RESPONSE OF EARLY WEANED PIGS TO AN ESCHERICHIA COLI CHALLENGE AND THEIR ABSORPTION OF OVALBUMIN OR XYLOSE AS INFLUENCED BY CREEP FEEDING

by

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(ABSTRACT)

The effect of exposure to creep feed at 10 d of age vs no exposure to creep feed or sow's feed on the ability of pigs weaned at 21 d of age to respond to an oral challenge of E. coli or to absorb ovalbumin or xylose was investigated. Eighty pigs (45 exposed, 35 control) were orally challenged 24 h after weaning with $3x10^{11}$ organisms of E. coli (0157:H88AC:H43); control pigs tended ($P<.10$) to scour more than the exposed pigs (46% vs 27%). Ovalbumin absorption was similar for both exposed and control pigs ($P>.10$), but absorption for both treatments increased ($P<.001$) from d 1 to d 4 and decreased to d 13 ($P<.001$). Xylose absorption was less ($P<.02$) at d 1 for exposed pigs compared with control pigs (0.781 vs .825 mmol) with no differences ($P>.20$) occurring thereafter. Lowest xylose absorption for both treatments occurred on d 7. Creep feeding conferred some protection to the E. coli challenge, but had little effect on intestinal absorption of ovalbumin and xylose.

Key words: (Creep Feeding, Egg Albumen, Xylose, Intestinal Damage, Escherichia Coli)

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CHAPTER I

INTRODUCTION

Poor postweaning performance coupled with scouring are major problems for swine producers in a 3 to 4 week weaning system. During the transition at weaning from sow milk with its protective antibodies, to a dry nursery starter diet, reduced feed consumption, low disease resistance, and environmental factors are known to make the pig more susceptible to infection by microorganisms which are normal environmental inhabitants. Postweaning scouring is normally associated with Escherichia coli, but E. coli in itself is unable to induce scouring; an additional stress (e.g. weaning, rotavirus infection, poor environmental conditions or dietary hypersensitivity) is needed to reduce the resistance of the pig and predispose it to E. coli proliferation and thereby effecting the resulting scouring..

A group of British scientists recently offered an explanation for some of the poor postweaning performance and scouring of early weaned pigs. Newby et al., (1983) proposed that a transient dietary hypersensitivity is the underlying cause of postweaning scouring. They suggested that the immune system of the pig is primed to certain feed antigens, via sow feed and(or) creep feed, prior to weaning, and that subsequent exposure to these dietary antigens in the nursery diet causes a severe immune response. This immune response is characterized by intestinal inflammation

and tissue damage that leads to low weight gain and scouring. These scientists further explain that if enough of these feed antigens are consumed prior to weaning via creep feed, the pig becomes tolerant to the feed antigens and no hypersensitivity response is evoked.

This theory may be of practical significance to the swine industry because small amounts of creep feed normally are consumed when pigs are weaned at 3 weeks of age. Nursing pigs are exposed to creep feed in an effort to encourage them to eat so that they will be adapted to the starter diet at weaning; thereby, improving the generally poor performance that occurs during the first week after weaning. Creep feeding was a recommended practice when late weaning, at 6 to 7 weeks, was the norm, and pigs would consume sufficient quantities (> 400 g per pig) of creep feed before weaning. However, the practice of creep feeding early weaned pigs is questionable because the small quantity of creep feed consumed may lead to a gastrointestinal hypersensitive reaction after weaning.

The objective of this research was to assess the influence of creep feeding in a practical farrowing-weaning system on the development of the hypersensitive reaction. Hypersensitivity was indirectly measured in pigs weaned at three weeks by measuring the intestinal absorption of ovalbumin or xylose, or by the pigs ability to cope with a postweaning challenge of E. coli.

CHAPTER II

REVIEW OF LITERATURE

Creep feeding

Creep feeding enables swine producers to supplement sow milk with a conventional dry diet. The basic idea for creep feeding is to allow the nursing baby pigs access to a highly digestible diet that provides energy, protein and other nutrients. The benefits of creep feeding have been well established in late weaning systems, i.e. greater than 5 weeks, but the benefits of creep feeding pigs weaned at three weeks of age are less clearly defined (Okai et al., 1976). Milk production of the sow peaks after about 3 weeks of lactation, and then gradually declines. The energy requirements of the baby pig for maximum growth rate steadily increase and surpass the ability of the sow to provide this energy at about 4 to 5 weeks of lactation. Thus baby pigs need an additional source of energy, via sow feed or creep feed, if they are to meet their energy needs. Creep feeding during the first 3 weeks, when the sow is more than able to provide energy for her young, is questionable.

Creep feeding is also beneficial in cases when the sow develops mastitis, metritis, or agalactia (MMA), and the milk supply is limited. Creep feeding late weaned pigs can lead to heavier, more uniform litters at weaning. The litter has access to extra nutrients and the runts of the

litter which may not be receiving enough milk are able to compensate for their deficiency by consuming creep feed . A larger pig is better able to cope with the stresses of weaning. Also, producers desire uniform litters to help facilitate an 'all in-all out' system of management.

There is some evidence that consumption of creep feed prior to weaning can induce intestinal enzymes needed to properly digest a corn-soybean diet (Okai et al., 1976). At birth, lactase activity is high and sucrase activity is low, but as the pig matures the activity of these enzymes reverse; sucrase is high and lactase is low (Kenworthy and Crabb, 1963). The intake of creep feed may hasten this normal transition of activities as well as induce the production other digestive enzymes. This provides the pig with a more mature gastrointestinal tract that is better suited to the digestion and absorption of the starter diet.

The underlying cause of malabsorption is that the gastrointestinal tract is immature and is unable to sufficiently digest and utilize the ingested feedstuffs. However, other factors beside immaturity of the gut can lead to a state of malabsorption. Gorging is another underlying cause. After weaning, a low feed intake usually occurs, then after a few days the, now famished, pig gorges itself on the starter diet (Goodwin, 1959). This excessive intake overloads the digestive capacity of the gut and therefore malabsorption and scouring can result. High levels of

protein have been implicated in malabsorption. The digestive enzymes necessary for the breakdown of proteins are not fully developed in the newly weaned pig. Low levels of protein and restricted intake have been beneficial in controlling malabsorption (Miniats and Roe, 1968; Bertschinger et al., 1979).

Ideally creep feed should be highly digestible, palatable, and highly nutritious. This is moderately achieved with a 20% crude protein diet consisting of corn and soybean meal supplemented with 20% dried whey. As stated, the digestive capacity of the pig is limited, therefore much of the energy and protein should be in the form of easily digestible milk by-products, like dried whey (Armstrong and Clawson, 1980). These milk by-products offer the pig a source easily digested nutrients. The use of sweeteners like sugar, molasses and flavors to make the feed more palatable and thereby entice consumption have met with variable results (Kornegay et al., 1979). Small amounts of creep feed should be offered frequently, because old feed may become spoiled or moldy and the pigs will refuse to eat it. More importantly, the pig may develope an aversion to the feed if spoiled or moldy feed is consumed.

The best method of offering the creep feed is to provide it in amounts so that the pigs will eat it within a few hours, and then repeat this a few times a day. It is important to have fresh, clean water readily available at

all times to encourage consumption of the dry feed. The form of the creep feed, whether it be a mash, pellets, paste or liquid, depends upon the producers preference, facilities and costs.

Three week weaning

Swine producers