising an ovarian steroid, androstenedione, linked chemically to a carrier protein (human serum albumin) was released for commercial use in sheep (Scaramuzzi et al., 1983; Geldard et al., 1984a, 1984b). Immunization with Fecundin leads to the production of antibody against androstenedione and associated increases in ovulation rate and lambing rate. The increase in lambing rate varies widely according to breed, body weight and the antibody titre at mating (Scaramuzzi et al., 1983). An average of 25 extra lambs per 100 ewes mated with a range of about 8 to 40 (Scaramuzzi et al., 1983; Geldard et al., 1984a, 1984b) could be considered typical.

Litter size in pigs is also determined by ovulation rate although, unlike the ewe, it has been assumed that the sow produces more ova than she is capable of maintaining during pregnancy and through to parturition. Historically a curvilinear relationship between ovulation rate and litter size has been assumed with maximum litter size in pigs occurring at 12-14 ova (Hughes and Varley, 1980; Blichfeldt and Almlid, 1982). Low ovulation rates were therefore not generally recognized as a significant problem in pig production as sows generally have ovulation rates of greater than 12-14 ova. However the results of recent comprehensive studies on the relationship between ovulation rate and litter size have been reviewed by Johnson et al. (1985) and indicate that maximum litter size occurs at ovulation rates of greater than 18 ova. Furthermore King (1987) has collated data on the ovulation rates of gilts and first litter sows in Australia (Table 1) which showed that the ovulation rate of young sows was low and likely to be limiting litter size. Consequently, methods of increasing the ovulation rate of gilts and young sows would have practical application in pig production.

Initial attempts to increase ovulation rate in gilts by immunization against androstenedione were not successful (Walton, 1985) probably because of the very low titre responses obtained in

Figure 1. The frequency distribution of ovulation rate (top) and litter size (bottom) for two groups each of 100 ewes immunized against testosterone (hatched bars) or nonimmunized controls (open bars). R.J. Scaramuzzi, R.M. Hoskinson and D. Paull, unpublished results.

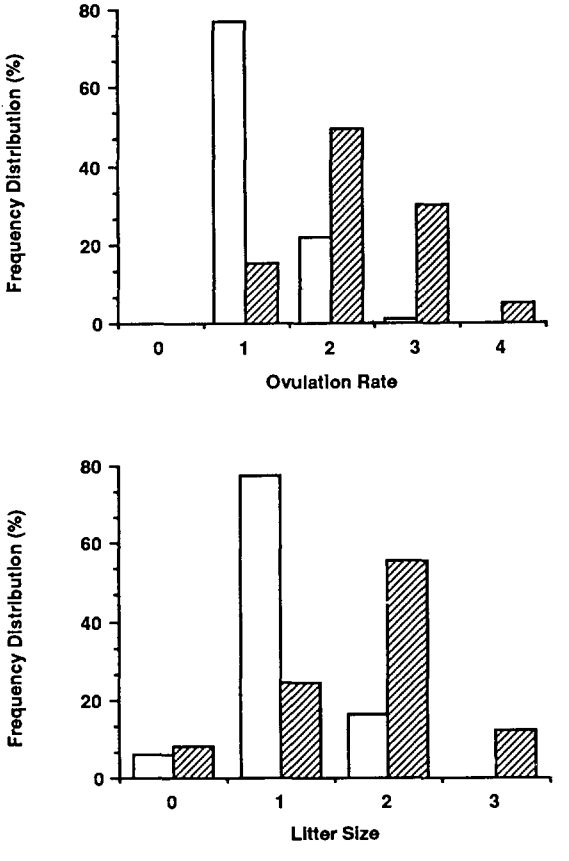

Table 1. Estimates of the ovulation rate of gilts and first litter sows in Australia.

Location	Year	State	Ovulation Rate* Gilts	and First Litter Sows
Abattoir	1971	NSW	9.4	14.0
Commercial Piggery	1979	NSW	—	12.0
Commercial Piggery	1980	WA	10.9	—
Commercial Piggery	1982	WA	—	15.4
Commercial Piggery	1984	VIC	—	11.0
Commercial Piggery	1986	NSW	12.7	14.8
Research Piggery	1985	VIC	—	13.1
Research Piggery	1986	VIC	11.3	12.4

* Data from Penny et al. (1971), Love (1979), Paterson et al. (1980), King et al. (1982) and King (unpublished results).

this work. In a subsequent publication an increase in ovulation rate was observed in a group of 10 gilts immunized against androstenedione-bovine serum albumin (McKinnie and Britt, 1985), but details of antibody responses and of the adjuvant used were, unfortunately, not provided in the abstract. Preliminary tests carried out by Glaxo Animal Health in the UK have indicated that

immunization with Fecundin can also increase litter size in gilts (H. Geldard, personal communication). In a small trial approximately 60 gilts were immunized with Fecundin using a slight modification to the protocol for immunizing sheep. Litter size of the immunized gilts averaged just under a piglet per litter greater than the corresponding litter size in non-immunized control gilts.

We are attempting to confirm the results obtained by Glaxo in a large trial involving 400 gilts on a commercial piggery in southern NSW. Half of the experimental herd have been immunized with Fecundin and the remainder were left as untreated control animals (R.H. King, R.J. Scaramuzzi and I. Walker, unpublished results). Fecundin was administered as a 4 ml subcutaneous injection 6 weeks prior to mating (primary immunization) and again 4 weeks later (booster immunization). A small sample (20) of the immunized gilts were bled just prior to (day 0) and at 3, 20 and 35 days after booster immunization. Final farrowing results are not yet available, however preliminary results of androstenedione antibody titre measurements have shown that the level of antibody response peaked at about 20 days after booster immunization and that the peak response was about only 1/20 of that seen in Fecundin-treated ewes.

These preliminary results together with those of Walton (1985) indicate that pigs are less responsive than sheep to immune stimulation with steroid- protein conjugates. They suggest a need to develop modified immunization schedules that produce antibody responses similar to those produced in Fecundin-treated sheep before we can fully evaluate the effects of steroid antibodies on ovulation rate and litter size in sows. The use of powerful adjuvants such as Freund's complete adjuvant and of different haptens such as oestrone or testosterone, which are known to produce higher antibody titres than androstenedione, are two of the possible alternatives. Another approach would be the use of passive immunization to produce the desired level of circulating antibody (Webb et al., 1984). Ideally a monoclonal homologous antisera would be preferable, although polyclonal antisera would also be effective.

ELIMINATING BOAR TAINT

Castration of male pigs reduces their growth rate, leanness and food conversion efficiency but the procedure is still carried out, in many overseas countries, on virtually all male pigs raised to bacon weight (90-95 kg) or heavier. The fully functional, that is post-pubertal, testis of the boar secretes increasing quantities of 5a-androst-16-en-3-one (5a-androstenone), the "boar taint" steroid, which it synthesizes from testosterone precursor. This steroid accumulates in fat, producing an unpleasant odour on cooking. Attempts to reduce or eliminate the undesirable effects of the boar taint steroid by immunization against 5a-androstenone (Claus, 1975; Shenoy et al., 1982; Williamson et al., 1985) or against Gonadotrophin Releasing Hormone (GnRH) (Falvo et al., 1986) have only been partially successful, and of the two approaches the latter appears more promising.

Immunity to the boar taint steroid results in the production of antibody which binds 5a-androstenone. This then has two effects; one is to block the biological action of the 5a-androstenone, and the other is to delay the metabolic clearance of the steroid. Consequently in the immune animal the level of 5a-androstenone in the circulation is much higher than in normal animals (Shenoy et al., 1982). These elevated levels of 5a-androstenone are reflected in elevated tissue levels which are released on cooking and may exacerbate the problem of boar taint (Shenoy et al., 1982).

Immunization against GnRH (Falvo et al., 1986), luteinizing hormone (Wickings and Nieschlag, 1984) or testosterone (Thompson et al., 1985) would all reduce the supply of freely available testosterone. Since testosterone is the precursor of 5a-androstenone, immunization of boars against any of these hormones would lead to reduced tissue levels of boar taint. However, these treatments would also reduce the anabolic effects of testosterone on carcass composition, and therefore the benefits of reduced levels of boar taint may be offset by reduced growth rates in immunized boars.

SEASONAL INFERTILITY

In many mammals the pineal gland, which secretes the indole amine melatonin, is responsible for the regulation of seasonal phenomena dependant upon photoperiod. Immunity to melatonin may therefore be expected to alter the manifestation of such phenomena. Seasonal infertility in pigs, as its name implies, is associated with the seasons and may be influenced by photoperiod (Claus and Weiler, 1985; McConnell and Ellendorff, 1987) and therefore melatonin. Elevated temperatures and high relative humidity are known to increase the level of seasonal infertility (Paterson et al., 1978) and may be more important than photoperiod in the aetiology of seasonal infertility. Melatonin has proved to be a molecule of low inherent immunogenicity and early efforts to study immunological neutralization of its biological activity have been held back by the poor immune response obtained with melatonin-protein immunogens. Nevertheless immunity to melatonin remains a worthwhile approach to explore.

BODY COMPOSITION

The growing consumer resistance to the consumption of animal fats has resulted in a need for leaner carcasses in our sheep, cattle and pigs. The need to produce leaner carcasses has stimulated considerable research into the control of adipocyte function. Immunization of sheep and cattle with crude membrane preparations of rat adipocytes has been reported to produce a leaner animal (Coghlan,1985). The use of a crude preparation of adipocytes, while not a viable commercial prospect, demonstrates an important principle. The isolation and identification of the specific antigens in membrane preparations will hopefully lead to a defined vaccine capable of inhibiting hypotrophy and/or hypoplasia of adipocytes.

The ß-agonists, such as clenbuterol, provide an effective means of reducing fat content (Ricks et al., 1984) and several pharmaceutical companies are actively pursuing the registration of ß-agonists for reducing carcass fat. Doubts concerning residues of the ß-agonists in tissues may well prevent their registration in some countries. A novel alternative to the use of ß-agonists is the use of an anti-idiotype vaccine to produce antibodies which will stimulate the ß-adrenergic receptor and so mimic the action of the ß-agonists on fat deposition (P.R. Carnegie, personal communication). Such an approach using endogenous immunoglobulins will avoid the problem of tissue residues of the ß-agonists.

Male animals produce less fat than females, an effect usually attributed to the male sex steroids. Immunization against the sex steroids testosterone and 17ß-oestradiol may therefore be expected to influence fat deposition. Researchers at the USDA Meat Animal Research Laboratory, Clay Centre, Nebraska, have reported that immunity to testosterone in ram lambs prevented the normal anabolic effects of this steroid on growth and fat deposition (Schanbacher, 1982) and that immunity to 17ß-oestradiol in the heifer lead to increased efficiency of feed conversion (Wise and Ferrell, 1984).

GROWTH

Growth is a complex process controlled in part by a number of different hormones. Immunization against any of these hormones may therefore be expected to alter growth. Somatostatin is a small peptide produced by the hypothalamus which acts on the pituitary gland to inhibit the release of growth hormone. Immunity to somatostatin would therefore be expected to lead to elevated levels of growth hormone and possibly to increased growth. Attempts to improve growth by immunity to somatostatin have produced mixed results. Some authors have reported growth responses of up to 18% in somatostatin-immune lambs (Spencer et al., 1983), while others have been unable to stimulate growth using this approach (Varner et al., 1980; Hoskinson et al., 1986a) even though elevated levels of circulating growth hormone were produced in response to immunization.

The immunological manipulation of growth remains an important goal for immunophys

iology, but further understanding of the control of growth and the development of new strategies for its manipulation are required. The use of polyvalent immunogens, resulting in the simultaneous immunoneutralization of several hormones, may help overcome the normal homeostatic control of growth which probably limits the effectiveness of immunity to a single hormone as a growth stimulant.

CONTROL OF APPETITE

Appetite as well as a number of other behavioural drives (thirst, libido, aggression) are now thought to be controlled by discrete nuclei within the hypothalamus and limbic system (Baile et al., 1967; Weston and Poppi, 1987). The chemical mediators of these drives have been tentatively identified, and are usually amines or small peptides (Morley et al., 1984; Baile et al., 1986; Weston and Poppi, 1987). The potential therefore exists for the modification of these basic drives by immunological means. The value of such methods to production would be enormous and sufficient to justify research into a still highly speculative field because one of the major limitations to protein deposition in growing pigs is voluntary energy intake.

IMMUNOCASTRATION

Intact boars produce leaner carcasses more efficiently than castrated boars. The occurrence of boar taint (see above), and of behavioural problems that are difficult to manage, has prevented the widespread marketing of intact boars throughout the rest of the world. The marketing of bull beef has been faced with a similar problem; the natural libido and aggression of the animals has proved difficult to control, is dangerous, and may result in bruising damage to the carcass. Immunity against GnRH has been shown to reduce or even eliminate libido and to produce a more docile animal (Robertson et al., 1982; Schanbacher, 1984). The carcass composition of such animals, while not as lean as the carcass of an intact male, was considerably leaner than carcasses from comparable steers. Immunity to GnRH reduced the synthesis of testosterone to very low levels resulting in decreased aggression and libido, but sufficient testosterone was still secreted to allow for some anabolic action on carcass composition. Immunity to the gonadotrophin luteinizing hormone, or to testosterone itself, produces similar effects on libido, aggression and carcass composition (Haynes and Southee, 1984).

Immunocastration is also a potential alternative to surgical castration since GnRH-immune animals would be infertile. This infertility is reversible, which in some circumstances would be an advantage, and therefore would need to be maintained by regular booster immunizations. The potential for an immunocastration vaccine in the domestic pet market, in the control of feral pests, and in the management of our fauna is quite enormous and, not surprisingly, this field has attracted a great deal of commercial interest.

GAZING INTO THE CRYSTAL BALL

The manipulation of animal production by immunization has to date centred on removing the effects of inhibitory hormones. If a hormone has a normal function in limiting a production trait, then by removing its activity the production trait is stimulated. Using such an approach any hormonally-regulated production trait is a potential target for immunological manipulation. Reproduction, growth, lactation and fibre production all fall within the scope of immunophysiology. Anti-idiotype antibodies extend the scope of this technology even further to include any production trait that is modulated by hormones.

Immunoadjuvants: The lack of inexpensive and suitable immunoadjuvants is a major obstacle to the development of immunological methods for manipulating animal production. Freund's complete adjuvant, although used in many experiments to demonstrate a principle, is generally regarded as unsuitable for commercial use. Muramyl dipeptide, derived from the cell wall of mycobacterium, is a potent immunostimulant (Nash et al., 1985), almost as powerful as

Freund's complete, but its present cost is too high for its widespread use in a productivity vaccine. Peptides related to muramyl dipeptide have been described and may be suitable for use in productivity vaccines (Allison and Byars, 1986; Nash et al., 1986). Water soluble polyionic adjuvants, such as DEAE dextran (Wittman, 1970) which is used in Fecundin, have a short duration of response and are not suited to many applications requiring long term persistent immunity, for example, immunocastration.

Peptide fragments: Many of the hormones involved in the expression of production traits are peptides and proteins which are often difficult and expensive to purify. Recent studies of a contraceptive vaccine based on immunization against human chorionic gonadotrophin (hCG) has demonstrated that the whole molecule is not required (Stevens, 1976). Fragments as small as 34 amino acids, when used as immunogens, were capable of neutralizing the biological activity of hCG (Griffin, 1986). The use of fragments, apart from the obvious economic advantages, also imparts higher specificity to the immune response. In the case of hCG, the objective was to induce antibodies specific for hCG, which did not cross react with the closely related luteinizing hormone.

Fusion proteins: Immunogens, in addition to the normal routes of chemical synthesis can also now be produced as fusion proteins. The nucleotide sequence coding for the hormone or hormone fragment of interest is linked to the nucleotide sequence for an heterologous protein to produce a hybrid "fusion protein". Immunization with the fusion protein produces antibodies which may bind the hormone. The advantages of fusion protein antigens are considerable; they allow total control over the immunogen, and also allow the immunogen to be coupled directly to peptide adjuvants. In a recent experiment using fusion proteins, the alpha chain of bovine inhibin was linked to bacterial ß-galactosidase and used to immunize ewes; the antibodies formed were directed against inhibin and led to significant increases in the ovulation rate of the immunized ewes (Forage et al., 1987).

Anti-idiotypes: An anti-idiotype is in essence an antibody to an antibody.They may form naturally, or they may be induced by immunization with a specific immunoglobulin; for example, a purified antibody to the ß-antagonist alprenolol was prepared from the serum of immunized rabbits and then used to immunize another set of rabbits (Schrieber et al., 1980). The second set of rabbits produced antibodies against the purified antibody to alprenolol. Some of these new antibodies displayed the property of mimicing the biological action of alprenolol, the original antigenic hapten (Schrieber et al., 1980). The advent of monoclonal antibodies has opened the way for highly specific anti-idiotype antibodies to be produced. It has been suggested that an anti-idiotype vaccine to the ß-adrenergic receptor could be used as an alternative to the ß-agonists for the stimulation of protein deposition. The action of other hormones could also be mimiced by anti-idiotype vaccines.

Recombinant DNA: This technology is not directly relevant to the immunological manipulation of animal production, but it is important for the production of fusion proteins (Forage et al., 1987) and in establishing the amino acid sequences of proteins of interest to animal production. The identification of peptide fragments suitable for use as immunogens will also be greatly assisted by the techniques of recombinant DNA.

Monoclonal antibodies: Monoclonal antibodies, in the few years since their discovery (Kohler and Milstein, 1975), have had a significant impact in research, in diagnostics and in vaccine production. A monoclonal antibody to progesterone was able the prevent pregnancy when administered to mice over 5 days starting on the day of mating (Wright et al., 1982). Their value as immunological agents to enhance animal production has not been as marked, but nevertheless their potential is very great.

CONCLUSIONS

Immunophysiology is the name given to the rapidly developing field of manipulating physiological processes by means of the immune system. The immune system normally needs to cope with a wide variety of of pathogenic organisms and consequently has evolved into an extremely responsive and highly flexible defence mechanism. Immunophysiology takes advan-

tage of the flexibility and specificity of immune responses to modify the action of selected hormones. The use of immunization against hormones in animal production is limited by two principal factors. First, the lack of a suitable array of immunoadjuvants which would allow the antibody response profile to be matched to a particular set of requirements. Second, our understanding of the basic endocrinology of the economically important production traits such as ovulation rate, growth, and the formation of adipose tissue.

Productivity vaccines present their own particular set of problems. With vaccines to control disease, generally the greater the response the more effective the control; but with productivity vaccines this is not necessarily so. A controlled low level of response or a short duration of response may be required to modify a particular production trait. Immunogen and adjuvant must be combined into a package which will produce the desired response, both in terms of peak response and the pattern of response with time. The long term use of a productivity vaccine must also be considered since its effect from year to year must be reproducible. Adjuvants that produce increasing anamnestic responses from year to year are undesirable. The development of local inflammation at vaccination sites and the harmful effects of the formation of immune complexes in chronically immune animals also need to be prevented.

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THE POTENTIAL OF TRANSGENIC PIGS AND RELATED TECHNOLOGY FOR THE PIG INDUSTRY

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INTRODUCTION

The dramatic advances in molecular biology and in reproductive technologies which have occurred in recent years have provided the animal producer with a powerful new means for genetic improvement by direct gene transfer. The feasibility of this approach was first demonstrated by the successful incorporation of a number of foreign or transgenes into the mouse genome (see review by Brinster and Palmiter, 1986).

The 'transgenic' mice resulting from these experiments were able, in many instances, to transmit the transgenes to their offspring in a manner consistent with chromosomal integration, thus allowing the establishment of breeding lines with specific new characteristics. As genes from any living species may be transferred in this manner, the potential usefulness of this technology to both producer and researcher in improving and investigating animal physiology, health and production is limited only by our imagination (Wagner, 1985; Ward et al., 1986; Brinster and Palmiter, 1986).

This breakthrough was consequential on the recognition that it was possible to create a transgene which comprised the structural gene of interest fused to the regulatory sequence or promotor from another gene chosen to specify the tissue in which the gene would be expressed and the factors which would determine its expression. This was dramatically demonstrated by Palmiter et al. (1982) when they created transgenic mice using a transgene comprising the rat (rGH) or human growth hormone (hGH) structural gene fused with a zinc-sensitive promotor from the mouse metallothionein gene. The transgenic mice they produced grew dramatically due to the rGH or hGH produced by the liver, the normal site of expression of the metallothionein gene, with gene expression being regulable by controlling dietary zinc.

Whilst these experiments can rightly be claimed to have heralded a new era of potential for the animal breeder, initial experience in studies aimed at extending the technology to species other than the mouse proved disappointing. In an heroic series of experiments in the rabbit, sheep and pig, Hammer et al. (1985) injected approximately 5,000 eggs with an hGH gene construct they had proved in mice. Of these, 500 resulted in foetuses or neonates, but only 10% of the rabbits and pigs born proved transgenic and only one of 73 lambs tested showed evidence of gene integration. Furthermore, and in contrast with their experience with mice, none of the transgenic offspring showed evidence of increased growth rate although serum hGH levels were found to be markedly elevated in over half of the transgenic rabbits and pigs.

As it had been previously established that daily injections of hGH had no demonstrable effects on either the size or growth rate of pigs (Baile et al., 1983), the lack of response was probably to be expected. However, growing pigs have been shown to respond to porcine growth hormone (pGH) with significant improvements in growth rates, lean tissue mass and food conversion efficiency (Turman and Andrews, 1955; Henricson and Ulberg, 1960; Machlin, 1972; Chung et al., 1985; Rebhun et al., 1985) indicating that further studies aimed at the introduction of extra copies of homologous growth hormone gene into the pig germ line was justified.

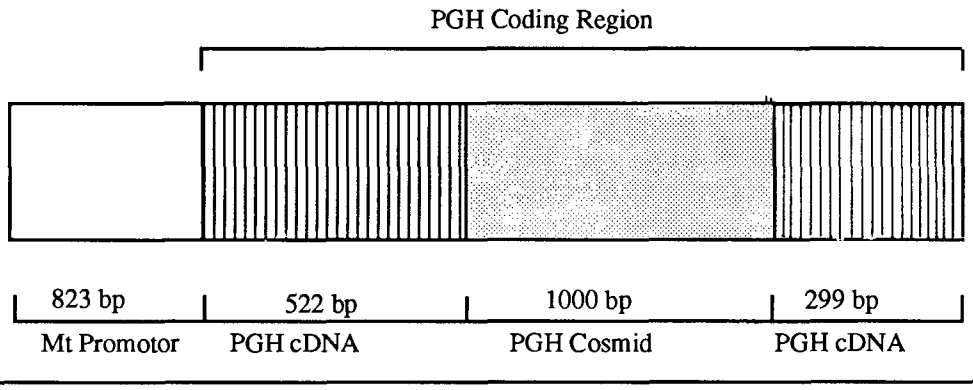
The present paper reviews our preliminary experience in a study aimed at achieving this goal.

THE TRANSGENE

The initial objective of our project was to build on the mouse studies and create new pig breeds with increased growth efficiency by incorporating extra copies of controllable pGH genes into the genome. There were good reasons for wanting to control the activity of these genes *in vivo*. First, from a producer's point of view, excessive growth of stud transgenic animals could lead to handling and husbandry problems but more importantly, high uncontrolled levels of circulating GH caused infertility in female transgenic mice (Palmiter et al., 1982).

To achieve practical control of the introduced pGH gene a control sequence was included which was obtained from the human metallothionein gene. This gene produces a metal binding protein in cells challenged by excess heavy metals such as zinc, copper or cadmium, and by isolating the metal sensitive sequences and substituting these sequences for those which would normally control the expression of the pGH gene, it was anticipated that a transgene would be created that would remain inactive until the pig was exposed to zinc through dietary supplementation. If not, sufficient structural information was known about the human metallothionein promotor to allow adjustment of its regulatory activity through genetic manipulation (Karin and Richards, 1985).

Figure 1. Porcine growth hormone (PGH) transgene construct.



The final design of the transgene employed in this study is shown diagramatically in Figure 1. The transgene consisted of the human metallothionein-IIA (Mt) promotor, fused to a hybrid pGH gene consisting of pGH cDNA sequences encoding amino acid residues 1-158 of pre-pGH, and pGH genomic sequences encoding pre-pGH residues 158-216. The transgene also contained approximately 700 bases of pGH gene 3^1 non-coding sequences to ensure the efficient polyadenylation of the hybrid message (Vize, unpublished data). This gene was proved by its successful incorporation into the mouse genome with 14/18 (80%) of transgenic animals showing dietary controllable expression of pGH and markedly increased growth rates (up to 1.9 fold) (Michalska et al., 1986b). The stable inheritance of the gene was proven (to F4 generation) and homozygous breeding lines have been established (A. Michalska, unpublished data).

TRANSGENESIS

To obtain recently fertilized pig eggs in large numbers, a low cost system was set up through collaboration with Metro Farms, a major South Australian pig producer. At weekly intervals, donor multiparous Large White sows culled on the basis of their age, were given intramuscular injection of 750 i.u. Pregnant Mare's Serum Gondotrophin (PMSG) at 1200 h on the day following weaning (3-week weaning). The time of ovulation was controlled by injecting 500 i.u. Human Chorionic Gonadotrophin (hCG) 44 h later. Treated sows in oestrus on the evening of the next day, and/or on the following morning, were hand-mated to fertile Large White boars. Animals were slaughted by

exsanguation after electrical stunning 52 h after hCG treatment. Reproductive tracts were collected within 10 min of stunning. The oviducts were cut at the utero-tubal junction and flushed with 20 ml of warm (37°C) phosphate buffered saline (PBS) supplemented with 1% v/v heat inactivated human serum (HIHS). One-cell and 2-cell embryos were recovered immediately from the flushings and washed in transport medium. Embryos were stored at 35°C and transported 40 km to the laboratory within 1.5 h of collection.

In our initial experiments (Michalska et al., 1986a) embryos were collected from 54 sows with the overall mean (S.E.) ovulation rate of 23 (±7) per sow. From 1245 ovulations, 863 1-cell embryos were recovered, for a recovery rate of 69%. Eight-eight eggs were classified as degenerated and the remaining 775 were used for the experiment.

The aim of the micro-injection procedure is to introduce about 600 copies of the gene of interest into the male pronucleus of the recently fertilized egg. This is relatively easily achieved in the mouse eggs as the male pronucleus is easily visualized. However, due to opaque lipid inclusion bodies within the cytoplasm of the pig ova, no nuclear structures were seen even with medium Nomarski microscopy and it was necessary to stratify the cytoplasm of the eggs by centrifugation (Wall et al., 1985). This allowed pronuclei to be observed in 442 (57%) of the eggs. One pronucleus of each of these 442 eggs was microinjected with about 600 copies of DNA in 2 picolitres of PBS. The remaining 333 eggs in which pronuclei were not seen after centrifugation, were put in culture in Minimum Essential Medium (MEM) to assess their fertilization and developmental ability. Most of these eggs did not divide and after fixing in acidic ethanol and staining with 1% aceto-orcein were found to be unfertilized.

After overnight culture, 423 injected embryos (276 1-cell and 147 2-cell or more) were transferred into oviducts of 14 recipient sows (30 ± 6 S.E. embryos transferred per recipient). Six of the recipients returned to oestrus during the fourth week following the transfer and four recipients were culled for bacterial infection (not connected with the embryo transfer procedure). Four sows farrowed litters giving the total of 13 piglets (four, four, five and four piglets per litter).

Tissue and blood samples were taken from the 13 born piglets, and evidence of transgenesis sought (Table 1). Four piglets, two females (numbers 177 and 295) and two males (numbers 180 and 736) were found to be transgenic with three, 15, six and six numbers of gene copies, respectively. No difference was evident in the growth rate of transgenic piglets compared with non-transgenic litter mates up to 20 kg live weight. Subsequently, however, one of the two transgenic females (no. 295) began to grow at a substantially faster rate than her transgenic sister (no. 177) or other litter mates.

Gilt 295 had elevated pGH levels at birth and grew without zinc supplement to the food as did mice generated with the same gene construct. The metallothioneine control sequence has now been genetically engineered to be less sensitive to zinc (Vize, unpublished data) and we are hopeful that with the transgenic pigs presently being created, the transgenes can be controlled to the extent that they will only be expressed in growing stock destined for the market.

Neither of the transgenic males produced in these initial litters showed evidence of increased blood growth hormone levels and it is difficult to assess whether they had enhanced growth rate as there were only two non-transgenic males produced in these initial litters that could be used as controls.

Two of the transgenic pigs (gilt 177 and boar 180) have since been mated with non-transgenic stock and their offspring examined for the inheritance of the transgene. In the first litters, six of eight of the boar's offspring and one of five of the sow's offspring expressed the gene indicating that, as with the mice, the generation of stable lines of transgenic pigs is feasible. When tested in a commercial boar testing unit, evidence of increased growth and food conversion efficiency were obtained with two of the F1 animals, but the investigations are incomplete. If enhanced growth rates are obtained in the F1 as opposed to their parents the data parallels our experience with mice where several breeding lines showed enhanced growth only in the F1 and subsequent generations and not in foundation stock (Michalska, unpublished data).

Table 1. Daily body weight gain (between 20 and 90 kg) of transgenic pigs fed *ad libitum*.

Animal	Sex*	Number of gene copies	Plasma pGH at birth	(m i.u./l) at 50 days	Weightgain (g/d)
Transgenic pigs:					
177	F	3	3	10	758
180	M	6	4	15	845
295	F	15	30	30	1273
736	M	6	1	11	646
Non-transgenic litter mates:		0	6.5	11.3	781±44
Average commercial Large-White pigs:		—	—	—	650-700

* F-female, M-male

CONCLUDING REMARKS

The impressive, albeit unregulated, growth performance of one transgenic gilt (295) has exciting research and commercial implications. The pig is established as an important experimental model in medical research and the potential availability of transgenic animals promises to allow experimentation which will yield new insight into gene regulation during normal and aberrant development and in various disease states (Brinster and Palmiter, 1986; Wagner, 1985; Ward et al., 1986).

Several groups have now reported successful production of transgenic pigs (Brem et al., 1985; Hammer et al., 1985). In a recent report by Hammer and his associates (1986), five of six transgenic pigs successfully transmitted the hGH gene construct to one or more progeny. Three progeny from a boar that expressed hGH also expressed the foreign gene. However, under the dietary regimes imposed, the rate of growth was not enhanced in any of the transgenic pigs in comparison to their litter mate controls although the expressed bovine growth hormone and hGH exerted definite biological effects in the transgenic pigs as was evident from the significantly decreased back fat measurement, elevated levels of IGF-1, stimulation of mammary development (by hGH) and reduction in endogenous pGH to non-detectable levels in the plasma.

There is compelling data showing that growing pigs will respond to exogenous porcine growth hormone with significant improvements in growth rates, lean tissue mass and food conversion efficiency (references cited above). In these studies, growth hormone was administered as a daily single injection and improvements in growth rates of 10-17% were achieved. If the data obtained with gilt 295 is a reliable guide, the gains due to pGH would have been even more impressive if the growth hormone had been administered in a continuous rather than episodic manner. This data provides further encouragement for the development of slow-release formulation to allow commercial use of the pGH already produced via recombinant DNA technology from bacterial or yeast sources.

Few firm conclusions concerning the timing of the commercial production of transgenic pigs can be derived based on the performances of the few animals so far created. Each transgenic animal created using the current technology is unique and requires individual assessment. Because of its uncontrolled level of expression, the present line of transgenic pigs are of experimental interest only and we impatiently await the birth of the piglets carrying the second generation gene constructs.

All animals in experimental litters performed better than commercial piglets reared under similar conditions for reasons which are not entirely understood but may relate to the small litter size. It is worth noting that gilt 295, the only animal to show clear evidence of elevated plasma GH levels, performed exceptionally well in achieving her target production weight (90 kg) in a remarkable 17 weeks compared with 21 weeks taken by the two other gilts produced in the same litter and reared under identical conditions and the 25-30 weeks targeted for commercial stock of

the same genotype. If the gene can be controlled so that it remains latent within breeding stock and if its controllable expression with consequential enhanced production characteristics can be sustained through the germ line, stock created in this manner will have an important impact on the efficiency of the pig meat industry.

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The data reported was obtained in a collaborative project between researchers at the Centre of Gene Technology and the Department of Obstetrics and Gynaecology, University of Adelaide. As may be readily appreciated, success in such a project is only achieved through the active and willing participation of many skilled people. The transgenes utilized by us were developed at the Centre of Gene Technology under the direction of Dr Julian Wells with special contributions by Drs Vize and Robins. Anna Michalska, a PhD student in the Department of Obstetrics and Gynaecology, did the microinjections and Dr Rod Ashman the surgery. Dr Barry Lloyd and his staff of Metrofarm Piggery at Wasleys provided and still care for the numerous animals involved.

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GENETIC IMPROVEMENT OF TRANSGENIC PIGS

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The transfer of extra genes for growth hormone (GH) into pigs promises to produce a faster growing leaner animal. A number of steps are required before the animals can be integrated into a commercial production system. A line of homozygous animals, in which the inserted sequence is stably inherited and its expression can be regulated, needs to be established. Extensive evaluation is required to determine the genetic and physiological impact of the expression of the inserted genes and the correlated changes in production characters. These investigations can be facilitated by the use of transgenic mice as a model for transgenic pigs. This paper outlines some of the problems faced in the integration of transgenic pigs into a production system and the role of transgenic mice in solving them. We plan to conduct a number of experiments utilizing both transgenic mice and our own mice, which have been bred for high growth rate or high reproductive performance, in order to probe these problems.

It is misleading to consider transgenic pigs simply as a new genotype which will replace existing ones in our pig herds. An increase in the level of GH in transgenic mice has deleterious effects on female reproduction, a situation which can be expected to persist in pigs. This precludes the breeding sows from expression of the inserted genes and necessitates the use of a nucleus herd system.

The problem in the implementation of a breeding programme is that sows must be selected on the value of traits when the insert is not expressed and yet the breeding objective is to increase these traits in the growers when the genes are expressed.

Breeding programmes in the pig industry currently concentrate on mass selection for growth rate, feed conversion efficiency and leanness. These are traits which are expected to be increased in the transgenic animals.

Unless progeny testing is adopted it will be necessary to estimate the correlation between the value of these traits in the "normal" and in the GH-enhanced environment. Gains made when the traits are selected in the absence of GH may not be additive with the increases in them when the genes are "switched on", because much of the variance in these traits may be accounted for by variance in the level of GH. The first experiment plans to investigate this by introducing the gene insert into a line of mice selected for high growth rate. It will be possible to determine by comparison with a control line, whether the response to selection for growth rate is additive with the increase in growth rate due to the rise in GH level.

Transgenic mice will be invaluable in assessing the gains to be made through selection for other traits such as meat quality in transgenic animals. Current work investigating the relationship between muscle fibre histology and meat quality may allow selection for desirable muscle characteristics.

If the response in growth traits of heterozygotes and homozygotes is the same, the role of the transgenic pig will be as a terminal sire. In a terminal sire programme the potential gains made from the heterosis between the transgenic and non-transgenic lines need to be investigated. Lines of mice which have been selected for high growth rate and high reproductive performance will be utilized in a series of crosses with transgenic mice to investigate the heterosis present for a variety of traits.

In order to exploit the full potential of the transgenic pig many questions need to be answered. The transgenic mouse will provide clues to many of the answers and therefore reduce the time it takes to introduce transgenic pigs into a commercial production situation.

GENETIC IMPROVEMENT OF GROWTH AND REPRODUCTION

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The major costs of pig production are determined by the growth rate, feed efficency and reproductive efficiency of the pigs. Current breeding programmes concentrate selection on efficient, lean growth. Attempts at improving female reproductive performance have emphasised selection on component traits, such as ovulation rate (Johnson et al., 1984), but gains have not translated significantly into increases in the breeding objective. This paper presents the preliminary results of a selection experiment which aimed to increase both growth rate and litter weight weaned, a more complete measure of reproductive performance.

Two lines of mice were established, each consisting of thirty breeding pairs, from a heterogeneous population. They were housed in a facility maintained at between 21 and 25 °C and supplied *ad libitum* with a commercial pelleted feed and water. They were mated,in single pairs, at 9 to 12 weeks of age. Young were weaned at 3 weeks of age and reared in single-sex groups of 7 mice per cage. They were individually identified and weighed at 3 and 6 weeks of age and at mating.

Selection in line A was on growth rate to puberty (6 weeks). Mice in line B were selected on growth rate to puberty given equal weight in an index with reproduction (weight weaned in the first litter by its dam). As a counter to the negative maternal effects operating in large litters (Robison,1981), litters in the selected lines were standardized to eight young at birth.

In an attempt to more closely model the situation in a commercial pig herd, mice in the selected lines (A and B) were allowed a breeding lifetime of up to 5 months (opportunity for 5 litters) and only replaced earlier if better progeny were available. In the selected lines males remained with the females continuously until they were culled for age or replaced by a superior mouse.

Table 1 presents the mean values for growth and reproductive traits for mice born between December 1986 and March 1987. The figures in brackets indicate the value for these traits in the first generation of the experiment (January 1986). All data are for the first litter performance only.

Table 1. Mean values of growth and reproductive components for selected lines.

Line	Numbers Born	Numbers Weaned	Total Wt Weaned(g)	Average Wt Weaned (g)	6 Week Weight (g)
A	8.3^{a}(8.6)	5.6^{b} (5.2)	87.1^{b} (68.3)	16.0^{a}(12.1)	30.1^{a}(27.4)
B	9.7^{a} (9.3)	6.8^{a} (5.1)	100.0^{a} (55.6)	14.7^{b} (10.8)	28.9^{b} (27.5)

Significant differences between the lines are indicated by dissimilar superscripts ($P<0.01$). No traits were significantly different in the first generation.

Selection for growth rate alone (line A) was successful and also led to an increase in total weight weaned, via an increase in average weaning weight. Selection for growth and reproduction (line B) achieved a greater increase in total weight weaned by an increase in the number and individual weight of mice weaned, while still attaining an increase in growth rate to puberty. The results suggest progress towards the breeding objective can be enhanced by including a more complete measure of reproductive performance in the selection criteria.

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SELECTION FOR REPRODUCTIVE EFFICIENCY

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Female reproductive efficiency is affected by a number of different physiological processes. While these processes can be altered to some degree by selection, adverse genetic and phenotypic correlations between the different traits has meant that successful changes in any trait, e.g. ovulation rate, has not necessarily resulted in improvements in overall effeciency (Johnson et al., 1984). In this paper we discuss some preliminary results of a selection experiment for a more complete measure of reproduction, i.e. litter weight weaned per day.

Three lines of mice, each consisting of twenty-eight pairs, were established from a heterogenous population. In all lines eight mice, where possible four males and four females, were selected from seven litters. In two of the lines, S1 and S2, selection was based on the total litter weight weaned in the first two litters of the dam divided by her weight at nine weeks and her age at the weaning of her second litter. The litter weight was divided by the dam's weight to overcome any adverse maternal environmental effect. In the third line, C, selection was random. Mice were mated in single pairs at nine to eleven weeks of age. Males were removed just prior to the females' second litter. Young were weaned at three weeks of age and reared in single sex groups and weighed at 3, 6 and 9 weeks. Throughout the experiment a commercial pellet feed and tap water were supplied *ad libitum*. The temperature in the mouse room ranged from 21 °C to 25 °C.

Table 1 shows the mean values over two litters of a number of reproductive traits for the mean of the selected lines and the control line after five generations of selection.

Table 1. Means of reproductive components for selected and control lines in generation 5.

Line	Conception rate %	Number born	Survival %	Average wean wt.	Parturition interval	Index value
S	91[a]	9.2[a]	91[a]	10.7[a]	29.9[a]	0.112[a]
C	73[b]	8.4[a]	89[a]	10.0[a]	30.4[a]	0.084[b]

The mean performance of the selected lines was superior to the control for all reproductive traits, although only in conception rate and overall performance, i.e. index value, were the differences significant. The pattern of results is similar to that obtained when selection was based only on litter weight weaned in the first litter (Luxford, 1987). Both sets of results are encouraging with respect to the likely success of this approach in improving female reproductive efficiency.

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GENETIC MANIPULATION OF THE GROWTH CURVE

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Animal breeders usually assume that selection done in males will produce results equally in both sexes of progeny. On the other hand, secondary sexual traits show different patterns of morphology and behaviour in the two sexes. Also, evolution has created different degrees of sexual dimorphism in size in different species. This paper reports experiments with mice in which either males only, or females only, were selected for weight at 9 weeks, or in which males and females were selected for different growth curves in the same population.

Non-inbred mice, in rooms heated to 21 °C, were fed commercial dog-cubes. They were mated, 1 male to each female, at 10 to 12 weeks of age. Males were removed before parturition. Young were weaned at 3 weeks of age and reared in singlesex groups of 7 or 8 mice per cage. They were individually identified and weighed at 3, 6 and 9 weeks of age.

Two unselected control lines (1:20 pairs mated; 1a:32 pairs mated) were bred by systematically moving males between families to avoid inbreeding. This can only be done in the first 5 generations in populations of this size (Beilharz,1982). However, rate of inbreeding in the selected lines must be higher, and responses to selection for greater bodyweight are, if anything, underestimated, because the selected lines had smaller population sizes. Lines 9a and 9b were established by random selection from line 1. In each line, 10 pairs were mated per generation. Selection was for 9-week bodyweight, but in males only in line 9a and for females only in line 9b. The other sex was selected at random. The first 14 and 13 generations were analysed in 9a and 9b, respectively.

Line 19a was established by random selection from line1 a. In each generation, 10 males and 10 females were selected on formulae combining weights at 3, 6 and 9 weeks but differing for the two sexes. The formulae aimed to hold down 3-week weight and raise 6-week weight in both sexes. Thereafter males were to increase, and females to hold down 9-week weight. Ten generations were analysed. Table 1 shows selection responses expressed as regression coefficients of a trait on generation number for selected traits. Data were analysed as actual values, and as logs (base10) to remove scale effects on variance. Weights are absolute values, while sex-differences are expressed as differences from the sex-differences of the relevant control population.

Table 1. Regression coefficients, with standard errors in parentheses of selected production traits on generation number.

Data	Line	3-week weight		9-week weight		Sex-diff. at 9 weeks
		M	F	M	F	
Actual	M 9a	.33(.098)	.28(.076)	.72(. 1 18)	.44(.098)	.23(.076)
	F 9b	.05(.065)	.11(.058)	.25(.087)	.36(.079)	-.08(048)
	19a	.02(.187)	-.06(.135)	.87(.203)	.46(.165)	.41(.160)
Log 10	M 9a	1.02(.323)	.83(.312)	.70(.123)	.51(.118)	.19(.096)
	F 9b	.37(.290)	.52(.248)	.32(.097)	.50(.104)	-.17(.067)
	19a	-.01(.810)	-.32(.675)	1.05(.260)	.65(.240)	.40(.208)

Sex-difference at 9 weeks increased in 9a and 19a, while it decreased in 9b. 3-week weight simply followed 9-week weight except in line 19a where it was selected so as not to increase in either sex. Hence, the assumption that selection in one sex will automatically produce the same response in both sexes does not hold. Moreover, line 19a has shown that growth curves can be changed differentially in the two sexes of the same population. We must now exploit selection for different growth patterns in the two sexes, in order to obtain greater efficiency of meat production.

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EFFECTS OF FEEDING LEVEL AND EXOGENOUS PORCINE PITUITARY GROWTH HORMONE (pGH) ADMINISTRATION ON THE PERFORMANCE OF PIGS FROM 25 TO 55 KG

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Exogenous porcine growth hormone (pGH) administration has been reported to improve the growth rate and feed:gain and reduce the carcass fat content of barrows offered fed *ad libitum* from 60 to 100 kg (Etherton et al., 1986). However, the modes of action of pGH remain unclear and there is little information on the responsiveness of younger pigs to pGH therapy or on how the responses in growth and body composition elicited by pGH might be modified by energy intake.

To investigate these aspects of pGH administration on growth and development, forty barrows were allocated at 25 kg to an initial slaughter group comprising four pigs and among six treatments in 2 x 3 factorial array. The respective factors were pGH administration (0.0 (Excipient) and 100 ug/kg/d) and three levels of feeding of a single protein adequate diet (*ad libitum*, 1.64 and 1.38 kg/d) to 55 kg live weight. The pGH (USDA-Lot B1) was solubilized in a bicarbonate buffer (2.5 mg/ml, pH 9.4) and administered daily by i.m. injection into the extensor muscles of the neck. Excipient pigs were injected daily with bicarbonate buffer. The results are summarized as follows:

Feeding level (kg/d)	Ad libitum		1.64		1.38		S.E.M.
pHG (µg/kg/d)	0	100	0	100	0	100	
Energy intake (MJ/d)	33.6	29.4	23.9	23.9	19.7	19.7	0.31
Daily gain (g)	905	1051	670	842	543	681	20.3
Feed:gain (g)	2.57	1.96	2.45	1.92	2.54	1.92	0.03
Body fat (g/kg)	258	188	230	153	196	140	6.10
Protein deposition (g/d)	109	151	84	127	77	106	3.20
Fat deposition (g/d)	283	192	171	126	107	76	10.40

Growth rate and the rates of deposition of the chemical components of the empty body increased linearly with food intake on both excipient and pGH treated pigs. However, at each level of feeding pGH increased growth rate 16-23%, rate of protein deposition by as much as 50% but reduced fat deposition by 26% and feed:gain by 22-26%. Body fat content also increased with feed intake and was reduced on each of the feeding treatments by 30-33% in response to pGH. Growth hormone administration also reduced voluntary energy intake by 12% ($P<0.01$) and increased maintenance energy requirement by 18%.

The GH-induced changes in growth and body composition were similar in magnitude to those which have been reported for heavier pigs (Etherton et al, 1986) indicating that the endocrine system and endogenous GH secretion in particular is a major factor limiting the pig's growth potential during most of its post-natal development. Results indicated that pGH acts either directly or possibly via the somatomedins to stimulate protein and water accretion in muscle tissue and that this is the mechanism by which pGH therapy improves growth performance. The decline in fat deposition on the other hand, appears to be a consequence of the concomitant reduction in energy available for lipogenesis. Results indicate that the higher energy maintenance requirement of pGH treated pigs further reduces energy available for fat accretion and accentuates the effect of pGH therapy on body fat content.

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A SYMPOSIUM: THE CONTROL OF PRE AND POSTWEANING DIARRHOEA IN THE PIG

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INTRODUCTION

The pig producer, in order to remain economically viable, has to keep ahead of rising costs by producing more kilograms of pork per year from the same number of sows. This means that he has to aim to wean more pigs/sow/year and get these to market weight earlier. As a result, there is tremendous pressure placed on housing and animals to achieve this aim, i.e. the national average weaners/sow/year is at present 17 and the target on many farms is 22.5.

Colibacillosis is a disease of intensification in both animals and humans. While the epidemiology of neo-natal colibacillosis is quite clear, the factors associated with postweaning colibacillosis are not. For instance, it is not known what factors turn a postweaning mortality rate of less than 1% into one of 10% to 25% almost overnight.

Diet is known to be an important factor, as the incidence of the disease has risen directly in proportion to the availability of good quality, high energy feedstuffs. It is important to maximize growth rate of pigs up to 10 weeks of age, because 1 kg gained in this period is worth 3 kg at the finisher stage. To achieve this, it is necessary to feed weaners *ad lib* with a good quality high energy diet. Unfortunately, not only does this create ideal conditions for overt clinical colibacillosis, but it is becoming more apparent that subclinical colibacillosis may prevent weaners from fully utilizing their diets. Prevention of postweaning colibacillosis is possible by restricting feed intake. However, the economic penalties of this are equal to, or greater than, the cost of the disease.

As piglets are weaned earlier to accommodate a faster turnaround time of the sow and farrowing facilities, the maturation of their digestive processes is a limiting factor. Producers in the UK have sought to overcome this problem by feeding a very expensive highly digestible diet which is not economically feasible in Australia.

Antibiotics have been relied on for years to mask the problem of colibacillosis. That era is at an end, not only due to consumer pressure, but also because of the ability of *E. coli* to circumvent the efficacy of antibiotics by development of multiple resistance. However, the use of non-antibiotic growth promotants may be enhanced by alternative control measures for *E. coli*, as this may allow full expression of growth promotant potential.

Vaccination would seem to be the most attractive control measure. However, scientists have been attempting to produce effective vaccines for as long as the disease has been a problem. The literature reports vaccine trials purporting to give good results. However, the final arbiter is the market place and to date only two products have been commercialized for neonatal colibacillosis [Neogard (Elanco Products Company, West Ryde, NSW 2114) and EcoVac (Ausvac Pty. Ltd., PO Box 558, Bendigo, Vic. 3550)], and two for postweaning colibacillosis [Autovac (Ausvac Pty. Ltd.,) and Intagen (BOCM/Silcock Ltd., Hampshire, U.K.)]. The long term effectiveness of these products is yet to be established.

The papers in this symposium present a multidisciplinary approach to colibacillosis. Fahy and others deal with epidemiology, pathogenesis and immunity of pre and postweaning diarrhoea, while Hampson reviews nutrition and the environment. The paper by Chandler and Luke on "Alternative approaches to treatment of diarrhoea" represents a break away from the traditional approach to the problem and may be the type of lateral thinking needed to provide a solution.

PREWEANING COLIBACILLOSIS

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INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is the major cause of scouring in unweaned pigs in Australia. Although many agents have been reported to cause scouring in sucker pigs (Table 1), it is fortunate that most of these are exotic or have not emerged as major pathogens under Australian conditions. For more detailed information on enteric pathogens the reader is referred to the following: Anon. (1980);Leman et al. (1981); Buddle (1985); Tzipori (1985); Taylor (1986).

It is important to differentiate between neonatal colibacillosis and postweaning colibacillosis. The *E. coli* involved in these diseases usually have different toxins, attachment fimbriae and haemolysin levels. Neonatal strains are usually non-haemolytic, carry K88, K99 or 987P fimbriae antigens and often produce STa toxin. Postweaning strains are usually haemolytic, may carry K88 fimbriae and often produce STb toxin. In our opinion much confusion arises from authors attempting to discuss them under the broad heading of *E. coli* induced scouring.

Table 1. Agents cited as causes of scouring in unweaned piglets.

Causes of scouring in unweaned pigs worldwide:

1. Transmissible gastro-enteritis virus.
2. Porcine adenovirus.
3. Porcine epidemic scouring (Coronavirus).
4. Rotavirus.
5. Enterotoxigenic *E. coli* (ETEC).
6. *Clostridium perfringens* types A & C.
7. *Salmonella sp.*
8. *Coccidia sp.*
9. *Campylobacter sp.*
10. *Candida sp.*
11. *Strongyloides ransomi*
12. Aujeszky's disease.

Causes of scouring in unweaned pigs in Australia:

1. Enterotoxigenic *E. coli* (ETEC).
2. Rotavirus.
3. *Coccidia sp.*
4. *Salmonella sp.* (rare).
5. *Candida sp.* (rare).
6. *Campylobacter sp.* (not proven).

At birth the piglet enters a complex microbial system. Its first encounter with micro-organisms is in the uterine cervix and vagina when the foetal membranes rupture. However, it is soon deposited in the most contaminated part of the farrowing crate. The normal piglet will soon start nuzzling the skin of the sow searching for a teat. This can take up to 60 min to occur, especially with early born piglets before milk let down has occurred (Spicer et al., 1986). In this search for a teat the piglet is ingesting particles of manure from the heavily contaminated skin and hair of the dam. Therefore, even before the piglet ingests colostrum, it has ingested the wide range of micro-organisms which inhabit the intestine of the sow. Normally, the piglet is protected from the adverse effects of the micro-organsims by antibodies in the colostrum, derived in part by the gut-mammary

gland link.

NEONATAL COLIBACILLOSIS

Neonatal colibacillosis can be seen between 12 and 72 h from birth with an average age at onset of 48h. The disease is often seen first in one or two pigs, but usually involves the whole litter. Scour becomes progressively more fluid until, at its peak, the faeces are profuse, watery and contain flecks of undigested milk. Piglets become dehydrated and emaciated. Those piglets which survive have a reduced growth rate (Tables 2 and 3) (Cutler et al., 1986).

Table 2. Effect of time of onset on subsequent growth rate.

Age at onset of scour	Number of pigs	Number died	Growth rate to weaning (g/day)	Carcass growth to slaughter (g/day)
No scours	1648	103(6.3%)	182	393
Day 1	125	7(5.6%)	174	384
Day 2-4	123	27(22.0%)	170*	376*
Day 5-7	77	77(13.0%)	159*	391
Day 8-11	64	4(6.3%)	163*	391
Day 12	64	2(3.1%)	145*	391

* Significantly different ($P<0.05$) from those piglets with no scours

Table 3. Production penalty associated with decrease in growth rate occasioned by neonatal colibacillosis (based on $1.85 per kg dressed weight).

Duration of scouring (days)	Number of pigs	Carcass growth (g/day)	$ loss if all pigs go to market at 165 days of age
No scours	1648	393	0
1 day	275	388	420
2 days	90	380	357
3 days	59	377	288
4 or more days	34	370	239

Total pigs = 2106. Total loss = $1304.
Therefore, average loss per pig = 62c
For an average litter of 10 piglets, this represents a loss of $6.20 per farrowing.

In Australia, enterotoxigenic *E. coli* (ETEC) are the usual organisms responsible for scouring during the first five days, but not all piglets scour. This may be because they have not ingested ETEC, or because the delicate balance of microflora in the intestine has not been upset; we suspect the latter.

Ordinarily, ETEC have to compete against other bacteria, antibodies and bacteriocidal factors in colostrum and milk, and even the acidity of the stomach, before they can proliferate and gain the critical mass required to produce a concentration of toxin which can markedly affect fluid secretion into the intestine. This is not an "all or nothing" effect, as seen in piglets with a transient (24 h) scour which resolves without the need for intervention. Probably the most important factor predisposing to scour is (relative) lack of protective elements in milk. This may result from either of two reasons: (a) Insufficent exposure of the dam to ETEC, so that the levels of protective antibodies in her milk and colostrum (gut-mammary gland link) are not sufficient to prevent attachment and proliferation of ETEC, or (b) sufficient antibodies are present but the piglets are not able to gain adequate access to the milk (usually a physical impairment). The latter may be split into two categories: 1) **Sow factors** - agalactia, restless sow and insufficient or injured teats; 2) **piglet factors** - splayleg, too small to compete (relative), periparturient anoxia, infections (such as

arthritis) and hypothermia. Where a moderate level of scouring exists on a property, colibacillosis may be responsible for more than 60% of deaths in the preweaning period (Table 4).

Table 4. Causes of preweaning mortality on a piggery with a moderate scour problem.

Cause of death	Number of cases	Percentage of total
Scours	16	
Small, emaciated, dehydrated (secondary scours)	10	
Total of above	26	40.6
Overlay	11	17.2
Splayleg	6	9.4
Anaemia	4	6.3
Bacterial septicaemia	3	4.7
Necrotic enteritis	2	3.1
Ruptured bladder	2	3.1
Cold exposure	2	3.1
Atresia ani	2	3.1
Hydrocephalus	1	1.6
Cleft palate	1	1.6
Congenital absence of kidney	1	1.6
Nil diagnosis	3	4.6
Total	64	100.00

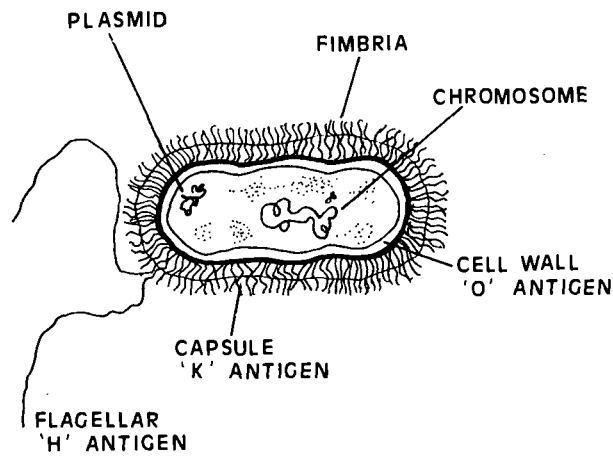
E. coli are part of the normal gut micro-flora and are only capable of causing disease under certain conditions. In intensive piggeries conditions are ideal for a problem to erupt. In nature a sow would not dung in the same area as she would farrow, but confinement in a crate allows her no choice. The following is a brief description of *E. coli* which hopefully will make the pathogenenis of the disease more clearly understood.

E. coli have a capsule which is composed of polysaccharide (Figure 1). Pathogenic *E. coli* have an A type capsule which is heat stable, mucoid and of high molecular weight. This characteristic makes their identification on MacConkey plates more evident as they appear as smooth colonies compared with the dry appearance of *E. coli* colonies which do not posses the A type capsule. Only smooth colonies are considered of diagnostic significance and these are picked off primary plates for further characterization. Hence a laboratory report that states the growth was predominantly smooth, non-haemolytic *E. coli* would be considered significant in the context of neonatal colibacillosis. The polysaccharides of the capsule impart a hydrophilic character to the bacterial cell and it is thought that this may act as a repelling factor to prevent them adhering to the enterocytes as these cells are hydrophobic.

There are at least 70 antigenically different types of capsules in *E. coli*. On serotype the capsule is recorded as K (from the German word for capsule, Kapsule). Therefore, a typical laboratory report would be non-haemolytic *E. coli* serotype O8:K85:H27.

The O or somatic (body) antigen is part of the cell wall and lies just beneath the capsule. The O antigen is a lipopolysaccharide portion which gives different antigenic determinants for serotyping. For *E. coli* there are164 different O antigens. The O antigen is also a virulence factor, the lipid A component of the lipopolysaccharide being responsible for endotoxic shock should it gain access to the systemic circulation.

Fig.1. The important components of *E.coli.*



ADHESION FACTORS

Many *E. coli* have adhesion factors on their outer surface which allow them to anchor to the enterocytes lining the small intestine. These small hair like particles, composed of protein, are referred to as pili or fimbriae. Because of historical precedence, the term fimbriae will be used in this paper.

Fimbriae are regarded by some authors as essential virulence factors, and it is considered that *E. coli* which do not possess them are non-pathogens. In evolutionary terms, the fimbriae allow bacteria to occupy their niche in the intestinal ecosystem by anchoring them to the intestinal wall to avoid flushing from the system by the strong gut peristalsis. Many different genera of bacteria have been shown to produce adhesion factors, e.g. *Escherichia, Salmonella, Pseudomonas, Shigella, Enterobacter, Klebsiella, Proteus,* and *Bacteroides.*

Once anchored to the intestinal villi, *E. coli* cause non-invasive diarrhoea by production of toxins which affect the target epithelial cells, causing a gross imbalance in the water flux of the intestine resulting in a nett fluid loss. *E. coli* which cause diarrhoea via this mechanism are referred to as enterotoxigenic *E. coli* (ETEC). [This classification separates them from the enteropathogenic *E. coli* (EPEC) which actually cause damage to the brush border or invade the enterocytes.]

Five fimbrial types are recorded in veterinary medicine: Type 1; K88; K99; 987P; and F41. The nomenclature assigned to *E. coli* serogroups is rather unfortunate, e.g. a laboratory report which states that an *E. coli* isolated from a scouring neonate serogrouped as O9:K35,K88 will cause confusion where discussion of adherence factors is concerned. Historically the confusion arose when K88 was first discovered. Studies on the *E. coli* laboratory strain E88, in the early sixties, showed that this strain had a surface component in addition to, and different from, the polysaccharide capsule. As it was thought that this was another capsule it was given the standard capsular nomenclature and designated K88. Since then it has been determined that K88 is different from the classic K antigens, the most significant difference being that it is composed of protein rather than polysaccharides (as is true for all fimbriae). However, the nomenclature has remained and been continued with the K99 fimbriae. In the case of 987P, this was recognized as being non-capsular and designated as the pili (fimbriae) first discovered on the laboratory strain of *E. coli* called 987, hence 987P. Similarly, K41 was so called when fimbriae were discovered on lab strain 41. A lot of confusion would be avoided if the nomenclature was changed, e.g. O9:K35, K88 would become O9:K35, F88.

We have stated that there are five recognized fimbriae on *E. coli* of veterinary importance: Type 1; K88; K99; 987P; and F41. These adhesion factors are quite different from those which are important in human medicine, where the fimbriae are designated as colony forming antigens (CFA)

and three types have been found: CFA/I; CFA/II; and CFA/III. A fourth adhesion factor has been named E8776. In addition, other adherence factors have been described on invasive human *E. coli* strains, e.g. those causing pyelonephritis, and meningitis in newborn infants.

Type 1 or common fimbriae: This adhesion factor was discovered by Guyot in 1908 when it was observed that certain strains of bacteria agglutinated red blood cells. It was found that this haemagglutination could be prevented by the addition of mannose to the reaction mixture. The reason for this is that the receptor on the red blood cell is a mannose containing glycoprotein, and addition of mannose to the reaction will saturate the *E. coli* fimbriae, thus preventing attachment to the red blood cells and consequent agglutination.

E. coli expressing type 1 fimbriae adhere to enterocytes of various animal species, but are regarded in the main as non-pathogens. Over half of the members of the family Enterobacteriaceae possess Type 1 fimbriae. An important attribute of fimbriae is that they are hydrophobic. This is in contrast to the capsule and cell membrane 'O' which are hydrophilic and thus unlikely to combine with the hydrophobic cell wall of enteroctyes. It is presumably the hydrophobic nature of fimbriae which facilitates adhesion.

K88 fimbriae: As mentioned previously, these were first discovered on *E. coli* strain E88. Although K88 strains of *E. coli* will agglutinate guinea pig erythrocytes, the reaction cannot be prevented by addition of mannose (mannose resistant agglutination), i.e. the receptor on the enterocytes does not contain mannose.

It is timely to digress and discuss the nature of the receptors on the enterocytes which allow *E. coli* to adhere. Almost all mammalian cells possess an outer, thin lining of carbohydrate containing material, the so called 'cell coat' or glycocalyx. The glycocalyx consists chiefly of sialic acid, containing glycoprotein. It is this carbohydrate portion of the cell coat which provides receptors for the *E. coli*. In the case of Type 1 fimbriae the carbohydrate receptor is probably a D-galactoside. It seems certain that the receptor for each type of fimbriae is different and this would explain the different species susceptibility to *E. coli*, e.g. the common veterinary ETEC are not known to cause diarrhoea in humans and viceversa.

In this regard there are pigs which are genetically resistant to infection with K88 ETEC, as they lack the receptor on enterocytes which allows adhesion. Colostrum and fat globules from milk contain glycoproteins that are similar to the receptors for K88 fimbriae and these glycoproteins no doubt compete with the enterocytes for K88 ETEC. There are serologically different types of K88 fimbriae designated K88ab, K88ac and K88ad. The "a" portion of each type is common and is thought to be the portion responsible for adherence to the receptor on the enterocyte.

K88 ETEC do not cause diarrhoea in lambs, calves or foals. Human volunteers fed K88 ETEC did not develop diarrhoea but did excrete the organism for some months. Therefore, K88 ETEC can be regarded as pathogens of pigs only. K88 ETEC can affect piglets from 1 day to 12 weeks. There is no age resistance other than that acquired by active immunity.

K99 fimbriae: K99-positive ETEC were originally thought to be pathogens only of calves and lambs, however, they are now recognized as being important pathogens of neonatal piglets. There does seem to be an age resistance to this ETEC as, unlike K88 strains, it is unusual to find K99 ETEC associated with pigs older than 2 weeks of age. Expression of the receptors for the K99 fimbriae on epithelial cells of the lamb appear to be influenced by levels of thyroid hormone present at the time of birth. Increased thyroid hormone secretion by the lamb, prior to birth, is thought to decrease the number of receptors. For this reason, premature lambs are thought to be more susceptible to diarrhoea caused by K99 ETEC. A similar mechanism may operate with pigs.

K99 is plasmid encoded and expression *in vitro* is diagnostically important. As with other fimbriae they are not expressed if grown between 18-30°C. Additionally they require growth on special media (E agar or Minca) for expression of fimbriae. *E. coli* possessing K99 will agglutinate erythrocytes, this agglutination is mannose resistant. Sialic acid is thought to be the receptor on the cell membrane to which K99 ETEC attach.

As with other fimbriae, K99 is hydrophobic and overcomes the hydrophilic nature of the bacterial cell wall and capsule. Whereas K88 ETEC are reported to colonize the whole of the small intestine, K99 and 987P ETEC are found to colonize only the distal small intestine, i.e. lower

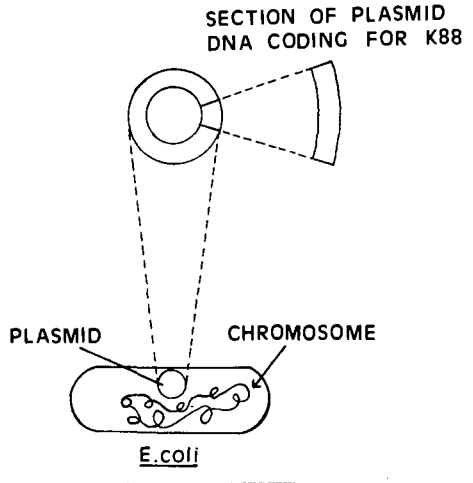
jejunum and ileum. This may be an important virulence factor for K88, as the anterior small intestine is supposedly more susceptible to the action of enterotoxins.

F41 fimbriae: Early studies on K99 caused some confusion as it appeared to consist of 2 sub-units, one migrating to the cathode in electrophoretic studies and the other to the anode. The anionic component is now known to be a separate fimbriae named F41, so named because the *E. coli* strain being studied was lab strain B41. Strains expressing F41 are almost totally restricted to the O9 and O101 serogroups. While ETEC strains expressing only F41 are isolated from pigs, in calves and lambs, F41-positive isolates also possess K99.

There is a certain amount of confusion regarding the ability of ETEC to express more than one fimbrial type. In most cases there appears to be only one mannose resistant fimbrial type expressed. However, *E. coli* expressing up to three fimbrial types have been reported. Expression of F41 requires growth on E or Minca medium at 37°C.

Plasmids: Mammalian (eukaryote) cells contain a number of individual chromosomes in the nucleus, e.g. humans 46. The bacteria (prokaryotes) contain a single, circular chromosome but unlike eukaryotes, some also contain small circular DNA, separate from the chromosome. These circular DNA are referred to as plasmids (Figure 2). Plasmids often contain genetic information for functions that contribute to virulence, e.g. antibiotic resistance and possession of fimbriae. The genes responsible for K88 expression are plasmid encoded. The relevance of this is their ability to lose the plasmid or fail to express it in the laboratory, e.g. the K88 fimbriae is expressed only when the bacteria are grown at 37°C; growth between 18-30°C does not allow expression. The preceeding adhesion factors are all plasmid encoded; however, 987P fimbriae differ in that they are encoded on the chromosone.

Figure 2. Plasmid.



987 fimbriae: A strain of *E. coli* isolated from a neonatal pig with diarrhoea was found to be negative for K88 and K99. Further studies on this strain, designated 987, showed that it adhered to the enterocytes of piglet ileum and jejunum. Subsequently, strain 987 was found to possess a previously unknown fimbria now termed 987P (P for pilus). There is no report of this having been isolated from other species. It is very difficult to obtain expression of 987P in the laboratory. This may be due to the fact that it is encoded by the chromosomal DNA rather than a plasmid DNA.

ENTEROTOXINS

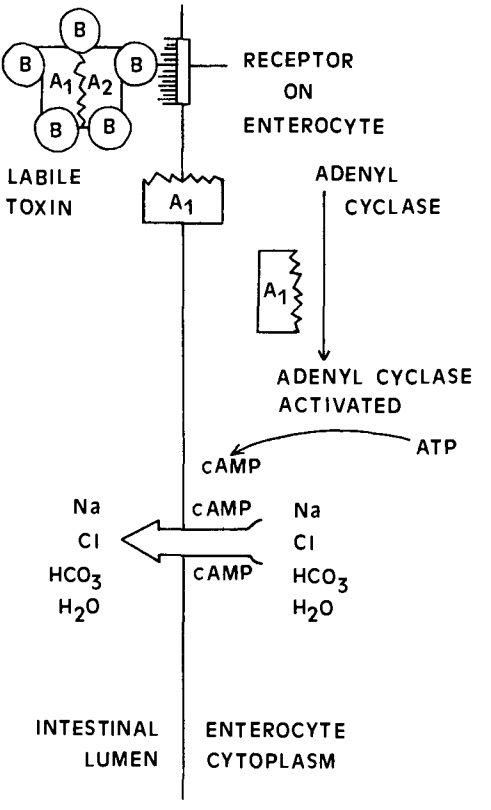
It is generally believed that for *E. coli* to cause diarrhoea they must not only adhere to the intestinal mucosa, but also be able to secrete toxins. These toxins upset the normal water and electrolyte flux of the small intestine. This secretion - absorption imbalance is manifest as diarrhoea

only if the ability of the large intestine to absorb the excess fluid (from the small intestine) is exceeded.

There are three types of toxins elaborated by porcine ETEC: 1) ST or stable toxin, named because of its ability to withstand heat inactivation (100°C for 30 minutes). ST is a small protein (2000 MW) and is non-immunogenic; 2) LT or labile toxin, a large protein (91,000 MW) which is immunogenic and readily inactivated by heat; 3) Vero toxin or VT, named because of its cytocidal activity when cultured with vero cells. VT is thought to be a neurotoxin and may be an active factor in oedema disease of weaners.

The mechanisms of action of LT is better understood than that of ST, no doubt due to its similarity to cholera toxin (CT). Like CT, LT is composed of two sub-units, A and B (Figure 3). The B sub-unit binds to a ganglioside receptor on the cell membrane. Once bound, the A portion enters the cell and activates the enzyme adenyl cyclase, which in turn stimulates the conversion of ATP to cyclic AMP. The nett effect is excessive accumulation of cyclic AMP at the cell membrane, which causes hypersecretion of Cl, Na+, HCO_3 and H_20 into the lumen of the intestine. If the hypersecretion is severe enough, the end result is dehydration, metabolic acidosis, hyperkalaemia, hypovolaemia and eventually death.

Figure 3. Labile toxin and its action.



The nomenclature concerning ST is totally confused. The following is intended to clarify the situation. The hypersecretion occurs from the cells in the villous crypt. In contrast, the effect of excess cyclic AMP on the mature villous cells is to inhibit absorption. The effect of ST is to cause the accumulation of cyclic GMP at the cell membrane. This excess GMP is not thought to cause hypersecretion, but affects the absorption of electrolytes and water from the intestine.

STa (also known as STI, ST_1, ST mouse) is active in infant mice and piglets less than 2 weeks of age. STb (also known as STII, ST_2 and ST pig) is active in pigs of all ages but not infant

mice. STb is only found in *E. coli* of porcine origin, whereas STa is found in *E. coli* of human and porcine origin. However, the gene which codes for human STa (STaH) is different from that which codes for pig STa (STaP).

The frequency of isolation of toxin is as follows; STb>STa>LT. More than half of *E. coli* isolates produce more than one toxin, the most common combination being STb-LT (Moon et al., 1986).

VACCINATION AGAINST ETEC

Vaccination against ETEC induced diarrhoea has had a chequered history, nowhere more so than in Australia. Several vaccines that did not contain K99 antigen were trialled here in the late seventies and did not appear to perform well. There is now little doubt that vaccines which contain the proper range of antigens are highly effective in controlling ETEC induced neonatal diarrhoea.

History of vaccination against porcine ETEC

Stevens and Blackburn (1967) reported that feeding live cultures of ETEC to sows prior to farrowing was effective in controlling diarrhoea in neonatal pigs. This was confirmed by Kohler (1974) using gnotobiotic pigs. Kohler subsequently developed this technique as a standard on-farm practice in North America. This method is still used.

Kohler's technique involves collection of the small intestine contents from a typically affected scouring piglet and culturing this overnight (18-20 h) in milk. Pregnant sows are fed 200ml of this culture on three occasions. This vaccination regime is exploiting the now well -known gut-mammary gland link. This link works in the following manner. Given that the neonate will be exposed to the same repertoire of intestinal pathogens as the dam, it would be of benefit if specific antibodies to these pathogens were present in colostrum and milk to protect the offspring whilst they are developing their own active gut immunity. Whenever the lymphocytes in the intestinal wall of the dam contact a pathogen, they are stimulated to divide; many of the daughter cells resulting from the division migrate from the intestine to the blood. The majority subsequently return to the intestines where they differentiate into plasma cells and excrete antibody (predominantly IgA) which is actively transported across the wall into the mucus gel lining the intestine, thus protecting the whole of the intestine from further attack by the provoking antigen. If the dam is pregnant, an hormonal influence causes a percentage of the lymphocytes, which migrated to the blood stream, to migrate to the mammary gland rather than the intestine. These cells rest in the intralobular connective tissue abutting the epithelial cells of the alveoli. Once settled, they produce specific antibody against the enteric pathogens which incited their formation and, as in the intestine, this antibody (mainly IgA and IgM) is actively transported into colostrum and milk for the duration of lactation.

Parenteral vaccines: These vaccines are used in North America and Australia. They can be either whole-cell-killed vaccines, or genetically-engineered vaccines containing purified pilus. In Australia both seem to work equally well, provided that they cover the ETECs causing the problem. Table 5 shows results obtained using both types of vaccine. The genetically engineered vaccine available is Neogard^{R} (Elanco). A whole cell vaccine EcoVac^{R} (Ausvac) is currently pending registration.

Vaccinated groups showed significant reduction in mortality, severity and incidence of neonatal scouring.

All components of ETEC, with the exception of the flagella (H) antigen, have been shown to be capable of inducing protective immunity, either alone or in combination, following vaccination. Porter's data relating to the protective nature of anti-O antibody have been dealt with. In the case of fimbriae, antibodies prevent adhesion by two methods: 1) Steric hinderance; 2) The hydrophilic nature of the secretory immunoglobulins, found in intestinal secretions, colostrum and milk, will cause repulsion of coated carbohydrate receptors on the enterocytes. However, antibodies directed against fimbriae alone may not be as efficient as those directed against all

components of ETEC, e.g. vaccination of pregnant ewes with K99 fimbriae resulted in specific antibodies to K99 in colostrum. When their offspring were challenged with a mucoid (heavily encapsulated) strain of K99 bearing ETEC, these animals were less protected than those challenged with a non-mucoid K99 ETEC. Similarly, Smith (1972) is of the opinion that even though K88 antigen is important, capsular and somatic antigens play some role since the best protection occurs when the vaccine and challenge strain are homologous.

Table 5. Effects of vaccination using whole cell and purified pilus vaccines on deaths and severity of scour.

	Treatment		
	Whole Cell vaccine	Purified pilus vaccine	Control
Number of sows	97	97	94
Piglets born alive	944	906	902
Litters with diarrhoea (%)	19(19.6)[a]	21(21.6)[a]	47 (50)
Scour days (scour days/litter)	125(1.3)[a]	166(1.7)[a]	523(5.6)
Treatment for diarrhoea (litter average)	133(1.4)[b]	145(1.5)[b]	1162(12.4)
Severity (litter average)	54(0.6)**a	76(0.8)**a	387(4.1)
Scour related deaths (% of total)	3(5.0)[a]	2(6.1)[a]	11(22.2)

Significantly different from controls: [a] X^2, $P<0.001$.
[b] t-test, $P<0.01$.

Non-fimbriated E. coli

Apparently non-fimbriated *E. coli* have been isolated from scouring neonates. The dams of these neonates had been vaccinated with K88 and K99 containing vaccines and although there was some reduction in the level of neonatal scour, it was still at an unacceptable level. Further swabbing of scouring neonates consistently led to the isolation of 0:8, 0:9 or and 0:101 non-haemolytic *E. coli*. These isolates were shown to produce toxin by gene probe assay. Inclusion of these three serotypes in the vaccine led to a dramatic reduction in the incidence and severity of scouring. Three other piggeries have been shown to have neonatal colibacillosis which responded to this 5-strain vaccine. The use of vaccines seems to repress the most dominant *E. coli* serotypes and allows emergence of other 'non-fimbriated' strains. Results from scouring outbreaks on properties using a neonatal scour vaccine are given in Table 6. Table 7 illustrates the range of adhesion factors and toxin types possessed by ETEC isolated from scouring neonates.

Table 6. Isolates from scouring outbreaks on properties using neonatal vaccines.

Number of properties	79
Fimbriated strains only *	7
Non-fimbriated strains	31
Mixed fimbriated/non-fimbriated	32

* Fimbriated strains = K88, K99, 987P

Similar experiences have occurred in other countries and for other species, e.g. Soderlind et al (1982) in an extensive study over a four year period in Europe found that 15% of ETEC isolated from vaccinated and unvaccinated piglets were negative for K88, K99 and 987P adhesins. They recommend that the antigenic composition of vaccines should be adapted to provide protection, not only against the most prevalent ETEC, but also against those most likely to emerge in a population by selective pressure. In North America roughly one third of neonatal scour problems do not respond to fimbrial vaccines and the Kohler autogenous vaccine is still widely used for this reason (L. Saif, personal communication). In humans approximately 60% of outbreaks of infant

diarrhoea are caused by *E. coli* which have no recognized attachment factor (B Rowe, personal communication). Links et al. (1985) have presented data which show the wide variation in antigenic composition in Class 2 ETECs (Table 8). (There is a distinction made between ETECs on the basis of certain characteristics, e.g. Class 1 ETECs are haemolytic, non-mucoid, belong to classical serogroups and toxin production can be detected using the gut loop assay in 6-8 week old pigs. In contrast, Class 2 ETECs are non-haemolytic, mucoid and belong to O serogroups 8, 9, 20, 64 and 101. Toxin production can be detected in the gut loop of pigs less than 2 weeks of age but not in older pigs.)

Table 7: Gene probe typing of non-haemolytic *E. coli* isolated from scouring neonatal pigs.

K88	K99	LT	STa	S	Number of isolates
+					26
+		+			2
+				+	22
+		+		+	2
	+				21
	+	+			3
	+		+		4
	+		+	+	1
		+			39
			+		10
				+	48
		+	+		1
			+	+	1

(Monckton, R. P. and Hasse, D., unpublished data).

Class 2 ETECs are in fact STa producers only and it appears that pigs develop resistance to STa with age. STa is the most prevalent toxin produced by ETEC isolated from scouring neonates (Moon et al., 1986).

Perhaps the most important non-fimbriated *E. coli* is O141:K85ac which is the common cause of postweaning scours in Australia. This *E. coli* is haemolytic, mucoid and invariably produces STb. Many isolates from different farms also produce LT and STa.

Table 8: Class 2 ETEC serogroups reported in the scientific literature (from Links et al.,1985).

Serogroup	Serogroup	Serogroup
O8:K85ab,K99:HNM	O9:K103,K88ac	O101:K27,F41:HNM
O8:K85,987:HNM	O20:KSNT,K88ac:HNM	O101:K28,K99
O8:K85:H27	O20:K101,987P:HNM	O101:K30
O9:K35	O20:K'L238':HNM	O101:K30,K99:HNM
O9:K35,K88ac	O64:K?	O101:K30,F41:HNM
O9:K35,K99:HNM	O64:K'B142',K99:HNM	O140:K99
O9:K35,987P:HNM	O64:K'V142',K88ac:HNM	O141:O987P
O9:K103	O101:K?,K99	O141:K-,987P:HNM
O9:K103,987P:HNM	O101:K?,F41	O'X46':K103

In summary, present day vaccines work well. If a neonatal scour does not respond to vaccination, chances are the serotype causing the problem is not in the vaccine or has emerged as a result of selective pressure due to vaccination. The approach to take in these situations is to swab scouring piglets and forward these swabs to a veterinary laboratory for isolation and serotyping. The Regional Veterinary Laboratory, Bendigo will provide such a service if local facilities are not available.

E. coli endotoxaemia-septicaemia: The acute endotoxic shock/septicaemia syndrome seen in calves does not often occur in pigs. Endotoxin (O antigen) liberated in the intestine appears to be flushed out with little if any absorption. However, it is not uncommon to isolate *E. coli* from organs other than the intestine of piglets which have died from scour. This is most likely to be a terminal or agonal event, the primary cause of death being due to hypovolemic shock, from excess intestinal fluid loss.

We have not looked closely at these *E. coli* to determine if they are of the same serotype as those isolated from the intestine of such pigs. In this regard, many of the *E. coli* causing systemic invasion in humans have adhesion factors for the P blood group of human red blood cells. This "P" adhesion is different from Type 1 fimbriae in that it is mannose resistant.

Uropathogenic *E. coli* of human origin possess a further adhesion termed the "K" adhesion factor and are isolated from cases of pyelonephritis.

OTHER CAUSES OF SCOURING IN SUCKER PIGS

Clostridium perfringens type C: The presence of haemorrhagic scouring during the first 2-3 days of life is highly suggestive of Clostridial enteritis. Although *C. perfringens* enteritis has been suspected on a few occasions, the disease has not been confirmed in Australia (Buddle, 1985). This disease will readily cause necrosis of the mucosa and the presence of characteritic rod-shaped, gram positive bacteria surrounding ghost like necrotic villi allow differentiation from colibacillosis.

Rotavirus: Rotavirus should be considered as a possible cause of scour in suckers over 7 days of age, and in newly weaned pigs. Tzipori (1986) is of the opinion that, in Australia, rotavirus causes occasional scouring in 2-3 week old pigs. The disease may be defined as an acute infection of the small intestine characterized by anorexia, occasional vomiting, and development of scouring (scouring is caused by maldigestion and malabsorption).

The incidence of infection in young humans, calves and piglets is very high (90%) and can be considered a normal phenomenon. This is probably the reason that the disease is not more troublesome, i.e. the exposure levels to the sow in the environment are so high that adequate levels of protective antibodies are available in colostrum and milk. When these levels drop, i.e. around 14-21 days, piglets would be at risk. Usually however, most piglets would have been exposed to rotavirus prior to this time and will have developed active immunity while protected by milk antibodies. Evidence for this theory has been provided from experiments on early weaning of pigs (2 days of age), where removal of piglets from the sow and placing them on milk diets has resulted in devastating outbreaks of rotaviral scouring. Rotavirus was first discovered in Australia as a result of an early weaning trial.

Rotavirus scouring can be differentiated from colibacillosis on the basis of age of onset, pH of faeces, villous atrophy of the small intestine and demonstration of the virus. In this regard it is important that samples be collected within the first 24 h of the onset of scouring, as the virus becomes increasingly difficult to find presumably due to lack of target enterocytes and the rapid onset of immunity (within 48-72 h).

Coccidiosis: In herds where the disease is enzootic, oocysts have been detected in the faeces of pregnant sows. The organism replicates in the epithelial cells of the villi and causes a maldigestion, malabsorption scouring due to necrotic enteritis. Although the disease can occur in pigs as young as 3 days, it generally occurs in the 7-21 day age group. This may relate to decreasing levels of milk antibodies or it may relate to the incubation period, which has been shown to be 4-6 days in SPF pigs. The predominant clinical sign is scouring, which persists for 4-6 days; the faeces are pasty or fluid and yellow to white in colour. Morbidity may be high, and although mortality is usually low, it may approach 75%. A Canadian study showed that 20% of faecal samples from scouring piglets contained oocysts. The inflammatory reaction of the small intestine may be minimal or may lead to severe necrotizing enteritis. Coccidiosis can be differentiated from colibacillosis on the basis of damage to the villi of the jejunum and ileum.

In one outbreak investigated by the Bendigo RVL, it was necessary to kill some affected 2

week old suckers to make a diagnosis as the mortality rate from the disease was so low. Gross lesions in these pigs ranged from none to a thick, yellow, fibrinonecrotic pseudomembrane in the distal small intestine. On histological examination, endogenous stages of coccidia could be seen in villous epithelial cells. Merozoites were readily demonstrable in impression smears from the lesions, stained with new methylene blue. No action was taken to treat animals on this large piggery as the cost of treatment of sows was far greater than the cost of the disease.

Scour occuring after the first week of life: Although the 2 main danger periods for ETEC induced scouring are during the first few days of life (non-haemolytic ETEC) and the first 2 weeks postweaning (haemolytic ETEC), they may be involved in what is loosely called the 14 day scour. This syndrome is fairly common, but fortunately morbidity and mortality are usually low. The cause is probably multifactorial and can involve any or all of the following: ETEC (both haemolytic and non-haemolytic); rotavirus; coccidia; and *Campylobacter spp.* The scouring is of a pasty to fluid consistency, yellow to white in colour. We frequently see what we call the "toothpaste syndrome" at 10 days of age. Affected litters are healthy but a pasty white collection of manure is seen in the dunging area. The cause of this may be dietary as the milk production of the sow may well be in excess of the digestive and absorptive capacity of the piglets.

One suggested explanation for the 14 day scour is that milk antibodies are declining rapidly and piglets are succumbing to enteric pathogens from which they were previously protected. One line of evidence for this comes from Europe, where following the introduction of ETEC vaccination into several herds, the main period of scour was transposed from the neonatal period to the 14-21 day period. This is preferred as older unweaned piglets are less likely to die or suffer a dramatic reduction in growth rate.

On several Victorian piggeries a 14 day scour appeared following the introduction of a 2 strain *E. coli* vaccine to control neonatal colibacillosis. Non-fimbriated strains were isolated from these pigs and the problem disappeared when these were included in the vaccine (D. Ross, personal communication). No controlled trials were done, so that there may have been a cause and effect relationship or it may have been coincidental.

We have seen a serious scouring outbreak in three week old suckers on one property. At its peak, morbidity was 90% and mortality 50%. The causative bacteria was a haemolytic ETEC serogroup O149:K88. It is unusual to find this particular ETEC causing problems in suckers as it is usually a pathogen of weaners. The problem lasted for about four weeks and disappeared spontaneously.

The role of *Campylobacter spp.* (probably *C. sputorum ssp. mucosalis* or *C. hyointestinalis*) as a cause of scouring in unweaned pigs should be kept in mind. Although this is predominantly a disease of grower pigs, lesions of necrotic ileitis have been seen in dead suckers which are highly suggestive of Campylobacter infection.

Salmonella: Salmonellosis may occur in pigs in early life and cause a severe and often fatal enteritis. However this organism usually affects older pigs and is an uncommon cause of scour in unweaned pigs. Differentiation from colibacillosis is made on the basis of gross and histological lesions and culture of the causative organism. In our experience, salmonellosis in pigs is rarely diagnosed.

POSTWEANING COLIBACILLOSIS

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INTRODUCTION

Colibacillosis is the most important cause of mortality in the weaner pig and is a major obstacle to more efficient production in the weaner. This paper examines the aetiology, epidemiology, pathogenesis and clinical signs of this disease, along with immunological procedures to control it.

AETIOLOGY

Cultures of faeces from typically affected scouring pigs or the intestinal contents of pigs, dying as a result of scouring, yield a pure or predominant growth of haemolytic *E. coli* (HEC). Twelve different serotypes of HEC have been associated with postweaning colibacillosis (PWC)(Table 9).

Table 9. Virulence characteristics of haemolytic *E. coli* isolated from postweaning colibacillosis (as reported in scientific literature).

Serotype	Isolation of oedema disease principle	Clinical oedema disease	Scouring (enterotoxins)	Endotoxic shock	Possession of fimbriae
O138	++	++	++	+-	-
O139	++	++	+-	?	-
O141	++	++	++	+-	+-
O98	ND	+-	++	?	+-
O2	ND	+-	?	?	?
O8	NEG	+-	++	+-	++
O45	ND	+-	++	?	-
O75	ND	+-	?	?	?
O121	ND	+-	?	?	?
O147	ND	+-	?	?	-
O149	NEG	+-	++	+-	++
O157	ND	+-	++		+-
O35	ND	+-	+-	?	-

Key to symbols:
++ = strong association.
+- = reported but inconsistent.
ND = not tested for.
? = no information available.
NEG = tested, but negative.

Whilst many authors prefer to regard oedema disease (OD) as a separate disease entity, we consider it as a systemic manifestation of PWC. However, to avoid confusion, it will be discussed under a separate heading in terms of aetiology and pathogenesis. Therefore, the diarrhoeic form of PWC will be termed enterotoxic colibacillosis and the oedema disease form, enterotoxaemic colibacillosis.

Enterotoxic colibacillosis: This is caused by infection of the small intestine with certain pathogenic strains of HEC. Under appropriate circumstances these HEC can proliferate and produce biologically active substances (exotoxins). In the case of enterotoxic colibacillosis, the toxins are referred to as enterotoxins and are the heat stable toxin (ST) and labile toxin (LT). The toxin repertoire of HEC isolated from scouring or dead pigs can vary greatly (Table 10). The toxins exert their effect on the lining cells of the small intestine (enterocytes) and cause a massive outpouring of fluid and electrolytes. The mechanism of action is the same as described in neonatal colibacillosis.

Table 10. Gene probe typing of haemolytic *E. coli* isolated from postweaning colibacillosis. (Hasse et al., unpublished data).

Serotype	Fimbrial type	STa	STb	LT	Number of isolates
O141					
	-	-	-	-	53
	-	+	-	-	3
	-	-	+	-	49
	-	-	-	+	1
	-	+	+	-	26
	-	-	+	+	4
	-	+	+	+	3
	88	-	-	-	2
	88	+	-	-	2
	99	-	-	-	1
				TOTAL	144
O149					
	88	-	-	-	23
	-	-	+	-	9
	-	+	+	-	4
	-	-	+	+	6
	-	+	+	+	2
	88	-	+	-	23
	88	-	+	+	4
	88	-	-	+	1
	88	-	-	-	10
				TOTAL	82
O139					
	-	-	-	-	13
	-	-	+	-	1
				TOTAL	14
Non-typable					
	-	-	-	-	9
	-	-	+	-	3
	-	+	+	-	4
	-	-	+	+	1
	88	-	+	-	1
				TOTAL	18

Adhesion factors: Whilst K88, 987P and F41 fimbriae have been found on HEC, most do not have demonstrable adhesion factors (Tables 9 and 10). The most common 'O' serotypes implicated in enterotoxic colibacillosis are O149 and O141. The O149 serotype is invariably associated with K88 fimbriae. The O141 serotype is rarely associated with a recognized fimbriae

type. However, there are reports of 987P and F41 being associated with O141 (Carghill, personal communication) and fimbrial like structures have been demonstrated on O141 isolated at this laboratory (Kennon, personal communication).

K88 positive HEC usually cause scouring commencing approximately 4 days postweaning. In contrast the non-fimbriated *E. coli* such as O141 do not cause disease until 7-10 days post-weaning (McKechnie et al., unpublished data). It is probably reasonable to assume that the presence of the K88 adhesive antigen allows these HEC to adhere and colonize much faster than the K88 negative HEC. However, there is evidence that some form of adhesive mechanism does exist in the non-fimbriated HEC, since the population in mucosal scrapings is higher than in the intestinal content of pigs with the disease (Smith and Halls, 1968).

Regardless of the method of colonization the HEC build up to high numbers, i.e. 10^9 organisms/g versus 10^3-10^6/g in normal pigs. The final outcome, transient or profuse scouring and recovery or death, will depend on the immune status of the pig.

EPIDEMIOLOGY AND PATHOGENESIS

Based upon the fact that HEC can be isolated from unweaned pigs and that occasionally HEC infection is diagnosed as a cause of death in unweaned pigs, it is probable that newly weaned pigs carry the causative HEC with them to the weaner house. This agrees with the observation of Miller et al. (1984) that initial infection with HEC can occur in the farrowing house.

Immediately postweaning there is a period of multiplication, shedding and reinfection by the faecal-oral route, so that the HEC reach levels of 10^9 organisms/g intestine and overt clinical signs are seen. Evidence for the faecal-oral cycle is circumstantial, though compelling. In our early work on vaccination, five piglets out of a litter of 10 were vaccinated. At weaning, the litter was weaned along with 20 other piglets into one pen while the remainder of the week's weaners were placed in 12 other pens of 30 pigs each. The subsequent mortality from enterotoxic colibacillosis in each of the 12 pens averaged 25% (range 14-32%). Mortality in the pen containing 5/30 vaccinated pigs was 0%. This was a statistically significant result ($P<0.001$) and suggested that an important component of the disease cycle (most probably faecal-oral recycling) was broken.

If it is accepted that HEC are carried in the intestine of unweaned pigs, why does the disease not occur until the postweaning period? Many theories have been put forward and each one, no doubt, forms part of the jig-saw puzzle constituting the epidemiology of postweaning colibacillosis:

1) Lactogenic antibodies. During the sucking period HEC may be held in check by specific antibodies in the sow's milk. At weaning the piglets are suddenly deprived of these, thus allowing the HEC to proliferate.

2) Diet. This is discussed by Hampson in this symposium. Briefly, pigs are abruptly weaned from a nutritionally complete, highly digestible diet, to one which is, at that time, largely alien to the digestive enzymes and processes of the piglet intestine. Therefore, undigested material will be available as substrate for HEC to utilize for growth. In the preweaning period there would be little free substrate available for rapid growth of HEC.

3) Temperature. This is probably one of the most critical elements in the epidemiology of postweaning colibacillosis. Wilson and English (personal communication) claim that outbreaks of colibacillosis can be prevented in North America and the U.K. by proper attention to temperature control in the weaner house. This is not always the Australian experience. Additionally, a large part of our problem is probably the high summer temperatures. Carghill (1982) has demonstrated that extremes of temperature and humidity have a deleterious effect on the population of lymphocytes in the gut wall and it is these lymphocytes which are secreting protective antibodies into the mucous layer of the intestine.

Chilling is known to reduce intestinal motility, thereby decreasing the peristaltic action which tends to flush enteric pathogens from the intestine. Thus chilling favours the proliferation of intestinal *E. coli* to the levels of clinical significance (Moon, 1974; Moon et al., 1979).

4) Stress. Dietary change, inadequate temperature control, handling, transporting, mixing

with new pigs and fighting are potential stressors of weaners. The latter is most important as apparently some pigs do not eat for 24-48 h while dominance hierarchies are being established, and then engorge (Cutler, personal communication). Stress is known to have an adverse effect on the immune system, mediated by increased levels of blood corticosteroids. Although this has not been demonstrated for weaners, an extrapolation can be made from the work of Barnett et al.(1987) showing an association in sows between elevated corticosteroid concentrations and a depressed immune response.

IMMUNITY AND POSTWEANING COLIBACILLOSIS

In earlier work at this laboratory to examine the toxin repertoire of HEC isolated from clinically affected weaners, we were consistently unable to reproduce the disease by oral innoculation of toxin-producing *E. coli*. The strains of HEC used, O141 and O149, were present on the farm from which the pigs were selected for the experiment. The level of mortality from scours on that farm was 1% and morbidity 10%. We concluded that the reason we could not produce the disease was that during the sucking period the piglets had received adequate exposure to HEC (or cross-reacting *E. coli*) so that their gut immune system was primed and therefore, they were able to mount an independent immune response when deprived of the sow's lactogenic antibody.

As the majority of farms do not experience a high level of mortality from postweaning colibacillosis (<5%) one may propose that most of the weaners have received adequate exposure to HEC during the sucking period. What factors are operating to interfere with exposure in the 5% of pigs which succumb? It is postulated that the most important determinant of exposure to HEC is the level of antibody in the sows milk; a high level of antibody interferes with exposure and medium to low levels allow adequate exposure. This fits with the observations of:

1) A litter effect, i.e. a litter sucking a sow with high levels of antibody would be less exposed than a litter sucking a sow with low antibodies. Therefore, one would expect the former to succumb as a litter.

2) The biggest pigs in a litter consume more milk (on an absolute basis) than their smaller litter mates. Therefore, they would consume more lactogenic antibody than their smaller litter mates, receive less exposure, and hence be more susceptible, postweaning, to HEC. The converse of this is that they eat more postweaning and hence have more undigested substrate available for proliferation of HEC.

Support for the "exposure theory" is the success of our vaccination program. This is designed to give controlled exposure to HEC serotypes that cause the problems at a time when antibody levels of the sow have dropped to a level which, although preventing overt clinical disease, still allow adequate exposure and hence stimulation of the piglet's gut-associated lymphoid tissue. This priming allows a rapid secondary immune response to HEC in the postweaning period and hence abortion of the disease process.

OEDEMA DISEASE (ENTEROTOXAEMIC COLIBACILLOSIS)

This form of colibacillosis is not very prevalent in Australia; the first experience with the disease at our laboratory was in 1985. However, it may be that more subtle or chronic forms of this disease go unnoticed if one is looking for classic oedema disease.

Oedema disease (OD) is caused by HEC which produce a toxin that is absorbed across the intestine and causes damage to blood vessels and hypertension. This results in leakage of fluid from blood vessels into the extracellular compartment of the body, i.e. oedema. OD was first described by Shanks (1938). The name "oedema disease", "bowel oedema" or "gut oedema" were first used because oedema of the submuscosa of the stomach and mesocolon was a prominent feature on autopsy. However, as some pigs which died from the disease showed none of these gross lesions of oedema, and because the disease was first reproduced by inoculation of pigs with fluid from the intestinal contents of pigs that died from the disease, Schofield (1953) suggested the

name"enterotoxaemia".

There are three syndromes of oedema seen at autopsy at our laboratory:

1) The classical oedema disease, with marked oedema of the stomach and mesocolon.

2) No marked oedema of the viscera but an excess amount of serous fluid in the peritoneal and thoracic cavity.

3) A chronic form in which there are no gross lesions on autopsy, but characteristic changes to blood vessels in the brain on microscopic examination.

Clinically the first two forms present as sudden death at the height of the disease outbreak in weaners. The third (chronic) form is first noted some time after the disease has passed. These latter pigs are invariable described by the herdsman as "poor doers" or "dippy pigs". They may have scoured during the outbreak, but not necessarily so. The chronic form is the most often diagnosed at the Bendigo RVL.

AETIOLOGY OF OEDEMA DISEASE

The majority of OD strains have been found to belong to relatively few serotypes, the most important of which are O138, O139 and O141 (Sojka, 1965; Sweeney, 1976; Awad-Masalmeh et al., 1982). These are the only three strains from which oedema disease principle (ODP) has been described. However, based on our own experience and from reports in the literature it would appear that other serotypes of HEC are also associated with OD (Table 9).

Oedema disease principle (ODP)

ODP is a biologically active exotoxin which is absorbed from the gut and causes disease by injuring small arteries and arterioles. Microscopically this is seen as a degenerative angiopathy. The lesions may occur in many organs and tissues. The angiopathy, in its early stages, is recognized by swelling of endothelial cells and pyknosis and karyorrhexis of smooth muscle, often accompanied by fibrinoid degeneration in the tunica media. Proliferative changes are seen in the tunica media and adventita in more chronic cases. Areas of malacia in the brain stem are often associated with these changes to the blood vessels.

Chronically affected animals are "poor doers" and invariably tilt their head to one side, the so-called "dippy pig". The reason for their failure to thrive is not clear. However, the hunger and satiety centres of the brain are located in the areas affected by malacic lesions and it could be that the pigs have a depressed appetite. Hydropic degeneration of hepatocytes has also been observed in these animals.

Since the observation that OD could be reproduced by inoculation of pigs with fluid from the intestine of pigs dying from the disease (Timoney, 1949), efforts have been made to isolate and characterise the toxin. The exact nature of the toxin is still unclear.

The *in-vitro* preparation of large amounts of ODP is difficult and hence complete purification has not been achieved. This has led to confusion in some cases as the reactions observed following inoculation of partially purified ODP were consistent with endotoxic shock. i.e. 'O' antigens. The fact that ODP is produced by a limited number of serotypes of HEC indicates that it is different from the 'O' antigen which is common to all gram negative bacteria.

Characteristics of ODP

ODP is heat labile, precipitable by acid or ammonium sulphate, has a molecular weight of 50,000-100,000, can be neutralized by specific antisera, has no identity with the capsular or somatic antigens, is possibly a lipoprotein or lipopeptide, is not an enterotoxin, and is lethal for pigs, mice and rabbits. ODP is cytotoxic for vero cells, which has led some workers to postulate that Vero toxin (VT) and ODP are one and the same. Goh (1983) in a study of HEC in Australia, found that all VT positive strains belonged to the serogroups commonly associated with oedema disease. However, Dobrescu (1983) found that, while ODP was cytotoxic for Vero cells, the pattern of cytotoxicity

was different from that produced by VT. Additionally, ODP did not cross-react with anti-VT or anti-LT antisera.

There are remarkable parallels between ODP and shiga neurotoxin (shiga neurotoxin is produced by some members of the genus *Shigella*), the cause of "Bacillary Dysentery" in humans. Apart from the enteric form, *Shigella dysenteria* causes delerium, convulsions and coma not associated with enteritis. In rabbits the shiga neurotoxin causes paralysis following parentral inoculation. The neurological injury is secondary to a vascular lesion. Shiga toxin, ODP and toxins isolated from human infant scouring (not enterotoxins) have all been shown to cause vero cell cytotoxicity.

ODP was first discovered following the reproduction of the disease using a fluid inoculation from the intestinal contents of pigs which had died from the disease. Cell- free extracts from cultures of OD causing *E. coli* reproduce the disease following I/V inoculation. The disease has also been reproduced by feeding to healthy pigs *E. coli* isolated from the intestine of pigs dying from OD. Neither intestinal contents nor *E. coli* from pigs dying of non-OD caused the disease.

The actions of ODP in mice and pigs have been described (Timoney 1949; Schimmelpfennig, 1970). After a latent period of 24 h mice develop fits and paralysis indistinguishable from those caused by *Shigella* neurotoxin. As in the natural disease, pigs first develop central nervous symptoms followed by oedema of the skin and mesentery.

Smith and Halls (1968) studied OD using an O141 HEC which produced both ODP and enterotoxin (hence scouring). After oral inoculation, scouring developed in 2-6 days and OD at 5-9 days. In most pigs, scouring had abated when OD occurred. This is consistent with findings that chronic OD occurs after the bout of scouring has passed and the observation of Gregory (1960) that the disease does not occur until 18-72 h after I/V inoculation.

Using purified ODP, Clugston and Nielsen (1974) found that acute hypertension developed 40 h post inoculation. This hypertension was associated with the appearance of neurological signs. It is postulated that the hypertension leads to loss of autoregulation of blood flow in the brain and that the hyperperfusion and consequent oedema causes injury to brain tissue.

CLINICAL FINDINGS IN ENTEROTOXIC AND ENTEROTOXAEMIC COLIBACILLOSIS

Regardless of the weaning age, the symptoms tend to occur at two definite times, around four days postweaning or 10 days postweaning. The earlier onset is associated with fimbriated *E. coli*, i.e. K88, and the latter with non-fimbriated *E. coli*. Although it occasionally occurs, it is unusual for weaners on the one farm to succumb to the disease at both periods, even though both fimbriated and non-fimbriated strains are present.

4-day syndrome

In this syndrome the predominant feature is scouring. Morbidity may be as high as 100%, with approximately 10% of affected animals dying if treatment is inadequate.

10-day syndrome

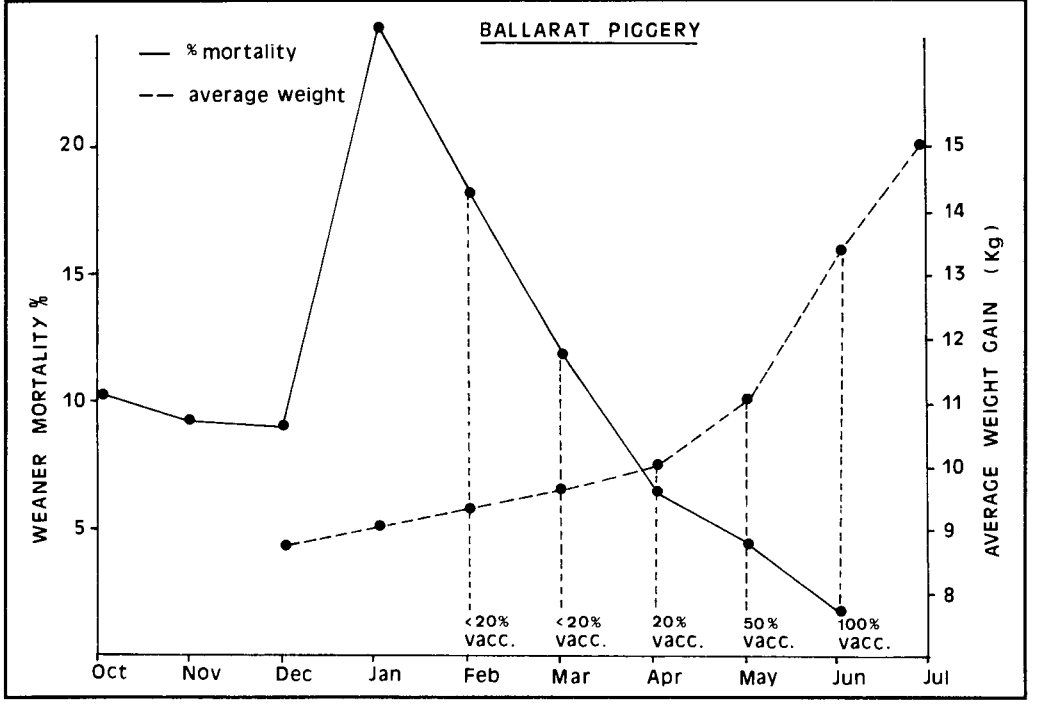
This usually presents in the first instance as sudden death, although some animals scour. Where oedema disease is a problem, animals may exhibit nervous symptoms some 48-72 h after the outbreak occurs. Animals exhibiting nervous symptoms at this stage invariably die. Some will die of oedema disease without being observed to exhibit symptoms. In the more chronic form of oedema disease, animals do not show overt nervous symptoms at the height of the outbreak but are observed some 7-10 days later to show subtle symptoms, such as head tilting and drooping ears, the so called "dippy pig". These animals fail to thrive and the majority invariably succumb to other diseases or are destroyed.

Mortality from the 10-day scour can be as high as 80%. Of the survivors, even on farms

where death rate is only 15-25%, there is a dramatic reduction in growth rate, with some farms recording a drop of 30-50% (Figure 4). With the exception of oedema disease, which will be discussed separately, the cause of death is due to loss of body fluids leading to dehydration, electrolyte imbalance and ultimately hypovolaemic shock. As with the neonatal form of colibacillosis, death supervenes when loss of body fluid exceeds 10%. In the sudden death syndrome, fluid is lost into the small intestine so acutely that the animal dies before the contents reach the large intestine and therefore no scour is evident.

A fourth syndrome (additional to scour, sudden death and oedema disease), of death due to endotoxaemia, has been reported in the literature (Schimmelpfennig 1970; Svendsen, 1979) but has not been observed on autopsy in this laboratory. In this syndrome endotoxins (the 'O' or somatic component of *E. coli*) are absorbed across the wall of the small intestine and gain access to the cardio-vascular compartment. Endotoxins activate autocoids and factor XII, causing an alteration of capillary permeability, and splanchnic and peripheral vascular resistance, resulting in shock (Oehme,1987). Affected animals are hypotensive, hyperthermic, and exhibit vomiting and tenesmus. Disseminated intravascular coagulation and leukopenia followed by leukocytosis are common findings. Animals will die rapidly from endotoxaemia and differentiation from other causes of sudden death can only be made on autopsy.

Figure 4. The effect of clinical colibacillosis on growth of weaners (4-7 weeks of age). Prior to onset of colibacillosis piglets averaged 15 kg at 7 weeks of age (not illustrated).



AUTOPSY FINDINGS

Enterotoxic colibacillosis

In all pigs dying of the intestinal form of the disease dehydration is a consistent finding. This is manifest as sunken eyes, loss of skin elasticity and dryness of the muscles and viscera. In scouring due to fimbriated strains, the stomach may contain food and the small intestine is flaccid. There may be an excess amount of fluid in the small and large intestine depending on the duration of scouring and level of dehydration.

In piglets dying from the "sudden death, non-fimbriated *E. coli*" syndrome, autopsy will

reveal the presence of food in the stomach and a flaccid small intestine containing copious quantities of fluid. The presence of fluid in the large intestine is variable, depending on the duration of the disease. The contents of the small intestine vary from cream coloured to a light pink, depending on the number of red blood cells present. At times the wall of the intestine is dark red and congested. Often deep red gastric infarcts are present.

Oedema disease (enterotoxaemic colibacillosis)

The mechanisms of action of oedema disease principle (ODP) are uncertain. However, it is known that affected pigs are hypertensive and ODP affects the walls of small arteries and arterioles throughout the body. Post mortem findings are consistent with exudation of fluid from the cardio-vascular compartment, i.e. subcutaneous oedema may be seen, especially around the eyes. Oedema in the submucosa of the stomach and mesocolon is quite common. The peritoneal cavity may contain strands of fibrin and a slight to marked amount of serous fluid. The pleural cavity may contain excess serous fluids and the lungs may display varying degrees of oedema. Laryngeal oedema has also been described. The pericardial cavity may contain excess fluid in which fibrin may be present, and epicardial and endocardial petechial haemorrhages may be seen. If the offending strain of *E. coli* produces an enterotoxin, excess accumulation of fluid in the intestine is seen.

In pigs affected by the chronic or subacute form of the disease, no gross lesions may be visible and diagnosis can only be made on microscopic examination of the brain. Characteristically there is a proliferation of mesenchymal elements in the blood vessels of the brain stem and an associated foci of malacia in the brain (Nielson, 1986).

Endotoxaemic colibacillosis

In addition to excess accumulation of serous fluid in the peritoneal and pleural cavities, there is splanchnic pooling of blood and ecchymotic haemorrhages on the diaphragm. Differentiation from oedema disease may require microscopic examination.

ORAL VACCINATION FOR PREVENTION OF ENTERITIS IN PIGS

Neonatal colibacillosis of pigs

Stevens and Blackburn (1967) reported that feeding sows with live cultures of *E. coli* prior to farrowing was effective in controlling scouring in neonatal pigs. This was confirmed by Kohler et al. (1975).

Porter and Lingood (1983) showed that feeding killed *E. coli* to pregnant sows from 50 days of gestation, followed by an intramuscular booster at day 100 of pregnancy, gave good protection against neonatal scours. The protective antibody was IgM, directed against the somatic 'O' antigen of *E. coli*. Both these regimes activate the gut-mammary gland link and result in circulation of specific anti-*E. coli* lymphocytes from the gut to the mammary gland. These lymphocytes are responsible for production of lactogenic antibodies.

Postweaning coliform enteritis (PWCE)

Since Pierce and Gowans (1975) demonstrated that the gut immune system of rats could be primed by oral administration of the antigen and subsequently boosted by an intraperitoneal injection of the same antigen, research workers have attempted to apply this technique to prevention of enteritis in farm animals. Husband and Seaman (1979) reported this to be successful in reducing PWCE from 23.4% to 6.4% on one farm. A similar regime has been tried on a 1000 sow farm experiencing a 5% mortality from PWCE with no effect (A. P. Kelly, personal communication).

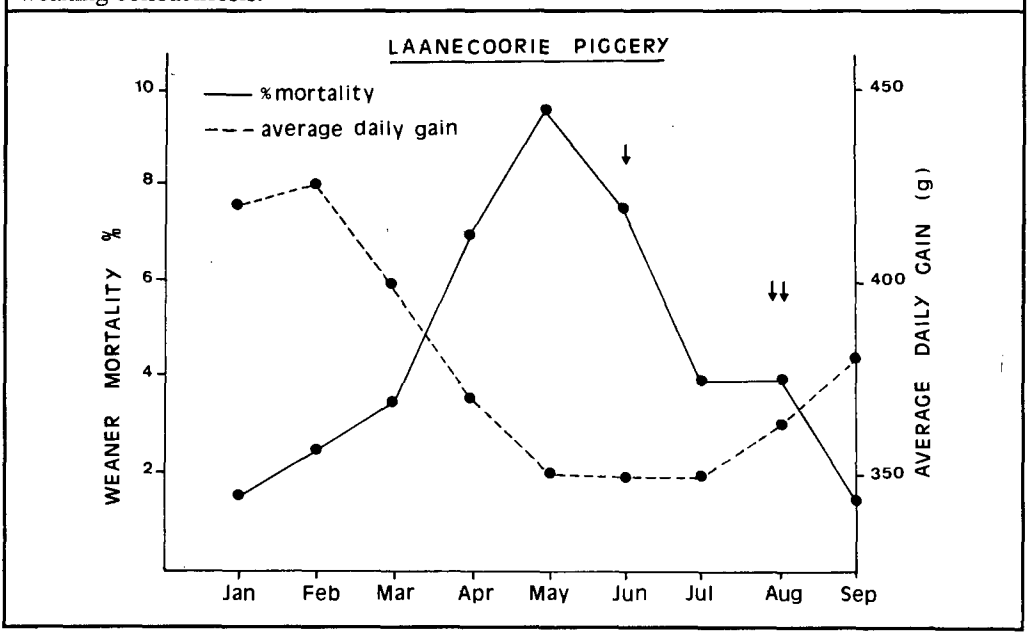
Porter et al. (1974) reported reduced mortalities using a killed *E. coli* vaccine which was mixed with creep feed and fed to suckers. Bertschinger et al. (1978) attempted to immunize newly weaned pigs by oral dosing with live *E. coli*. The vaccine was only successful when combined with a low energy diet. Piglets vaccinated and fed a normal energy diet died either from vaccination or from challenge. Their attempts to induce effective immunity by vaccination of sucker pigs were unsuccessful.

Awad-Masalmeh et al. (1984) reported successful trials using oral dosing of *E. coli* using both live and killed cultures. They were able to show resistance to experimental challenge and reduction in mortality in experimental challenge trials. On-farm studies using killed cultures showed reduced morbidity, mortality and improved feed conversion compared with controls.

The system developed at RVL Bendigo is an oral vaccine. Piglets are vaccinated with a single dose as suckers. The vaccine consists of significant strains isolated from the particular property and is now marketed by Ausvac Pty. Ltd. as Autovac^{R} (registration pending). Field trials with Autovac, on properties with severe to moderate levels of postweaning scouring, have consistently shown marked reduction in morbidity and mortality and increased growth rate. The use of the vaccine has allowed *ad libitum* feeding on many properties which previously could only control PWC by severe feed restriction. Results of field trials with Autovac are given below.

Trial 1 (Laanecoorie Piggery, Vic.): On this property postweaning mortality had been climbing steadily and growth rate decreasing, since January 1985 (Figure 5). Response to conventional therapy was nil. In May, two dead weaners were autopsied at the laboratory and a single haemolytic serotype isolated. A vaccine was instituted and mortality declined from 10% to 4%. This result was not as good as expected based on our previous trialling experience. However, further dead weaners were autopsied and an additional HEC serotype isolated. This was included in the vaccine and mortality fell below 2%. Growth rate also improved dramatically.

Figure 5. Decreased mortality and increased growth rate following vaccination against post-weaning colibacillosis.



Trial 2 (St Arnaud Piggery, Vic.): At the commencement of vaccination, morbidity was 80% and mortality 15%. Serotypes of HEC causing the problem were isolated and incorporated into a vaccine. A trial was designed whereby 50% of the pigs were vaccinated and the remainder were left as controls.

Amongst vaccinated piglets morbidity was reduced to 7% and mortality to 3% compared

with 80% morbidity and 15.5% mortality in the control (Figures 6 and 7). The farm management decided to abandon the trial when faced with these results and all pigs were subsequently vaccinated. Morbidity and mortality slowly drifted down to 3% and 1%, respectively, during the next month reflecting the decreased environmental contamination of the weaner accommodation.

Figure 6. Postweaning mortality on a 1000 sow piggery before and after vaccination.

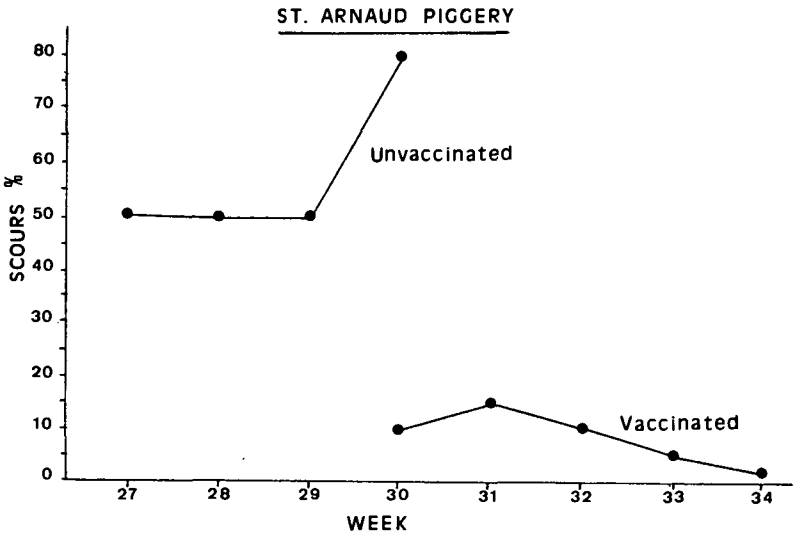
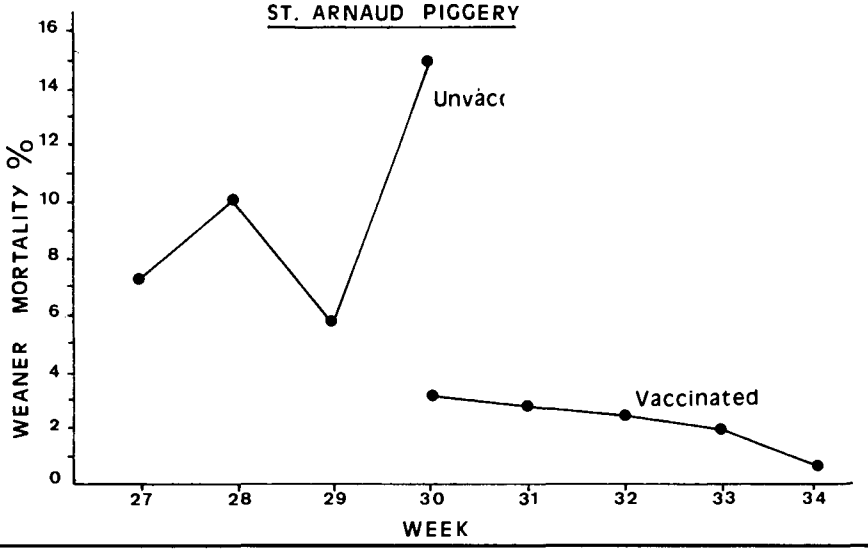


Figure 7. Postweaning morbidity on a 1000 sow piggery before and after introduction of a vaccination program.



Trial 3: During the latter half of 1985 there was considerable interest in raspberry cordial for the prevention and treatment of scouring in weaner pigs. The company which sold the cordial had no experimental evidence to support its claims, although they maintained that many farmers were using it with excellent results. In an effort to determine if raspberry cordial was as effective in controlling post weaning scours as vaccination, we asked for the co-operation of the St Arnaud

piggery and a Ballarat (Victoria) piggery in running controlled trials.

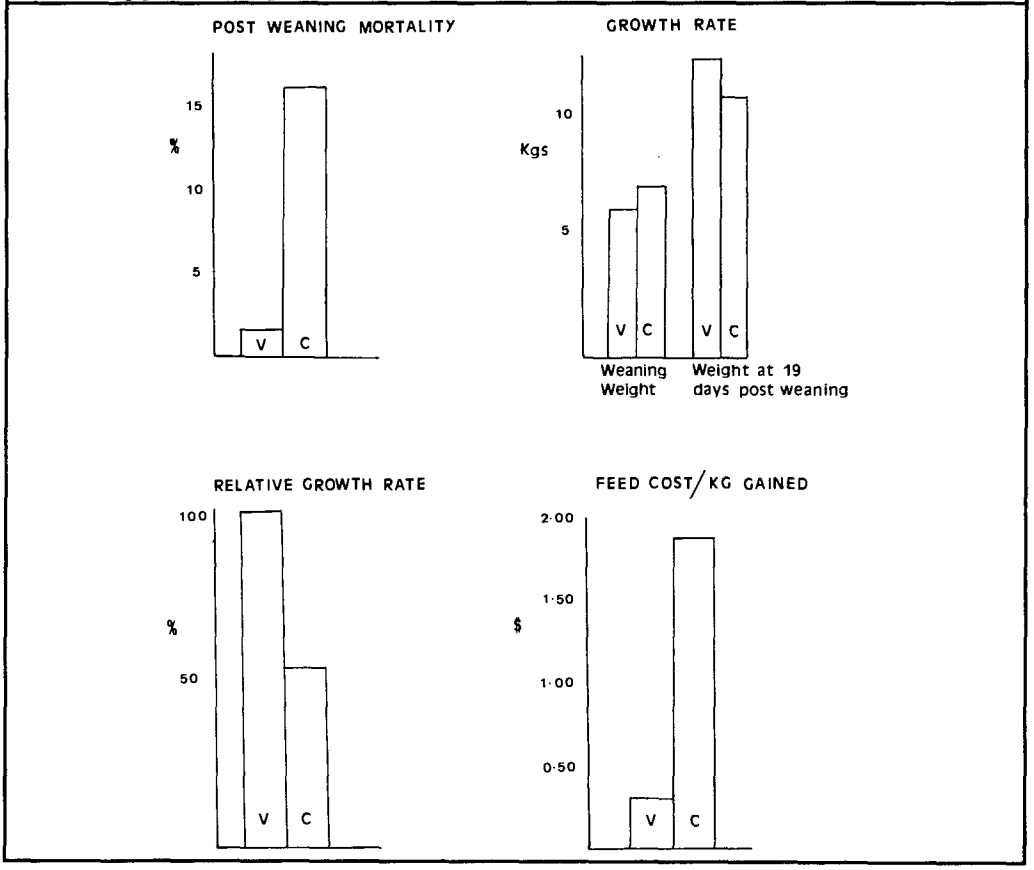
The design of the trial was that approximately half of the piglets were vaccinated and given water to drink postweaning. The remainder were not vaccinated and offered raspberry cordial *ad libitum* from weaning until 19 days postweaning, when the pigs were moved to the first stage grower shed. All pigs were weighed at weaning and again at 19 days postweaning.

The design of the trial was that approximately half of the piglets were vaccinated and given water to drink postweaning. The remainder were not vaccinated and offered raspberry cordial *ad libitum* from weaning until 19 days postweaning, when the pigs were moved to the first stage grower shed. All pigs were weighed at weaning and again at 19 days postweaning.

The mortality rate amongst the vaccinates was 1% on the Laanacoorie piggery (Figure 8); This pig died of mulberry heart disease. The mortality rate amongst the cordial group was 15% X^2 $P<0.01$). Twelve of the pigs were autopsied and all had died of coliform gastro-enteritis. The remaining three pigs were too badly decomposed to allow us to reach a conclusive diagnosis. However, they were scouring badly prior to death. The growth rate of the cordial group was 55% of that for the vaccinated groups. The cost, per kg gained, of the cordial group was $1.98 compared with 29c/kg gain for the vaccinates.

The results from the Ballarat piggery were very similar although the mortality rate was not as high. Statistical analysis of growth rate data was not possible as animals were not individually weighed.

Figure 8. A comparison of the efficiency of vaccination (v) and raspberry cordial (c) in controlling postweaning colibacillosis.



Trial 4 (Griffith Piggery, NSW): This farm had been experiencing a high mortality and severe growth rate retardation in pigs for 18 months prior to vaccination. The serotypes implicated were isolated and a vaccination trial commenced. The only results we have from this farm are for a small

trial (Table 11) as they elected to vaccinate all pigs after seeing the results on growth rate. Piglets were weaned at 4 weeks and moved to a grower shed 35 days later at 9 weeks of age.

Table 11. Growth performance in Trial 4.

Number of pigs	Treatment	Weaning weight	Weaner to grower weight	Growth rate (g/day)
25	Vaccinated	6.39 kg	23 kg	475*
25	Controls	7.74 kg	21 kg	379

* significantly different from control, P<0.05

Trial 5 (Mayfair Farms, Vic.): This 4,000-sow unit had been experiencing a 2.5% postweaning mortality, a 10% scour and reduced growth rate. Vaccination reduced mortality and morbidity (as determined by treatment rate) and increased growth rate (Table 12). All dead piglets were autopsied to determine cause of death. The additional profit from decreased mortality and increased growth rate (4-9 weeks of age) was approximately $1.50 for every pig weaned.

Table 12. Results of Trial 5.

Number of pigs	Treatment	% Mortality	% Mortality due to scours	% Treatment	Growth Rate (g/day)
875	Vaccinated	1.17*	0.47*	2*	478*
404	Controls	2.4	2.0	11	451

* significantly different from controls, P<0.001

Trial 6: This trial was supervised by Dr Ian Roth, who was at that time Acting/Special Veterinary Officer (Pigs) with the NSW Department of Agriculture, Tamworth. The results are shown in Table 13. By his calculations, the savings in reduced mortality and treatment, plus increased growth rate, resulted in a $200 saving for every 100 pigs weaned.

Table 13. Mortality and morbidity (pigs treated) results from a Tamworth piggery.

Number of pigs	Treatment	% Mortality	% Treatment
565	Vaccinated	0.53*	2.48*
487	Controls	2.87	26.50

* significantly different from control, P<0.001

Autovac autogenous vaccines are currently being used successfully on over 120 properties throughout Australia and we see this approach as being essential for the control of postweaning colibacillosis in an era when antibiotics are becoming ineffective prophylactically and unacceptable from a consumer perspective.

Trial 7: These results (Table 14) cover only mortality, and are from a property in Forbes , NSW.

Table 14. Mortality results of Trial 7.

	Month	Number of weaners	% Mortality	Average mortality
Pre-vaccination	May	142	22.5	
May-December	June	55	80.0	
1985	July	148	13.5	
	August	195	18.5	
	September	136	12.0	
	October	156	9.0	
	November	106	30.0	
	December	143	23.8	21%
Post-vaccination	January	145	5.5	
January-July	February	123	4.0	
1986	March	96	10.4	
	April	142	1.4	
	May	114	3.5	
	June	122	2.5	
	July	113	1.7	3.5%

DIETARY INFLUENCES ON PORCINE POSTWEANING DIARRHOEA

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INTRODUCTION

The occurrence of diarrhoea in piglets within three to 10 days of weaning (postweaning diarrhoea; PWD) has long been associated with the proliferation of certain serotypes of beta-haemolytic *Escherichia coli* in the proximal small intestine of affected animals (Richards and Fraser, 1961). Many of these *E. coli* isolates have been found to be enterotoxigenic [ETEC], producing either heat stable toxin 'a' [STa], heat stable toxin 'b' [STb], heat labile toxin [LT], or combinations of these; some strains also produce shiga-like vero cell cytotoxins [VT] (Smith et al., 1983; Morris and Sojka, 1985). Of these toxins, LT and STb cause fluid accumulation in intestinal loops of weaned pigs, but STa does not (Burgess et al., 1978). Forms of VT may be involved in the production of oedema disease in weaned pigs (Dobrescu, 1983; Smith et al., 1983), but their role in PWD is uncertain. Many ETEC from PWD lack the well-characterized adhesins found on *E. coli* from neonatal diarrhoea (i.e. K88, K99, 987P or F41), but may possess other uncharacterized adhesins (Nakazawa et al., 1987; Okerman, 1987). The haemolysin of *E. coli* isolates from PWD does not appear to play a part in the aetiology of the diarrhoea (Smith and Linggood, 1971), and non- haemolytic ETEC may on occasions also be recovered from natural cases of PWD (Hoblet et al., 1986). Initial infection with PWD-producing strains of ETEC can occur in the farrowing house, the organisms then being carried into the weaner house undetected in the intestinal tract (Miller et al., 1984a); alternatively the ETEC infecting weaned pigs can originate in the contaminated environment of the weaner house(Hampson et al., 1987).

Despite the strong association between infection with haemolytic ETEC and occurrence of PWD, oral dosing of weaned pigs with these bacteria does not invariably reproduce the condition (Smith and Jones,1963; Kenworthy and Allen, 1966; Armstrong and Cline, 1977). The organisms can also be found in the intestinal tract of healthy litter mates of pigs with PWD, although usually in lower numbers (Kenworthy and Crabb, 1963; Svendsen et al., 1974, 1978). These observations have led to a search for other factors influencing the occurrence of PWD, generally with the presumption that "predisposing factors" allow ETEC to establish themselves in susceptible portions of the intestinal tract in sufficient numbers to initiate diarrhoea. Although many such predisposing factors have been suggested, for example fluctuating environmental temperatures, chilling, crowding etc., the purpose of this paper is only to outline reported influences of diet and closely related factors on susceptibility to PWD, especially in relation to pigs weaned at three to four weeks of age ("early weaning").

Loss of sow milk at weaning

By definition, PWD only occurs in newly-weaned pigs. Therefore a common factor in its occurrence is recent withdrawal of sow milk. Milk has a variety of potentially important specific and non-specific protective effects against pathogenic *E. coli*. For example, if present in sufficient concentration, milk antibody may inhibit growth of *E. coli* (Wilson and Svendsen, 1971), block adhesion to enterocytes (Nagy et al., 1979) or neutralise LT (Brandenburgh and Wilson, 1973). Iron-binding proteins such as transferrin and lactoferrin may inhibit bacterial growth (Bullen et al., 1972), whilst fat globules may compete as receptor sites for certain *E. coli* adhesive factors (Atroshi et al., 1983). Withdrawal of such potential protection allows enteropathogens to proliferate, provided that they possess some selective growth advantage over other members of the intestinal flora. This protective influence of milk in pigs of weaning age has been demonstrated by Deprez

et al. (1986), who inhibited postweaning proliferation of haemolytic *E. coli* in weaners by supplementing their diet with sows' milk.

Influence of Weaner Diet

The weaner diet differs from the preweaning one in physical state, nutrient composition, availability, and the amount of it which is consumed. Since these differences have all been suggested as factors influencing susceptibility to PWD, and despite the fact that such aspects of the diet may be interrelated, an attempt will be made to address these facets separately.

Physical state of the weaner diet

Weaner diets are usually offered either as a dry meal or as dry pellets. A number of authors have found, however, that liquid weaner diets have benefits over dry ones; these include reducing the number of coliform organisms in the intestinal tract (Decuypere and Van de Hyde, 1972), improving food conversion ratio (Efird, 1982a), and preventing postweaning growth checks (Lecce et al., 1979). Therefore, physical transition from a liquid to a dry diet may predispose weaners to PWD. For example, Lawrence (1983) noted that wet feeding reduced gastric pH, thereby maintaining a greater bacteriocidal barrier to ingested ETEC, and influenced gastric emptying and the characteristics of the digesta and its movements in other parts of the intestinal tract. It is possible that in turn this could influence the extent of proliferation of ETEC at these sites.

Experiments have been undertaken comparing the influence of solid and liquid diets on PWD. Miller et al. (1984a) challenged one-week-old piglets with marked naladixic-acid-resistant ETEC, and then weaned them at three weeks of age onto a liquid diet of cows' milk fed twice daily. The piglets remained healthy and did not excrete ETEC. However, when the pigs were transferred two weeks later to a dry commercial diet fed *ad libitum* they developed PWD and excreted the ETEC in their faeces. Interpretation of these results is confounded by the possibility that the cows' milk may have had some non-specific antibacterial activity, and intake of the milk was restricted, whilst the dry feed was offered *ad libitum.*

Byrne and Halls (1984) noted that germ-free gnotobiotic piglets which were transferred from a liquid diet to a solid one developed a transient reduction in height of villi and a decrease in crypt cell production rate in the proximal small intestine. When Tzipori et al. (1980a) transferred gnotobiotic piglets from a liquid to a solid diet, they found that they became less susceptible to challenge with an ETEC, although more susceptible to challenge with rotavirus. Working with caesarian-derived SPF piglets and another strain of the same serotype of ETEC, Tzipori et al. (1984) found different effects. Firstly, piglets killed two or three days after being transferred from a liquid diet to a solid one showed few alterations in height of villi or depth of crypt in the small intestine. Secondly, when challenged with the ETEC, the piglets on the dry diet developed severe diarrhoea, showed heavy bacterial adherence to the intestinal mucosa, severe blunting and fusion of villi, and crypt hyperplasia; those on the liquid diet had only minor mucosal changes, little bacterial adherence and mild or no diarrhoea. Similar results were obtained by H.S. Chang and S. Tzipori (unpublished data, quoted by Tzipori, 1985) using four-week-old caesarian derived SPF piglets transferred from a diet of cows' milk to either a wet or a dry milk-based diet. The two diets were also offered to pigs on a commercial unit with a history of PWD, and again those on the liquid diet tended to do better, with less mucosal changes, lower bacterial counts in the lumen and less adhesion to the mucosa. The benefits of a liquid diet were not, however, observed when the experiment was undertaken using a soya-bean based diet. To summarize, the advantages of the liquid form of the diet appeared to depend on the type of diet used (soyabean based diets being detrimental whether fed wet or dry), were independent of gross effects on the mucosal structure, and apparently acted by reducing the usual postweaning proliferation and adhesion of ETEC. In piglets given dry weaner diet there was both greater proliferation and more extensive adhesion of ETEC, which in turn appeared to cause mucosal changes and initiate diarrhoea.

The relationship between the changes reported above in the intestinal mucosa of caesarian-

derived SPF milk-reared piglets suffering proliferation of ETEC, and those changes normally seen after weaning in conventionally-reared piglets, is not clear. In the latter animals, there are reductions in height of villi, increases in depth of crypts and decreases in certain enterocyte brush-border enzyme activities throughout the small intestine immediately after weaning (Gay et al., 1976; Kenworthy, 1976; Smith, 1984; Hampson and Kidder, 1986; Hampson, 1986a; Miller et al., 1986). Similar changes have been seen in conventional pigs on a number of diets, including a liquid one (Hampson, 1986b), with or without the occurrence of diarrhoea, and also with or without the presence of ETEC and/or rotavirus (Hampson et al., 1985; Hampson, 1986a). Changes in ultrastructure of the small intestinal epithelium of weaned pigs also occur, and these are greatest in pigs with severe PWD and a high proportion of ETEC in their intestinal tracts (Kenworthy et al., 1967). Although ETEC were originally thought not to alter small intestinal structure (Moon, 1974), STb has been shown to induce a degree of villus atrophy in piglet intestinal loops (Rose et al., 1986), and this activity might help to explain the mucosal changes seen in the SPF piglets. Further investigation is required into the relationship between liquid weaner diets based on milk and their potential inhibition of proliferation and adhesion of ETEC, and on the possible role of ETEC in causing mucosal changes.

Nutrient composition of the weaner diet, and ability to digest and absorb this material

Numerous studies have been directed at manipulating the nutrient composition of pig weaner diets, and most have been undertaken from the point of view of attempting to optimize growth rates in healthy pigs, rather than examining their influence on PWD. These trials have been conducted under different husbandry conditions, using animals of various breeds and ages, with a variety of different sources, combinations and levels of nutrients used. Not surprisingly, a variety of sometimes conflicting results have been produced. In general, the diet should be tailored to match the ability of the young pig to deal with it.

Digestive capacity of young pigs: A variety of mucosal carbohydrases (Kidder and Manners, 1980) and pancreatic enzyme activities (Corring et al., 1978) are incompletely developed in three-week-old pigs. Furthermore, certain enzyme activities may be reduced for a short period after weaning. For example, Hartmann et al. (1961) found depressed pancreatic lipase and protease activities after weaning at one week of age. In pigs weaned at 16 days of age Efird et al. (1982b) recorded reductions in pancreatic trypsin and chymotrypsin activities, but increases in intestinal chymotrypsin, and a greater proteolytic activity of the stomach compared to unweaned pigs. Lindemann et al. (1986) also found a general depression in pancreatic enzyme activities, but not in gastric proteolytic activity in pigs during the first week following weaning at four weeks of age. Pigs weaned at two, three or five weeks of age also show reductions in some, but not all enterocyte brush- border enzyme activities (Gay et al., 1976; McCracken, 1984; Kelly et al., 1984; Hampson and Kidder, 1986; Miller et al., 1986).

Absorptive capacity of young pigs: There is evidence suggesting that there may also be an overall loss of absorptive capacity in young pigs immediately after early weaning. As previously indicated, small intestinal villus height is usually reduced after weaning, there is a change in villus morphology (Plates 1 and 2), and an increase in crypt depth associated with a more rapid rate of enterocyte turnover (Gay et al., 1976; Kenworthy, 1976; Smith, 1984; Hampson, 1986a; Miller et al., 1986). The early-weaned pig shows a reduced capacity to absorb xylose (Miller et al., 1984a; Hampson and Kidder, 1986; Hampson and Smith, 1986), and villus enterocytes show reduced sodium-dependent alanine uptake (Smith, 1984; Miller et al., 1986). It is therefore not surprising that a "malabsorption syndrome" may be seen in newly-weaned pigs (Kenworthy and Allen, 1966a). The outcome of such changes in digestive and absorptive capacity depend on the total capacity of the intestines to absorb, and on the amount and digestibility of the diet consumed immediately after weaning.

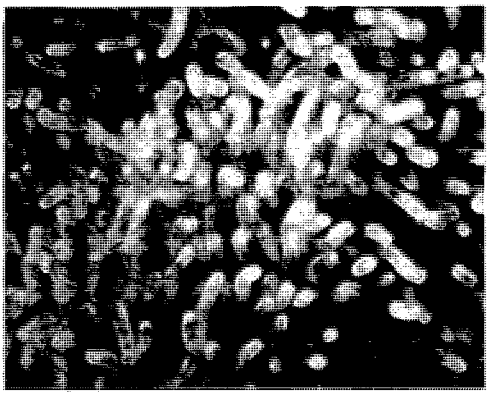


Plate 1

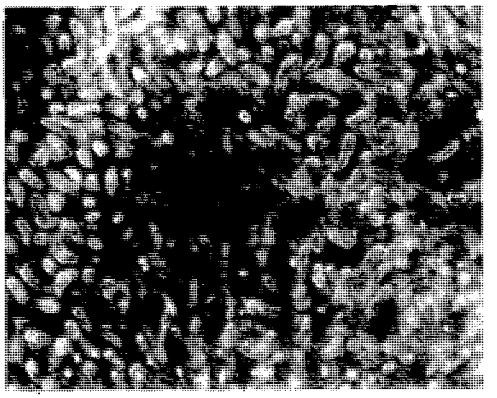


Plate 2

Dissection-microscope appearance of jejunal villi in an unweaned 26- day old piglet (Plate 1) and in a 26-day old piglet weaned for five days (Plate 2). Magnification X 12.

Protein levels and sources in the weaner diet: Three-week-old piglets need a high protein diet if their potential for deposition of lean meat is to be exploited in full. Campbell (1977) found that with a diet containing 15.12 MJ DE/kg, 19% protein gave optimal growth whilst 21.5% gave the best conversion to lean carcass by eight weeks of age. Where PWD is a problem, however, it has been suggested that reducing protein levels in the diet may be beneficial (Bertschinger et al., 1979; Prohaszka and Baron, 1980). Kornegay et al. (1974) noted a non-significant "trend" for faeces to be firmer in weaned pigs fed a 14% protein diet compared to others given 18%, and Namioka and Murata (1965) found that the intestines of pigs fed very high protein levels (29.59%) contained greater numbers of coliform organisms than those fed a lower level (20.86%). Prohaszka and Baron (1980) ascribed the increased susceptibility of pigs weaned onto a "high" protein diet (21%) to an inability to produce sufficient gastric acid to digest the protein. This in turn led to an elevation in gastric pH to a level which permitted selective proliferation of ingested ETEC. Other workers have noted elevated gastric pH values in newly-weaned pigs, but not necessarily associated with consumption of high levels of dietary protein [Thomlinson (1969) in the protected centre of dry-food boluses, and Lawrence (1970) after frequent feeding, or feeding finely-ground barley]. 1Thomlinson and Lawrence (1981) added lactic acid or bran to weaning diets to reduce gastric pH values and increase the "barrier" to passage of ingested ETEC to the intestines. These manipulations did appear to delay multiplication of *E. coli* in the intestines, and to reduce piglet mortality after weaning.

Armstrong and Cline (1976) demonstrated that the amount of protein in the intestinal contents of weaned pigs is related to the level in their diet. It has been suggested that the antigenic nature of this protein may influence the effects of *E. coli* in the pig (Kenworthy, 1970), and be the cause of structural changes in the small intestine, which in turn predispose to PWD (Miller et al., 1984a). The latter possibility will be discussed in the section on "creep food, intestinal structure and hypersensitivity to diet". It should, however, also be pointed out that many workers have found that dietary protein levels have had no influence on either coliform numbers in the intestinal tract or on incidence of PWD (Smith and Jones, 1963; Palmer and Hulland, 1965; Smith and Halls, 1968; Armstrong and Cline, 1976; Armstrong and Clawson, 1980).

The source of dietary protein for weaner diets has also received considerable attention. Products with high digestibility such as dried skim milk or whey have been recommended (Armstrong and Clawson, 1980; Lecce et al, 1985), but these are expensive and have not always proved beneficial (Meade et al., 1965; Kornegay et al., 1974). Pouteaux et al. (1982) compared buttermilk powder, soyabean meal and pea protein concentrate as protein sources for weaner diets, and found that they caused no differences in either faecal water content or incidence of diarrhoea. However, pigs fed herring meal may be more liable to develop PWD (Smith and Halls, 1968·

Bertshinger et al., 1979), and high levels of soya protein may also cause problems (e.g. Anon., 1987). H.S. Chang and S. Tzipori (unpublished data quoted by Tzipori, 1985) noted that soyabean meal protein adversely affected small intestinal mucosal morphology in weaned pigs challenged with ETEC, and feeding soya protein has been shown to be associated with villus atrophy, crypt hyperplasia and an increased flow of digesta through the small intestine of the calf, possibly as a result of hypersensitivity to globulin components of the soyabean meal (Kilshaw and Slade, 1982). Pigs fed soya-containing weaner diets usually have poorer growth rates than do those fed milk based ones (Lecce et al., 1985), and this may reflect a poorer digestibility of the soya protein (Wilson and Leibholz, 1981), or possibly the development of intestinal hypersensitivity to this material (Newby et al., 1985). Soya extracts alone may also cause fluid accumulations in piglet ligated small intestinal loops (Nabuurs, 1986), so it is not surprising that high levels of soya protein are unsuitable for newly-weaned pigs.

The number of protein sources used in the diet may also influence PWD, with most authors finding that simple diets result in less diarrhoea than do complex diets which contain many protein sources (Okai et al., 1976; Ball and Aherne, 1982; Etheridge et al., 1984b). However, Meade et al. (1965) found that diet complexity may make little difference to postweaning performance and complex diets have resulted in faster growth rates, mainly because they tend to cause increased consumption of the weaner diet (Bayley and Carlson,1970).

Energy levels and sources in the weaner diet: Sucking piglets receive approximately 60% of their dietary energy as fat, but they appear less able to utilize lipids after weaning, and relatively low levels are usually incorporated in commercial diets (around five to 10%). Optimal digestible energy levels for such diets are 15.12 MJ/kg where tallow is incorporated at 5.6% (Campbell et al., 1975). The energy source of weaner diets therefore consists mainly of a variety of carbohydrates, of varying complexity and digestibility. Although it has been said that diets that are high in energy may predispose to selective colonization of the small intestine with pathogenic *E. coli* (Moon, 1974), there unfortunately appears to be little direct evidence available on this topic. O'Grady and Bowland (1972) found that pigs weaned at two weeks of age had lower mortality on a high energy diet compared to a low energy one (16.0 and 11.8 MJ DE/kg, respectively), and inclusion of 5% molasses in the formulation reduced mortality. Tzipori et al. (1980b) found that a high-energy weaner diet contributed to earlier postweaning consumption of the diet, and a delay in onset of PWD compared to pigs fed a commercial diet. Once pigs have adjusted to the weaner diet, their voluntary intake (to 50 kg live weight) is mainly limited by their gut capacity, and not by the energy concentration of the diet (Campbell and Taverner,1986).

To test the possibility that ETEC are selectively able to utilize excess energy sources in the intestine, Vasenius (1969) orally inoculated weaned pigs with sucrose-fermenting ETEC, with or without dietary sucrose supplements (10%). Pigs receiving the sucrose developed diarrhoea, whilst those not getting it had only transient or no diarrhoea, and had somewhat lower numbers of ETEC in their faeces. These results suggest that the bacteria used the substrate, proliferated and initiated diarrhoea, although another interpretation could be that the sucrose itself started an 'osmotic' diarrhoea which in turn facilitated recovery of the ETEC. Even though most *E. coli* strains from PWD are capable of fermenting sucrose (Larsen, 1976), Palmer and Hulland (1965) found that the presence of 15% sucrose in a weaner ration did not influence proliferation of haemolytic *E. coli*. Bayley and Carlson (1970) found that supplementing a weaner ration with 5% glucose increased "digestive disturbances", whilst Armstrong and Cline (1976) noted no effects of 20% or 30% glucose in the diet. In contrast, Entringer et al. (1975) found that grower pigs offered glucose or lactose suffered more diarrhoea than those fed starch, and they ascribed this to the more rapid intestinal passage of the readily digestible disaccharides.

Dietary fibre in the weaner diet: There have been a number of reports indicating that both ETEC proliferation and susceptibility to diarrhoea after weaning may be reduced by adding crude fibre (generally derived from oats or barley) to the weaner diet (Richards and Fraser, 1961; Palmer and Hulland, 1965; Smith and Halls, 1968; Armstrong and Cline, 1976; Bertshinger et al., 1979).

However Rivera et al. (1978) found that adding oat fibre to the diet conferred no benefits either in terms of performance or diarrhoea, and English (1981) found less PWD in pigs fed a highly digestible diet with low fibre content (0.8%) than in those on commercial diets (3% crude fibre).

Assuming that ETEC do have the potential for selective utilization of unabsorbed nutrient in the intestinal lumen (as suggested by Kenworthy and Crabb, 1963, and Moon, 1974), the addition of fibre to the diet could act by both reducing the nutrient density of the diet and by increasing its rate of passage through the intestinal tract (Lawrence, 1983). Alternatively it is known that both the source and level of fibre in the diet may rapidly influence bacterial production of volatile fatty acids (VFAs) in the large intestines of growing pigs (Stanogias and Pearce, 1985). In the pig, VFAs are mainly produced by *Bacteroides spp.*, and these colonize the gut within two days of birth (Smith and Jones, 1963). VFAs constitute an important energy source in the pig, and their absorption in the large intestine may also facilitate water and electrolyte absorption (Crump et al., 1980). Their function in this capacity in the large intestine may be of great importance since this appears to be the major site for water and electrolyte absorption in the weaned pig (Hamilton and Roe, 1977). VFAs may also be beneficial because, in concert with pH and oxidation potential, they may have a suppressive effect on Enterobacteriaceae in the porcine intestine (Prohaszka, 1986). Weaned pigs do appear to have a much more active intestinal bacterial fermentation than do pigs remaining with the sow (Etheridge et al., 1984b). The protective role of fibre could therefore be mediated by its influence on VFA production by the normal intestinal flora. Such a protective role for the normal flora has been speculated upon by Palmer and Hulland (1965), who found greater proliferation of ETEC and more PWD in weaned pigs fed bacitracin than in those not receiving the antimicrobial. Oral chloramphenicol also increases incidence of diarrhoea in weaned pigs orally inoculated with ETEC (Cox et al., 1986), again presumably by supressing the normal flora.

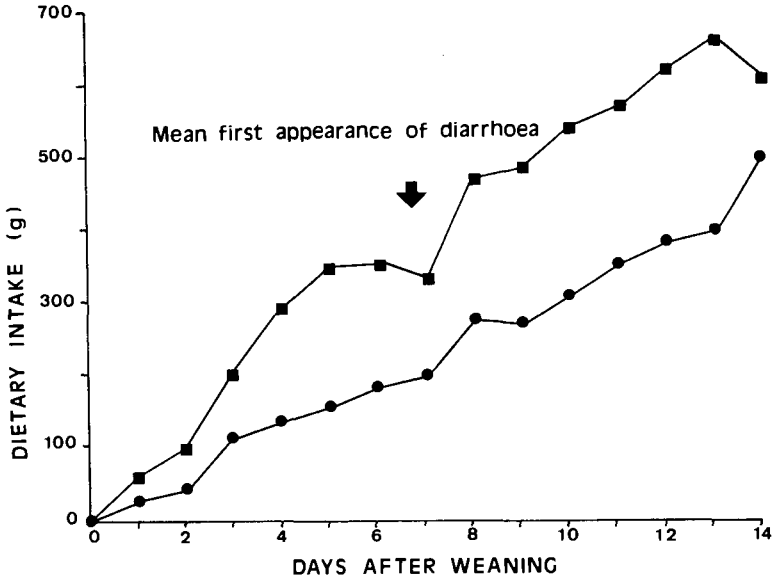
It should be noted however that Etheridge et al. (1984b) found that weaned pigs fed a corn-soyabean meal diet had greater intestinal bacterial fermentation, more VFA production and more diarrhoea than did animals fed a more digestible diet based on oat-groats and casein. Further work is required to observe the relationship between bacterial fermentation, VFA production and proliferation of ETEC in pigs on various diets over the critical postweaning period.

Availability and intake of weaner diets: It has been argued that the often poor performance of piglets immediately after weaning is a reflection of inadequate intake, and that regular feeding of a liquid diet might improve performance without overloading a limited digestive capacity (Lecce et al., 1979). This problem of limited digestive and absorptive capacity was demonstrated by Lecce et al. (1983) when they infected newly-weaned pigs with rotavirus and ETEC, and fed them either a diet containing 30% protein, 40% lactose and 20% animal fat fed in three equal meals over the day, or the same fed in 24 equal increments. Two other groups received one third of this ration, diluted with water to give similar quantities. The high nutrient intake fed three times a day resulted in the most prolonged PWD, the most persistent shedding of rotavirus, and the greatest colonization with ETEC. A less severe response was found in the high intake group fed in 24 instalments, and the groups receiving the low nutrient intake were least affected. In this experiment rotavirus was presumably contributing to the postweaning malabsorption.

Although these results do not identify what components of the diet were involved, they do suggest that feeding too much highly digestible, good quality nutrient to newly-weaned pigs may predispose to PWD, possibly by encouraging proliferation of ETEC. Feeding small amounts of the diet at regular intervals improved performance, presumably because this feeding method does not overwhelm the limited and temporarily reduced absorptive and digestive capacities of young weaned pigs. Ball and Aherne (1982) also found that pigs fed once a day suffered more diarrhoea than those fed *ad libitum*, whilst animals given a limit on total intake performed best. The greater incidence of diarrhoea in the time-limited group was ascribed to their more erratic feed intake and a tendency for pigs to gorge themselves. Where pigs on *ad libitum* intake did develop diarrhoea, this was more severe than in the other groups, and may have reflected large intakes by individual greedy animals. Hampson and Smith (1986) observed that it was individual animals with the greatest intakes of a group that developed PWD (Figure 9), although this effect was not observed

in later experiments using different diets (Hampson et al., 1988). Over eating may predispose to a temporary gastrointestinal stasis (Ruckebusch and Bueno, 1976), which in turn could favour proliferation of ETEC (Kenworthy and Crabb, 1963). However, although gastric stasis may precede scours in piglets (White et al., 1972), when opium tincture and spasmentral were given to weaned pigs to reduce gastro-intestinal motility, this was not followed by a higher incidence of PWD (Schulze, 1979).

Figure 9. Mean daily dietary intakes in weaned caged pigs ■-■, developing diarrhoea (n=18); ●-●, not developing diarrhoea (n=28) (data from Hampson and Smith, 1986).



Restriction of dietary intake after weaning has been beneficial in treatment or prevention of PWD (Palmer and Hulland, 1965; Smith and Halls, 1968; Svendsen, 1974; Bertshinger et al., 1979), but this procedure is mainly recommended for "cheap" diets of low digestibility; an alternative approach is to offer a better quality weaner diet (English, 1981; Etheridge et al.,1984a; Fowler,1985). It is generally presumed that restriction of intake or improving the digestibility of the diet results in less unabsorbed substrate being present in the intestine to support the growth of ETEC. Paradoxically, good quality diets based on dried skim milk appear to induce less production of enterocyte brush-border maltase activities after weaning than do those based on uncooked cereals (McCracken, 1984), and restriction of postweaning intake also reduces the normal increases in these enzymes (McCracken and Kelly, 1984). Restriction of postweaning intake may reduce growth rate temporarily, but it does not appear to have any significant effect on growth to 90 kg weight (Ball and Aherne,1982).

Influences of creep food on PWD

Potential benefits of creep food

Pigs weaned at six to eight weeks of age have traditionally been fed creep food to supplement declining milk production after three weeks of lactation. It is more difficult to justify the use of creep food for pigs weaned at three or four weeks of age, although English (1981) demonstrated that consumption of 610 g of high quality creep food before weaning at four weeks of age improved subsequent postweaning intake and performance. Judging from the weight gains of these pigs, the majority of the creep food was eaten in the last week before weaning; Friend et al. (1970), Okai et al. (1976) and Hampson and Kidder (1986) also found difficulty in getting pigs to eat much

creep food before three weeks of age. Madec and Josse (1983) found that consumption of less than 100 g of creep food before weaning was associated with increased variation in weaning weight, lower weaning weights, more preweaning diarrhoea, and an increased risk of developing PWD.

It therefore appears that even for pigs weaned at three weeks of age, offering creep feed may have some benefit, especially for litters where the sow is lactating poorly or unevenly (Fowler, 1985). Hampson and Kidder (1986) found that piglets fed creep food had a slight, but not significant increase in mean weight, with a smaller spread of weights at 20 days of age. However, brush-border lactase and sucrase activities were not influenced by consumption of creep food up to three weeks of age, and lactase activity declined in creep-fed piglets, but not those denied access to creep food, between 21 and 32 days of age. Friend et al. (1970) reported a 12% increase in pancreatic trypsin concentration in pigs fed creep food between one and three weeks of age, and Shield et al. (1980) found a higher pancreatic amylase but not protease activity in piglets fed creep food before weaning at four weeks of age. Aumaitre (1971) suggested that earlier development of sucrase, maltase, amylase and trypsin activities might be induced by encouraging earlier consumption of non-milk sugars, starch and protein. In general it remains debatable whether consumption of creep food before early weaning can significantly influence digestive development, and thus indirectly potentially reduce susceptibility to PWD.

Creep food, intestinal structure and hypersensitivity to diet

As previously indicated, piglets develop a series of changes in small intestinal structure and enterocyte brush-border enzyme activities immediately after weaning (Gay et al., 1976; Kenworthy, 1976; Smith, 1984; McCracken, 1984; Hampson and Kidder, 1986; Hampson, 1986a; Miller et al., 1986). Villus height can be reduced by 25% within 24 h of weaning, and continues to decline until around five days after weaning, after which time it stabilizes. This response could be initiated by a lack of a continuous supply of substrate in the intestine (McCracken and Kelly, 1984), and could possibly be mediated by a temporary reduction in crypt cell production rate as seen in gnotobiotic pigs weaned onto a dry diet (Byrne and Hall, 1984). However, this may not be the whole explanation. Hampson (1983) starved five pigs for two days after weaning, and compared these with other pigs of the same age which were either offered food after weaning or kept with the sow. The starved pigs did not show the usual postweaning crypt hyperplasia as occurred in the other weaned group, but both weaned groups showed similar significant reductions in height of villi and loss of lactase activity. Thus, consumption of food after weaning is necessary for crypt hyperplasia to occur, but lack of postweaning intake (and hence lack of crypt hyperplasia) may not be necessary for postweaning villus atrophy to occur. Crypt depth normally continues to increase steadily over an 11 day period after weaning, at a much greater rate than that seen in unweaned pigs. Continuous absence from the sow is required for these changes to occur, since pigs weaned for two days and then returned to their dam for three days show crypt elongation only equivalent to that of pigs weaned for two days (Hampson, 1983). The greatest relative elongation in crypts occurs in the distal half of the small intestine, and crypts remain long for five weeks after weaning, and probably remain so throughout adult life (Hampson, 1983). These changes almost certainly reflect an increased rate of enterocyte production and turnover (Al-Mukhtar et al, 1982a). Amongst the brush-border enzymes, the maltases (two and three) increase after weaning, alkaline phosphatase is largely unaffected, lactase declines, and sucrase declines to a minimum by five days after weaning and then increases. Sodium-dependent alanine transport into villus enterocytes is comparatively reduced after weaning at three but not at five weeks of age (Miller et al., 1986).

Such changes may temporarily reduce digestive and absorptive capacity, and may go a long way to explaining the increased susceptibility of the newly-weaned pig to diarrhoea. Changes in relative maturity of villus enterocytes in newly-weaned pigs may also explain their altered susceptibility to enterotoxins. Stevens et al (1972) demonstrated that 38-day-old pigs weaned for three days showed a greater secretory response to both LT and ST than did unweaned pigs of various ages. Furthermore, STa is active in the intestines of neonatal pigs, but not those of weaned piglets, whilst STb is active in both groups, but more active in weaned animals (Burgess et al., 1978;

Morris and Sojka, 1985). Alterations in surface properties of enterocytes after weaning may also influence the adhesion of ETEC; for example enterocytes of the newly-weaned rabbit show development of a receptor for the D-1-5 pili of *E. coli* RDEC, which is an enteric pathogen of the weaned rabbit (Cheney and Boedeker, 1984).

Many mammals appear to undergo increases in crypt cell production rates, increases in sucrase activity and decreases in lactase activity around the third week of life (Moog, 1979). These changes are generally thought to be under the influence of hormones of the adrenal cortex and thyroid gland, and in the young pig successful attempts have been made to induce precocious maturation of certain digestive enzymes by oral treatment with tri-iodothyronine and prednisolone (Bainter and Nemeth, 1982). The more marked alterations which occur within the first five days of weaning pigs when they are three weeks of age are probably superimposed upon normal developmental changes. An understanding of the aetiology of these changes could help in the formulation of control measures for PWD. Suggestions as to all or some of their causes have included the toxic action of bacterial metabolites such as those produced by microbial decarboxylation and deamination of amino acids in the diet (Hill et al., 1970; Kenworthy, 1976), a poor and irregular supply of nutrient after weaning (Kelly et al., 1984), the action of rotaviruses (Lecce et al., 1982), or intestinal hypersensitivity to dietary antigens.

The latter intriguing possibility was raised after Stokes et al. (1981) elicited a delayed-type hypersensitivity response to ovalbumen in the intestines of unweaned pigs, and noted villus atrophy and crypt hyperplasia similar to that normally seen after weaning. Subsequently, these workers formulated an hypothesis that the previously unexplained intestinal changes which occur after weaning are the result of intestinal hypersensitivity to antigenic material in the weaner diet, and obtained evidence supporting this proposition (Miller et al., 1982, 1983, 1984 a,b,c,d; Newby et al., 1983, 1985; Hampson, 1986b; Bourne, 1986; Bourne et al., 1986; Stokes et al., 1986). They suggested that small intakes of creep food before weaning "primed" hypersensitivity, whilst large intake (> 600g) prevented it by establishing a state of immunological tolerance to the dietary antigen. Abrupt weaning without prior consumption of creep
food was also protective, because the pigs were not primed to the dietary antigens. A brief summary of experiments and observations supporting this hypothesis are:

(i) Piglets which were exposed to creep food between seven and 10 days of age ("primed") developed earlier and more prolonged shedding of ETEC, more diarrhoea, and more depression in their ability to absorb xylose after weaning than did piglets given creep food from seven days until weaning at 21 days of age ("tolerized"), or others given no creep food before weaning ("unprimed").

(ii) Piglets were given cows' milk as a supplement from seven days of age, and were then weaned onto diets which had either casein or hydrolyzed-casein as the protein source. The hydrolysed-casein was shown to be considerably less antigenic than the native casein. Only pigs weaned onto the casein diet developed diarrhoea (in the absence of ETEC). When killed 10 days after weaning the pigs on the hydrolyzed-casein diet also had lesser increases in crypt depth at a site 75% along the small intestine, and greater brush-border sucrase activities in the distal small intestine than did pigs fed the casein diet.

(iii) Pigs killed five days after having been abruptly weaned onto a diet in which the protein source was hydrolyzed-casein (hypoallergenic) had shorter crypts (differences significant at sites between 50 and 75 per cent along the small intestinal length) and greater brush-border lactase and sucrase activities than did animals receiving a diet containing casein as the protein source (Figure 10).

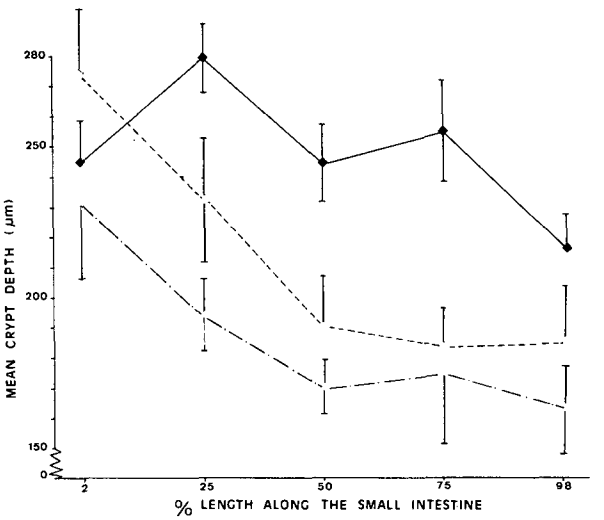
(iv) Pigs on a commercial farm where PWD was a problem performed better and had less diarrhoea after creep feed was no longer offered before weaning (abrupt weaning).

(v) Pigs weaned onto a diet containing a full-fat soya protein extract developed, by five days after weaning, a cutaneous delayed hypersensitivity response to soya extract injected intradermally into the ear; this coincided with maximal depression of ability to absorb xylose after weaning. Adequate feeding of the soya antigen before weaning apparently completely abrogated malabsorption, crypt hyperplasia and diarrhoea after weaning.

(vi) In weaned pigs, enterocyte brush-border alkaline phosphatase activity did not decline by five days after weaning, whereas disaccharidase activities did (Miller et al., 1986). This pattern of activity was similar to that seen in the intestine at sites where the gut immune system is activated (Smith, 1985).

(vii) The general observations that pigs weaned at three weeks of age are more susceptible to PWD than those weaned at five weeks. Young pigs may have insufficient time and opportunity to develop tolerance to dietary antigen, and also have limited proteolytic activity of the stomach and pancreas to cleave ingested proteins to small and less antigenic peptides and amino acids.

Figure 10. Crypt depths along the small intestine in 26-day-old piglets; Δ - Δ , unweaned not receiving creep food;, ♦ - ♦, weaned for five days onto a diet containing casein as the protein source; ◊ - ◊, weaned for five days onto a diet containing hydrolyzed-casein as the protein source (data from Hampson, 1986b). Error bar = 1 sd.



These results and observations have generated considerable interest, but some experiments can be criticized because of a lack of information about relative food intakes on the various treatments, and as yet there have been no direct measurements of hypersensitivity in the small intestine. This latter information may be difficult to acquire since the only indicators of intestinal cell-mediated hypersensitivity reactions may be increases in intra-epithelial lymphocyte numbers and increased rates of cell production, which are mediated by lymphokines (Mowat and Ferguson, 1981). Another problem is the lability of the small intestine, which adapts readily and rapidly in only a limited number of ways to a variety of different stimuli, whether they be microbiological, hormonal or immunological.

As regards the experiments with hydrolyzed-casein diets, other possible explanations for their protective effect on the intestinal mucosa besides reduced antigenicity must be considered. For example, hydrolyzed-casein is known to be more readily digestible than is casein, and less is likely to reach the distal small intestine and colon (Morin et al., 1980). Hence if small peptides and amino acids in the intestinal lumen were able to stimulate crypt cell production directly (the luminal nutrition hypothesis; Diamond and Karasov,1983), a similar outcome would result. Alternatively a hydrolyzed-casein diet could effect other aspects of intestinal physiology. For example, the partial hydrolysis of both milk and soya-proteins in the diet has resulted in reductions in trypsin and chymotrypsin activities in the duodenum of pigs to which the diets have been fed (Leibholz,1981); diets containing unhydrolyzed soya-protein, which result in poor performance after weaning, may do the reverse and increase trypsin and chymotrypsin activities in the intestinal contents (Efird, 1982a). Since pancreato-biliary secretions are believed to have trophic effects on the gastro-

intestinal mucosa (Altmann, 1971), the extent of stimulation of their release could be another mechanism by which different diets affect the mucosa after weaning. Another possible explanation for the results could be that the hydrolyzed-casein diets stimulated less release of enteroglucagon, which is a peptide-hormone acting on the intestinal mucosa. Food reaching the lower Ileum stimulates release of this peptide, which in turn increases crypt cell production rate (Al- Mukhtar et al., 1982b). A final possibility for the results obtained with the hydrolyzed-casein diets could involve insufficient substrate arriving in the distal small intestine for stimulation of "excess" production of bacterial products such as polyamines, which might normally stimulate enterocyte turnover (Hill et al., 1970). The latter explanation seems unlikely however, since oral te tracycline treatment to supress the normal microbial flora did not prevent crypt hyperplasia in weaned pigs (Hampson, 1986b).

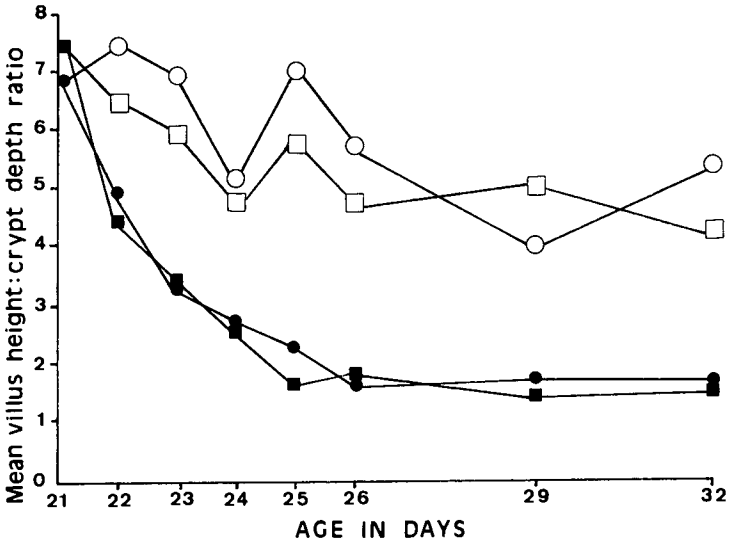
Other workers have cast doubt on the hypothesis of hypersensitivity to dietary antigens, at least as it stands at present. Fowler and Fraser (1985) used eight different preweaning feeding strategies in an attempt to either sensitize or tolerize piglets to their weaner diet, however the only treatment difference was a tendency for the abruptly weaned pigs to have diarrhoea for longer than any of those given creep food before weaning. Kelly et al. (1986) found that abrupt weaning did tend to lessen the severity of PWD, although a large creep intake before weaning at two weeks of age was not protective. Byrne and Hall (1984) "weaned" germ-free piglets onto a solid diet at 23 days of age, after having prefed one group with 45 g of this material at 10 -12 days of age ("priming"). Both weaned groups then showed decreased crypt cell production rates compared with milk-fed controls. The primed piglets developed low serum passive haemagglutination titres to soyabean antigens, and had higher numbers of immunoglubulin-containing cells in their lamina propria, but there was no evidence of a hypersensitivity response in their small intestines. The lack of crypt hyperplasia in the "weaned" germ-free animals, whether or not they were primed, suggests that a normal intestinal flora is necessary for mucosal changes to occur. This flora could act simply by increasing overall immune reactivity in the gut. Exposure of germ-free pigs to the normal microbial flora of a piggery is itself sufficient to cause villus atrophy and increased rates of crypt cell production (Kenworthy and Allen, 1966b).

In studies of the small intestinal mucosa and its brush-border enzyme activities in pigs after weaning, no differences were seen between groups of pigs which were either abruptly weaned or offered creep food before weaning (Hampson and Kidder, 1986; Hampson, 1986a). In other experiments where piglets were fed various levels of creep food before weaning, no postweaning differences were seen between groups in growth rate, excretion of ETEC or rotavirus, or in ability to absorb xylose (Hampson and Smith, 1986). PWD did not occur in abruptly weaned pigs, but this was associated with small postweaning dietary intakes on this regimen. In a further experiment in which pigs were either not offered creep food, or forcibly "primed" with one of two diets before weaning, group effects were again not seen in any of the above parameters, nor in villus height or crypt depth in the small intestine (Hampson et al., 1988). These results suggest that feeding creep food does not influence intestinal changes occurring after exposure to the weaner diet, and therefore suggests that the hypersensitivity hypothesis is incorrect, or requires modification.

In summary, whilst changes in small intestinal mucosal structure and enzyme activities after weaning seem likely to be involved in predisposing to PWD, there still remains doubt as to their precise aetiology. Although diet is important, work with germ-free gnotobiotic piglets suggests that a normal flora is necessary before a change of diet will stimulate intestinal changes, even if there is "priming" with creep food. Evidence for the intestinal reactions being "primed" by creep food in conventional pigs is equivocal.These results do not exclude the possibility of an interaction between diet and normal flora causing changes directly (as suggested by Kenworthy, 1976), and such changes could be mediated by immunological reactions to both components. The decline in villus height to crypt depth ratio ceases around five days after weaning, stabilizes (Figure 11), and then is followed by a "normal" postweaning increase in sucrase activity (Hampson and Kidder, 1986; Hampson, 1986a). A lack of reversion to preweaning values suggests that there is a stable transition to an 'adult-type' intestine after a brief hiatus associated with and exaggerated by weaning at an early age. As pointed out by Bourne (1986), this stable adult pattern could result from

a continuous low-level exposure to new antigenic dietary material in the lumen of the intestine.

Figure 11. Villus height to crypt depth ratio at a site 25% along the small intestine in pigs killed between 21 and 32 days of age; O-O, unweaned, offered creep food; □-□, unweaned, not offered creep food; ●-●, offered creep food before weaning at 21 days of age; ■-■, not offered creep food before weaning at 21 days of age (data from Hampson, 1986a).



CONCLUSIONS AND PRACTICAL IMPLICATIONS

PWD is a complex disease, essentially caused by the activity of certain strains of ETEC which proliferate in the small intestine after weaning. Very occasionally diarrhoea occurs in the absence of ETEC and other infectious agents, but it is usually mild and probably associated with malabsorption and/or changes in gastrointestinal motility. The ETEC which can cause PWD are certainly present in many piggeries, yet the occurrence of disease is very variable. There are many potential risk factors associated with the development of PWD, and where disease occurs all of these should be examined, and improvements made where possible.

Various aspects of the weaner diet can influence susceptibility to PWD. It should be borne in mind that young weaned pigs have a digestive system that is far from mature, with temporary reductions in this limited digestive and absorptive capacity occurring in the immediate post-weaning period. Under these circumstances it is not surprising that presenting large quantities of poorly digestible diet can predispose to diarrhoea. If the diet is not of high digestibility and there is reluctance to improve it, it appears that restriction of access to it is the most effective means of control of PWD. Improved growth rates can be obtained if the diet is highly digestible, milk-based and (ideally) fed in liquid form at regular intervals. Paradoxically, if PWD becomes a problem with such a diet because of overconsumption, then addition of some fibre may be beneficial. At present it would seem best to avoid high levels of non-milk sugars and of low quality protein in the weaner diet, and especially to be cautious with the use of soya protein in the first 10 days after weaning. If some form of hypersensitivity to diet (manifest as increased crypt cell production rates) does prove to be an inevitable manifestation of consuming an adult-type diet, it would seem sensible to avoid highly antigenic dietary material in the critical period immediately after weaning. As regards creep feeding, this procedure is probably not justified for pigs weaned at three weeks of age; creep food is expensive, largely wasted and appears to give little physiological response by three weeks of age. Its potential for priming intestinal hypersensitivity, although far from proven, must also be considered.

Further investigation is required into the relationship between form and type of diet at

weaning, proliferation of ETEC and occurrence of PWD. A better understanding of the condition will help when devising control measures, and the information may have wider relevance for reducing severity of other enteric conditions, improving utilization of the diet, and increasing growth rates. In particular more needs to be known about:

(i) The possible role of hypersensitivity to dietary antigens in predisposing to PWD.

(ii) Whether or not the small intestinal mucosal changes which occur after weaning can be modified by practical diets or dietary regimens.

(iii) If and how it is that intestinal structural changes occurring after weaning predispose to proliferation of ETEC (e.g. appearance of new receptors for the bacteria; malabsorption and selective use of unabsorbed substrate by ETEC etc).

(iv) Whether or not the normal intestinal bacterial flora has a role in mediating intestinal structural changes and/or regulating ETEC numbers after weaning.

(v) The mechanisms by which weaner diets which are based on cows' milk fed in liquid form may act to inhibit proliferation and adhesion of ETEC.

ALTERNATIVE APPROACHES TO TREATMENT OF POSTWEANING SCOURS

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INTRODUCTION

Attachment of bacteria to mucosal surfaces is often a key first step in pathogenesis (Beachey, 1981). Before bacteria are able to overcome the usual cleansing mechanisms of such surfaces, a complex array of physicochemical factors must be brought to bear. These range from non-specific electrical charge or hydrophobic interactions, to formation of very specific chemical bonds between appendages called adhesins on the bacterial surface, and coupling molecules called receptors on the mucosal surface (Jones and Isaacson, 1983).

The K88 adhesin of *E.coli* was first recognized as a surface antigen on strains of *E.coli* isolated from diarrhoeic pigs (Orskov and Orskov, 1966). Originally thought to be a polysaccharide capsular ("K") antigen, it was later shown to be a filamentous protein (Stirm et al., 1967) with a functional role as an adhesin (Jones and Rutter, 1972).

Subsequently it was demonstrated that some pigs lacked the receptor for K88 adhesin on their brush border membranes and that K88$^+$ *E. coli* were unable to infect these animals. Resistance to infection (lack of the K88 receptor) was also shown to be largely determined at a single allele, by a recessive gene (Sellwood et al., 1975; Rutter et al., 1975; Rapacz and Hasler - Rapacz, 1986).

It was easily recognizable that genetic resistance to K88$^+$ *E. coli* should enhance piglet survival. Persistence of the dominant gene (which blueprints expression of the K88 receptor and hence disease susceptibility) would therefore appear to be anomalous when the cause of the disease is common in the environment. Persistence of the dominant gene was proposed by Gibbons et al. (1977) to be linked with fluctuating immune responses to K88$^+$ *E. coli* within pig herds. As the immune status of the herd improves with respect to K88$^+$ *E. coli* (following disease outbreak or oral vaccination) the selective pressure in favour of the recessive gene is decreased. In addition, homozygous recessive (K88 resistant) dams mated to susceptible boars could have poor reproductive performance. Heterozygous offspring born to these dams receive reduced antibody protection, apparently because of lack of immune recognition of K88$^+$ *E. coli* by these dams (Sellwood, 1984a). This scenario may lead to selection pressure against the recessive gene. In addition to such natural selection based on survival, artificial selection based on differences in growth performance may affect persistence of the genes. Differences in growth parameters may result from the difference(s) in intestinal characteristics determined by these genes (Edfors-Lilja et al., 1986; Walters and Sellwood, 1984).

Until recently it has not been possible to determine the K88 phenotype of live animals without cumbersome techniques involving anaesthesia and possibly surgery (Snodgrass et al., 1981). If the K88 phenotype of boars could be identified easily and only boars of K88-non-adhesive phenotype used for breeding purposes, within only a few (3-4) generations, animals of K88-adhesive (susceptible) phenotype would virtually disappear. Such a breeding programme would avoid problems that may be associated with mating resistant sows to susceptible boars.

A research programme to investigate inherited resistance of pigs to K88$^+$ *E. coli* was commenced in 1981. The intention was to develop a practical method for determining the K88 phenotype of live pigs. The programme commenced with development of a new K88: receptor binding assay based on enzyme immunoassay and led eventually to the identification of two avenues whereby improved control of the disease may be achieved. These aspects are discussed below.

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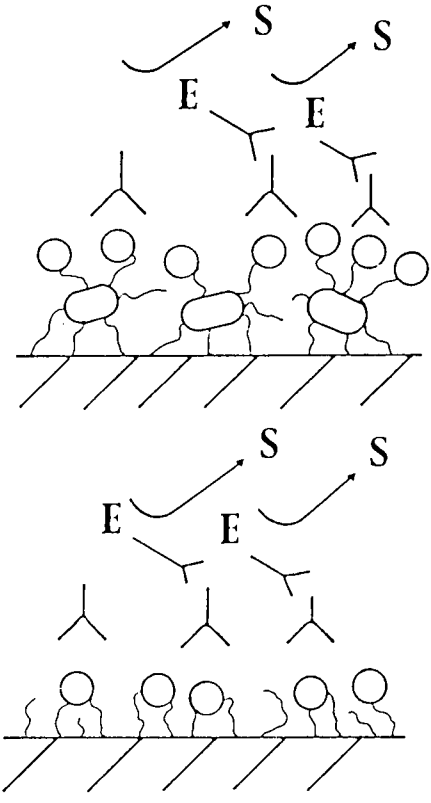
DETERMINATION OF K88 PHENOTYPE

Traditional techniques to determine K88 phenotype require tedious purification of brush border membranes, followed by incubation of these membranes with K88$^+$ *E. coli* and microscopic examination of the mixture for evidence of interaction between bacteria and membrane (Sellwood et al., 1975; Snodgrass et al., 1981).

Attempts to improve these cumbersome techniques proved difficult, although the concept of a simple field test capable of detecting the K88 phenotype of sloughed brush border material in faeces had great appeal. The mucous nature of the intestinal contents and hydrophobicity of the K88 pilus protein led to problems with non-specific binding in most solid phase assays. A technique which was found to give excellent results when intestinal scrapings were tested, was the indirect enzyme immunoassay outlined in Figure 12. The structure of this test is the reverse of that observed *in vivo*, as immobilized adhesin is used to bind free brush border material (as either membrane vesicles or "soluble" receptor). Shear forces exerted during washing steps remove loosely bound intestinal material, resulting in an assay that is both sensitive and accurate. This assay (designated KPEIA, Chandler, 1986) enables intestinal material from large numbers of animals to be phenotyped quickly and easily. The technique has also facilitated demonstration of some new properties of the K88 receptor. Unfortunately, when the faeces of K88-adhesive pigs were tested, no binding activity could be demonstrated.

Figure 12. An indirect EIA to confirm the K88 phenotype of brush border material using K88$^+$ *E. coli* or K88 pili on the solid phase.



STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF THE K88 RECEPTOR

The K88 adhesin has been demonstrated by many researchers to be rather promiscuous in its ability to adhere to cells. Attachment of K88 to red blood cells of several animal species has been

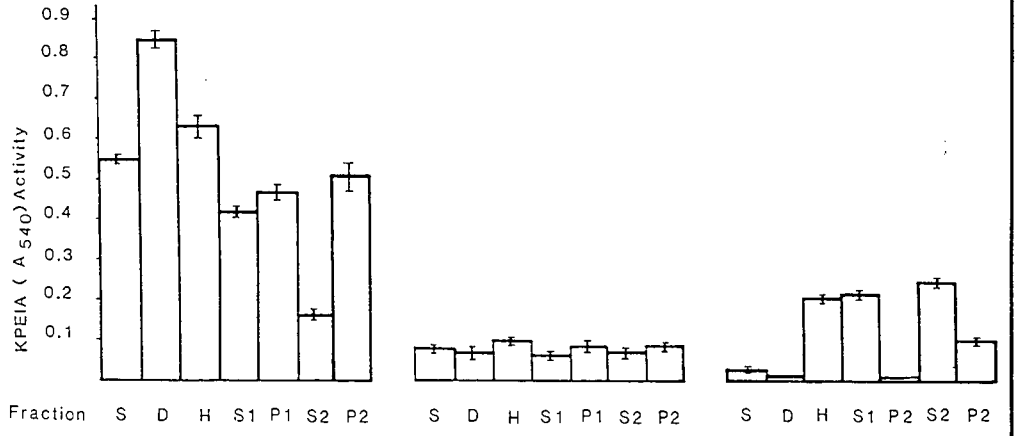
demonstrated, but this attachment has been shown to differ in various ways from binding to brush borders (Sellwood, 1980). Attachment of K88 to intestinal cells of other animals has also been reported (Runnels et al.,1980; Tzipori et al., 1984; Laux et al., 1984). It is not known whether binding in these cases is mediated by a receptor similar to the porcine intestinal receptor.

Several K88-adhesive components of the porcine intestinal mucosa have been isolated, including both glycolipid (Kearns and Gibbons, 1979; Nilsson and Svensson, 1983) and glycoprotein (Staley and Wilson, 1983) fractions. It appears that although a variety of sugar residues may be involved in receptor function, ß-D- galactosyl residues on a mucosal glycoconjugate are important (Jones and Isaacson, 1983; Laux et al., 1986).

Our studies have demonstrated almost complete loss of binding activity following receptor activity detected in the KPEIA is probably glycoprotein-associated. Sellwood (1980), Anderson et al. (1980) and Staley and Wilson (1983) made similar observations in binding studies using radio-labelled pili. When fractionating detergent-solubilized pig brush border membrane material, Staley and Wilson (1983) identified various molecular weight glycoprotein fractions to which K88 pili actively bind. They concluded the receptor was a glycoprotein, which in its native state exists in multimeric forms.

More recently, Laux et al., (1986) demonstrated binding between K88 pili and immobilized mouse intestinal material. K88 adhesin bound to both soluble and insoluble (membrane-bound) fractions from mouse small intestine. Our studies with pig intestinal material have also shown binding to both vesicle-containing fractions of membrane and "soluble" fractions (material not sedimented by centrifugation at 29,000 x g for 20 min). The latter fraction could be obtained from intestinal material taken from some pigs of the either K88 phenotype if the material had been subject to mechanical or osmotic disruption (Figure 13). Binding activity of intestinal material derived from animals of either phenotype was also apparent if the test was conducted at low pH. Material showing this non-specific (not phenotype related) binding was sedimented by further centrifugation of the test material at 85,000 x g for 1h.

Figure 13. Activity in the KPEIA of fractions collected during purification of brush border vesicles from three *pig intestines.



* Fractions were obtained from a K88+ adhesive phenotype pig (left-hand group) and two K88-non-adhesive phenotype pigs. Columns and bars represent mean absorbance (A_{540}) of the

substrate solution ± standard error of 6 wells/test.

** Fractions illustrated are as follows: S, intestinal scraping; D, following hypotonic disruption; H, following homogenizing; S1 and P1, supernate and pellet fractions, respectively, obtained following centrifugation at 2,900 x g for 30 min; S2 and P2, supernate and pellet fractions, respectively, obtained following centrifugation at 29,000 x g for 30 min (Chandler, 1986).

On the other hand, material obtained by extraction of K88-adhesive phenotype brush border vesicles with Triton X-100 detergent displayed phenotype-dependent activity in the KPEIA. Activity was retained following removal of the detergent and centrifugation at 85,000 x g. Receptor, solubilized in this manner, remained sensitive to protease. When affinity purified antibody, specific for the detergent-solubilized K88 receptor fraction was used for detection of brush border binding in the KPEIA, non-specific activity was not observed. Unexpectedly, this modified assay failed to show as K88-adhesive, some pigs that were clearly K88-adhesive by either the microscopic binding assay or the traditional KPEIA. Apparently, in some instances, less specific binding components are important in determining K88 phenotype.

Demonstration of more than one component in K88 receptor activity was consistent with binding and dissociation analyses performed by Sellwood (1980). These studies indicated either negative cooperativity in the binding reaction (i.e. decreased with increased receptor occupancy) or the existence of two receptors, one widely distributed and of low binding affinity and another less widely distributed and with higher affinity.

The recent report of K88 receptor polymorphism, and apparent variations in binding avidity for the three serological variants of the K88 adhesin (Bijlsma et al.,1982; Rapacz and Haster-Rapacz, 1986) further complicate the picture. These observations may help explain the occurrence "weakly adhesive" phenotype pigs (Sellwood, 1984b; Chandler et al., 1986). Such pigs may possess only low-avidity receptors. It appears that animals of this type are not susceptible to infection, but that some offspring of matings between "weakly adhesive" phenotype boars and K88-non-adhesive sows are of typical K88-adhesive phenotype (Sellwood, 1984b).

LOCATION OF THE K88 RECEPTOR

Studies designed to confirm the intestinal location of the K88 receptor by KPEIA revealed that while receptor activity was mainly confined to the small intestine wall, as expected, it was also present in some samples of small intestinal contents. Usually, receptor activity of scrapings was greatest where samples were taken from mid-small intestine (Chandler, 1986).

K88$^+$ *E. coli* infections have been reported to be initiated in the lower small intestine; usually a site of low receptor activity. The infections then progress in an anterior direction until the whole small intestine is colonized (Moon, 1980). It would appear that some factor(s) other than receptor activity encourages proliferation in the ileal end of the small intestine. Possibly differences in peristaltic activity in this zone (Kidder and Manners, 1978), or some other physical or chemical parameter of the lower small intestine contribute to colonization. Studies by Hinton et al. (1985) have indicated that in the absence of colonization by K88$^+$ *E.coli* the intestinal flora of weaner piglets contains a varied mixture of *E.coli* strains, some of which are readily isolated from the ileum. It remains unknown whether an autochthonous population of K88$^+$ *E. coli* in the large intestine, together with low gut motility, can contribute to pathogenesis, or whether proliferation in the lower small intestine can only follow recent ingestion of the pathogen.

In our studies about 50% of pigs exhibited some deviation from the common pattern of maximum K88 receptor activity in the mid-small intestine. Sometimes little or no receptor activity was apparent at one or more intestinal sampling sites of otherwise K88-adhesive phenotype pigs. It appeared that these areas of reduced receptor activity may have been related to zones of intestinal distention produced by food boluses. This observation led to the postulate that pancreatic proteases in the food boluses may inactivate the protease-labile K88 receptor *in vivo*.

Further studies with the KPEIA indicated that K88 receptor activity could be identified in material collected from the stomachs of about half the K88-adhesive phenotype pigs tested. All samples of stomach mucosa were neutralized before being tested. The activity displayed by

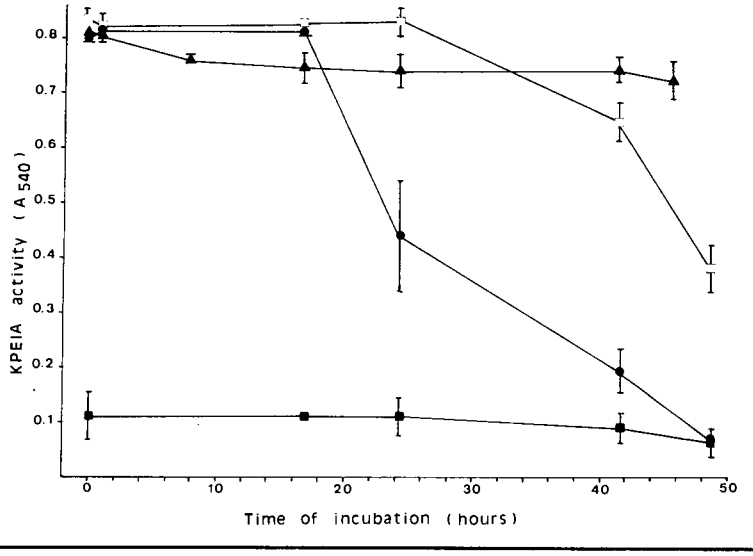
material from the stomach was originally attributed to reflux of receptor-containing small intestinal contents. However, the activity was later shown to mainly be confined to mucosal scrapings taken from the pyloric and (to a lesser extent) cardiac (mucus-secreting) regions of washed stomach linings. Laux et al. (1986) have identified K88 receptor activity in intestinal mucus and reported that such material can be bound so tenaciously to brush borders that it may appear to be an integral part of the membrane. It may be that unbound (mucus-derived) K88 receptor within the intestines of K88-adhesive phenotype pigs may protect the pig from infection by "blocking" the adhesin.

Extensive testing of contents or scraping material from caecum or large intestine failed to demonstrate any KPEIA activity except that some (erratic) receptor activity was observed in faecal material from preweaned pigs. Contact with faeces was shown to destroy receptor activity very rapidly. It is presumed that this inactivation reflects the high proteolytic activity of faecal microflora.

STABILIZATION OF THE K88 RECEPTOR

Experiments in which pancreatic proteases, faeces, or various exogenous proteases were incorporated in the KPEIA prior to addition of K88 receptor-containing material, confirmed previous reports of the protease stability of the K88 adhesin (Klemm,1985) The ability of immobilized K88 adhesin to bind receptor-containing material was not affected by prior exposure to any of these substances. However, when receptor-containing material was exposed to proteases prior to its inclusion in the KPEIA, severely reduced binding activity was observed. Surprisingly, when K88 receptor was bound to the immobilized adhesin prior to exposure of the adhesin:receptor complex to either protease or faeces, the bound receptor remained able to react with the detection antibody (Figure 14). Similar results were observed in test wells overlain with various protease suspensions. Apparently proteases are unable to affect the receptor binding site once the bond with the adhesin has been formed. In addition, sufficient structure appears to remain on the receptor-containing material for the detection antibody to recognize.

Figure 14. Residual KPEIA activity following incubation of diluted faeces* in test wells, after scraping material had been given time to interact with immobilized K88 adhesin. Symbols □ , ● and ▲ represent the reactivity of three K88-adhesive phenotype intestinal scrapings. The symbol ■ represents the reactivity of a K88-nonadhesive intestinal scraping. Symbols and bars represent mean absorbance (A_{540}) and standard error of six wells per sampling.



*Faeces were diluted 1/10 (w/v) distilled water.

FREQUENCY OF PIGS THAT LACK THE K88 RECEPTOR

A recent survey of intestinal scrapings from 4,000 bacon-weight pigs revealed that 25 per cent of pigs in Victoria appear to be of K88-non-adhesive phenotype (Chandler,1986). However, the percentage of K88-non-adhesive pigs within individual herds was found to vary from fewer than 10% K88-non-adhesive to about 40% K88-non-adhesive. Farms with few K88- non-adhesive pigs had recurrent outbreaks of postweaning disease (PWD) associated with K88$^+$ *E. coli.* Subjective evaluations by piggery managers indicated, that at the time of the survey, farms with a high percentage of K88-non-adhesive pigs (>35%) had few problems with PWD.

BACTERIAL ECOLOGY OF POST WEANING DISEASE

Hinton et al. (1985) observed that the normal complex pattern of *E. coli* strains in the lower bowel was lost when a haemolytic (K88$^+$) *E. coli* strain appeared in the intestinal flora of weaned piglets. The K88$^+$ strain rapidly dominated throughout the intestine. Colonization was often independent of clinical illness. The onset of disease was often attributed by these authors to transient hypersensitivity to dietary antigens.

Our observations on Victorian piggeries have indicated a similar, but slightly more complex picture. These observations include the following notable points:

(i) Usually more than one strain of haemolytic *E. coli* can be cultured from rectal swabs taken from weaned piglets suffering from PWD on Victorian piggeries, and usually one dominant K88$^+$ and one dominant K88$^-$ *E. coli* strain are apparent.

(ii) On some piggeries the dominant strains have remained similar over periods of many years.

(iii) When K88$^-$ haemolytic *E. coli* are present, they are commonly the only strain evident in faecal swabs taken from dead pigs.

(iv) Both types of haemolytic *E. coli* appear in the faeces within 24h of weaning (as observed by Hinton et al., 1985) and both may be excreted in large numbers by apparently healthy piglets.

(v) Despite the early appearance of both types of haemolytic *E. coli* after weaning, the pattern of growth of the two types thereafter is often quite different. Usually K88$^+$ *E. coli* exhibit the most rapid increase in number and are most commonly associated with disease that occurs within the first week after weaning. K88$^-$ strains, on the other hand, usually attain their greatest prevalence during the second week after weaning. These strains may be associated with deaths of piglets 6-7 weeks after weaning.

A rapid build-up in the prevalence of haemolytic (particularly K88$^+$) *E. coli* after weaning appears to correlate with subsequent outbreaks of PWD. If these results are verified, there may be scope for developing a simple means of predicting groups of weaner piglets at risk of PWD.

(vi) Disease symptoms associated with haemolytic *E. coli* vary considerably, probably because of expression of different toxins by different strains. K88$^+$ strains are usually associated with acute dehydrating scours. Symptoms associated with the K88$^-$ strains, on the other hand, may include chronic scouring or sudden death; sometimes death may occur without apparent scouring. Oedema and/or neurological disorders may be apparent prior to death.

(vii) Monitoring of *E. coli* excretion throughout the weaning period, and subsequent K88 phenotype testing of slaughtered pigs, have confirmed that phenotype has a profound influence on the faecal microflora following weaning. K88-non-adhesive animals invariably pass very few K88$^+$ *E. coli* (as expected). Excretion of K88$^-$ *E. coli* is apparently unaffected by K88 phenotype, but the incidence of scouring associated with this type of *E. coli* appears to be lower in animals of K88-non-adhesive phenotype. Recent studies of the K88 phenotype of piglets that died during an extremely bad outbreak of PWD on one commercial piggery has indicated that this observation may not apply in all cases.

It should be noted, however, that the assessment of K88 phenotype is always suspect in pigs that have died of PWD, as intestinal damage and blocking of binding by free adhesin or anti-adhesin antibody may affect the binding reaction.

(viii) Culture of intestinal material from piglets that have died from PWD has indicated a much higher incidence of dual infections by haemolytic strains of both K88$^+$ and K88$^-$ *E. coli*, or by haemolytic *E. coli* in conjunction with other intestinal pathogens, than had previously been indicated by culture of rectal swabs.

APPLICATION OF THE LOCAL RESEARCH FINDINGS

The PWD research programme to date has centred on the K88$^+$ group of haemolytic *E. coli*, largely because at the time of commencing the studies the impact of the K88$^-$ group was less fully appreciated. The research programme has been quite successful nevertheless, as two completely new directions for control, or even eradication, of the disease attributable to the K88$^+$ group have been indicated.

K88 resistance breeding

For the first time, the stability of the adhesin:receptor complex has been demonstrated, giving a potential mechanism for transporting serologically identifiable K88 receptor through the hostile environment of the large intestine. This knowledge has been exploited by development of an orally-dosed hollow plastic capsule which is able to trap and protect the K88 receptor. Test capsules are provided with small holes so as to allow entry of sloughed intestinal material (probably likely to contain sloughed K88 receptor, if this is present). The receptor is bound and stabilized on the internal surface of one end of the capsule by K88 pili which are in turn covalently attached to the plastic surface. The small entry holes in the capsules permit only limited entry of lower bowel material. An enteric coating over the external surface of the capsule prohibits entry of stomach contents. Capsules retrieved from faeces are opened, placed in storage buffer and, when convenient, tested for presence of bound brush borders by KPEIA procedures. Unfortunately this test yields many non-reactive and weakly reactive capsules from K88$^-$ adhesive phenotype pigs (false negatives in order of 30-70% can be expected). If sufficient capsules are retrieved from the faeces, a suitably reliable typing can still be obtained. False-positive tests (reactive capsules from K88-non-adhesive phenotype pigs) do not appear to be a problem.

Exclusive use of K88-adhesive boars for three or four generations would leave an insignificant number of susceptible animals within the herd and a permanent reduction in postweaning scours should be achieved.

Two problems remain to be solved:

(i) The commercial worth of K88-non-adhesive phenotype herd over a mixed phenotype herd is not known. The value of K88-non-adhesive pigs will only be known when a commercial "non-adhesive"" herd is established and evaluated. This will entail elements of risk, cost and inconvenience for the farm concerned.

(ii) Typing pigs with the "Heath-Robinson" capsules currently in use is labour-intensive. The cost of setting up a commercial K88-non-adhesive herd would be $3,000-4,000. The cost of manufacturing the plastic dies to mass produce capsules more suitable for the job is high, probably over $25,000. Once such a capsule is available, phenotype testing would cost very little.

Use of a protease feed additive

The protease susceptibility of the K88 receptor, whilst making it difficult to obtain identifiable receptor outside the animal, also presented us with a possible mechanism for destroying activity of the receptor *in vivo*. Protease activity is normally low in young pigs, rising to "normal" levels of pancreatic activity by about 5 weeks of age (Kidder and Manners, 1978). The low protease activity at birth facilitates uptake of active maternal antibody but leaves the bowel in a 'receptor-active' state. This may be a factor associated with the susceptibility of neonatal piglets to infection by K88$^+$ *E. coli*. In older pigs, intestinal (pancreatic) protease activity is initiated by entry of food boluses into the intestine. Food intake and intestinal motility are disrupted at weaning,

possibly again providing receptor-active environment within the intestine. It was therefore proposed that an exogenous protease, enteric-coated to protect it whilst in the hostile environment of the stomach, and fed as small particles to facilitate passage through the stomach, may be able to provide improved continuity of protease activity within the small intestine over weaning.

A product which meets the above criteria has been developed by a local pharmaceutical company, Vitapharm Pty. Ltd. The feed additive produced by Vitapharm includes immobilization of the protease on micro-capsules to provide greater enzyme stability. Preliminary evidence suggests that this additive will reduce the susceptibility of K88-adhesive phenotype weaner pigs to infection over the critical weaning period. The product, if proved effective will provide a vaulable alternative to antibiotic medication or resistance breeding.

The aim of current and future research in our laboratories is to extend the "host-based" research approach to the haemolytic K88^{-} strains of *E. coli*. Hopefully these studies will lead to further practical approaches to treating PWD.

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PROTECTION OF SUCKLING PIGLETS FROM DIARRHOEA CAUSED BY ENTEROTOXIGENIC ESCHERICHIA COLI BY VACCINATION OF THE PREGNANT SOW WITH RECOMBINANT DNA DERIVED PILUS ANTIGENS

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Enterotoxigenic strains of *Escherichia coli* (ETEC) cause severe fatal diarrhoea in neonatal piglets by colonizing the mucosa of the small intestine and producing exotoxins which disturb the fluid-electrolyte balance of the enterocytes. Bacterial surface proteins, called fimbriae or pili, mediate close attachment to enterocytes thereby enabling rapid absorption by the cell of the bacterial enterotoxin. A few pilus types have been associated with these ETEC and it has been demonstrated that immunity in the piglet to a limited range of pili (K88, K99, 987P) will confer resistance to a large number of ETEC serotypes (Rutter et al., 1976; Morgan et al., 1978). A purified pilus vaccine** has been developed in which the pilus anti-gens are derived from recombinant non-toxigenic K12 strains of *E. coli* (Clark et al., 1985).

In a challenge trial, commercial sows were inoculated intramuscularly twice during pregnancy with the pilus vaccine or a placebo vaccine (controls). Piglets born to these sows were allowed to feed naturally. The piglets were inoculated with 1.5-5.5 x 10^9 viable piliated ETEC within 12 hours of birth. Selected piglets from both vaccinated and control litters were necropsied 16 to 28 hours after challenge depending on presence and severity or absence of scouring.

Severe watery diarrhoea was evident in all control piglets within 12 hours of challenge. Most of these piglets became dehydrated, depressed and lost body condition within 36 hours. By contrast, the majority of piglets suckling vaccinated sows did not scour; of those that did, only a transient looseness was observed. The difference in incidence of scouring between the two groups was significant ($X^2_1 = 57.7, P< 0.001$). Coliform bacterial counts of mucosal scrapings of small intestine were significantly lower in vaccinated piglets ($t = 5.64, P< 0.01$).

Microscopically, extensive adhesion of coliform bacteria to the mucosal epithelium of all control piglets was apparent, while there was little or no coliform attachment in vaccinated piglets. No virus involvement in the disease syndrome was implicated on the basis of digestive enzyme (lactase) assays and histopathology. Specific anti-pilus antibody titres were significantly elevated in serum and colostrum of vaccinated sows and in serum of their piglets compared to the placebo-inoculated sows and their piglets (t-test, $P< 0.001$).

It is concluded from these results that piglets obtaining adequate amounts of colostrum from vaccinated sows will be protected from piliated ETEC colonization of the small intestine and from the resultant diarrhoea.

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FIELD TRIALS WITH A RECOMBINANT DNA SCOURS VACCINE

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Neonatal colibacillosis due to enterotoxigenic *E. Coli* is a disease of considerable economic importance to the pig industry. Infection in the first three days of life causes severe diarrhoea, dehydration and high mortality. Pathogenicity is primarily related to the organism's ability to adhere to the intestinal epithelium by means of attachment factors (pili), and to the elaboration of the toxins ST and LT (Bywater, 1981).

The organism is ubiquitous and newborn piglets have little protection against disease at the time of infection. The development of a genetically- engineered polyvalent vaccine providing high levels of passive immunity against pilus attachment antigens is a practical means of prevention of the disease in neonatal piglets (Clarke et al., 1985).

Adequately testing such a vaccine for registration is difficult due to the cyclic nature of disease prevalence, the need to maintain adequate contamination levels, potential genetic K88 resistance, the age of the sow and cross-fostering.

In these trials we attempted to minimise these factors by vaccinating only one-third of the available animals, and randomizing gilts, parity-one sows and older animals separately. No other forms of treatment or cross-fostering were allowed.

The results of the trial are presented in Table 1. Piglets were observed daily and scour incidence, severity and deaths were recorded, together with reproductive parameters of the sows. There were no differences between treatments for litter size or birthweight. Piglet scour parameters were significantly improved in the treatment group.

Table 1. Morbidity and mortality due to scours in suckling piglets nursing vaccinated or unvaccinated (control) sows. Results of 3 vaccine trials.

	Treatment	No.of Sows	Scour Days Per Litter	Scour Days Per Piglet	Mean Deaths Due to Scours Per Litter 0-3 Days Old	Mean Deaths Due to Scours Per Litter 3 Days Old To Weaning
Piggery A	Control	32	11.75[b]	1.17[c]	0.37	0.71[a]
	Vaccinated	18	0.05[b]	0.04[c]	0.00	0.06[a]
Piggery B	Control	60	10.24[c]	0.99[c]	0.02	0.15
	Vaccinated	30	2.07[c]	0.21[c]	0.00	0.07
Piggery C	Control	56	10.08[c]	0.98[c]	0.44[b]	0.17
	Vaccinated	29	1.14[c]	0.10[c]	0.00[b]	0.03

Values with similar superscripts are significantly different at the levels noted below:
[a] $0.01<P<0.025$; [b] $0.001<P<0.01$; [c] $P<0.001$

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STUDIES ON THE INCIDENCE AND INDUSTRY SIGNIFICANCE OF PORCINE LEPTOSPIROSIS

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Infection with *Leptospira interrogans* serovar *pomona* is widespread in Australian pigs and can cause abortions and stillbirths in susceptible sows. Other serovars may be involved to a lesser extent. Human leptospirosis is a zoonosis principally associated with abattoirs at which pigs are slaughtered. Some piggeries use leptospirosis vaccines to protect breeding females against reproductive losses.

Insufficient information is available about the incidence and economic importance of porcine leptospirosis in Australia, and about the practical effectiveness of vaccination programs. This is partly because the disease is frequently asymptomatic. It is also partly due to inadequacies in the available diagnostic procedures, and so we have set out to develop, evaluate and apply a number of new techniques. Those that have proved most valuable have been an IgM enzyme immunoassay (EIA) for antileptospiral antibodies (Ballard et al., 1984), DNA hybridisation (DNAH) for detecting leptospires in urine (Millar et al.,1987), and immunogold silver staining (IGSS) for visualizing leptospires in infected kidney (Skilbeck and Chappel, 1987).

A total of 842 sera collected from three Victorian abattoirs have been tested by IgM EIA and 13% were positive. Macroscopic kidney lesions, known as "white spotting", are widely regarded as indicative of leptospirosis. Of 63 sera from animals with such lesions, 41% were positive by IgM EIA, and 65% of 31 affected kidneys had leptospires demonstrable by IGSS. On the other hand neither the presence nor the absence of white spotting is a reliable indicator of leptospirosis status.

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COPPER RESISTANT ESCHERICHIA COLI AND POSTWEANING DIARRHOEA IN PIGS

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Piglets are commonly exposed to copper-supplemented feed after weaning and it may be that this practice exacerbates the problem of postweaning diarrhoea in some situations.

Plasmid-determined resistance to copper has been demonstrated previously (Tetaz and Luke, 1983). Some enterotoxigenic *Escherichia coli* (ETEC) isolated from scouring pigs have now been shown to be resistant and the determinants of resistance shown to be transferable *in vitro* to laboratory strains of *Escherichia coli.*

When 79 *E. coli* isolates from cases of suspected colibacillosis (Links, 1977) were examined by gene probe analysis and conventional procedures for copper resistance, toxigenicity and adhesin production, 12 hybridized with the copper resistance probe. Of these, three produced Shiga-like toxin (two also produced ST 1a). Nine of the 12 produced LT and/or ST 1a. Six of these nine produced K88 antigen (five isolates were of sterotype 0149:K91, 88ac : H10 and one of sterotype 08:K87,88ac:H19).

Given the association of copper resistance and virulence in some strains of ETEC, management implications of feeding copper-supplemented rations to weaners need to he considered. Aumaitre (1981) has reported that high levels of copper (250 ppm) are favourable for growing finishing pigs but appear to be inconsistently efficient for piglets up to 20 kg. Braude (1981) has claimed that there is no firm evidence available on the effect of feeding copper to pigs below 20 kg live weight. Considering the risk of selecting resistant pathogens at the critical time of weaning, it may be appropriate to omit copper from weaner diets. Investigations are continuing to determine whether the inclusion of copper in pig diets leads to a build-up of resistant organisms in the piggery environment.

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APRALAN - A NEW AMYNOCYCLITOL ANTIBIOTIC FOR THE CONTROL OF COLIBACILLOSIS AND SALMONELLOSIS

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Colibacillosis and Salmonellosis are severe, often fatal diseases affecting all animal species. In pigs they most often manifest as severe diarrhoea with high morbidity and variable mortality in neonates and weaners. Septicaemic and toxaemic forms are also recognised. Because disease control is often difficult, feed or water medication may be used to control clinical cases.

Apralan (apramycin, Elanco) is an aminocyclitol antibiotic produced by *Streptomyces tenebrarius*, and is related to tobramycin, kanamycin and neomycin. The activity of apramycin includes *Salmonella spp.*, *Escherichia coli*, *Proteus spp.*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. Apramycin is bactericidal, inhibiting bacterial protein synthesis (Perzynski et al., 1979) and is rapidly absorbed. It has a low order of toxicity and is not ototoxic in pigs. It is used in pigs and calves for the treatment of colibacillosis and salmonellosis.

A trial using Apralan feed premix was conducted in a large NSW piggery with a history of post weaning scours. Two hundred and forty pigs were randomly allocated to the treatment groups (Apralan 80ppm and control) in a randomized block design. Weight gain and feed conversion were recorded at the beginning and end of the 21-day treatment period. Scour incidence and severity were observed daily, and no other prophylactic or therapeutic medication was provided.

A summary of the results is given in Table 1. Scour parameters were significantly reduced in the treatment group, and growth parameters significantly improved ($P<0.01$).

Culture and sensitivity tests performed on swabs from piglets scouring during the trial period confirmed the presence of haemolytic and non- haemolytic *E. coli* sensitive to apramycin using a disc diffusion test. Feed assay results showed adequate apramycin levels.

Apramycin was effective in the control of weaner scours when used as an in-feed medication.

Table 1. Apramycin administration (80 ppm) in a large commercial piggery.

Treatment	Average Wean wt/ Pig (kg)	Average Fin.wt/ Pig(kg)	Average Gain/Pig (kg)	Piglets Scouring[a] (%)	Duration of Scour[b] (days)	Total Deaths	A.D.G.[c] (kg/day)	F.C.E.[d]
Control	5.8	8.5	2.7	13.5	8.7	1.0	0.127	2.6
Apramycin	5.8	10.8	5.0[e]	1.0[e]	2.0[e]	0.0	0.239[e]	1.5[e]

[a] Prevalence of scour in the treatment groups
[b] Average number of days when scours were noticed in the pen
[c] Average Daily Gain (kg/day)
[d] Feed Conversion Efficiency (feed: gain)
[e] Significant at $P<0.01$

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RECENT DEVELOPMENTS IN VACCINATION FOR PORCINE PARVOVIRUS

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Porcine parvovirus (PPV) is probably the most common aetiological agent of infectious reproductive failure in pigs. The virus causes embryonic death or death in foetuses which are not immunocompetent. These foetuses are usually less than 70 days gestation. A viraemia is a prerequisite for transplacental infection. To control the disease, the animals to be used for breeding need to have an active immunity to the virus. The safest and most efficient method is by vaccination. The cost effectiveness of vaccinating for this disease have been described by Parsons et al. (1986). They claim that even expensive vaccines are cost effective.

A project on vaccination for porcine parvovirus at James Cook University has resulted in the development of a range of diagnostic tests for monitoring the disease and the immune responses following vaccination.

Field trials have now been carried out on nine farms using a vaccine supplied by a local manufacturer. Immune responses and breeding performance in all herds have been closely monitored. Two vaccination strategies have been used : 1) where gilts selected for breeding are given two doses, one month apart at least two weeks before the first service; 2) an additional dose is given at each weaning. The most desirable strategy is one which keeps the costs down by using as few doses as possible.

The programme has now been running in excess of one year. The results in Table 1 indicate that animals are responding serologically to the vaccination. Animals are now being experimentally challenged to determine the ability of the vaccine to prevent transplacental infection. Natural outbreaks of PPV infection have not occurred in any of the herds under study.

Table 1. Serological responses following vaccination of two local herds.

Number of doses of vaccine	ELISA titre	HI titre (Log_2)
0	1.48	0.29
1	4.29	1.03
2	13.2	4.04

Results of work with other vaccines suggest that vaccinated animals with very low or even undetectable titres may be protected (Edwards et al., 1986). The vaccines apparently work by preventing the viraemia.

Work on the design of a vaccine which should satisfy all of the demands of the industry is well under way. An ideal vaccine should need few doses to produce high titre responses and be unaffected by maternal antibody. It should be inexpensive. Muscle damage should not be produced at the site of injection and it should be safe in all classes of vaccinated animals. Commercial development of this product will follow and it is hoped that it can be marketed internationally.

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SEROLOGICAL DIAGNOSIS OF PORCINE PARVOVIRUS BASED ON ELISA

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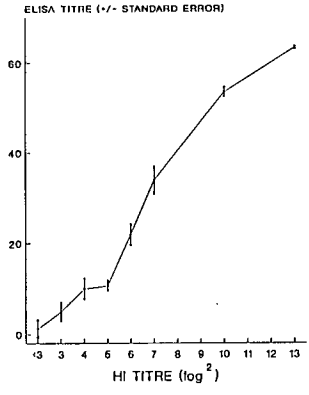
A competitive enzyme linked immunosorbent assay (ELISA) has been developed for the measurement of antibody titres to porcine parvovirus. The test uses a monoclonal antibody against porcine parvovirus structural proteins, conjugated to horseradish peroxidase to compete with antibody in pig serum for attachment to specific sites on the viral antigen.

This assay can be automated and hence can be used for the monitoring of the epidemiology of porcine parvovirus infection. Results for 635 serum samples tested in ELISA and the reference test, haemagglutination inhibition (HI) as outlined in Figure 1 show that the two tests measure similar immune responses.

Figure 1. Comparison of haemagglutination inhibition and ELISA titres



Because of the sensitivity and specificity of the assay, blood collected using absorbent paper may be used for measuring both high and low levels of immunity. This means that it is not necessary to collect serum samples to carry out the test. Typical results are shown in Table 1. Because of the small amount of the sample, the filter paper titres are lower than the corresponding serum titres. They still allow accurate identification of low titre samples which may indicate maternal immunity or vaccination responses.

Table 1. Comparison of serum HI and ELISA titres with filter paper titres.

Sample No	Serum HI (Log_2)	Serum ELISA	Filter Paper ELISA
1	7	59	22
2	7	53	19
3	6	6	6
4	4	9	7
5	4	2	2
6	3	7	1

A modification of the test can be used to demonstrate the presence of parvovirus antigen in mummified foetuses. It will give accurate results even on badly decomposed specimens.

These assays have advantages of sensitivity, specificity, price and convenience over the conventional procedures. Once sufficient quantities of suitable reagents are available, these tests can be introduced into routine diagnostic laboratories.

AUTHOR INDEX

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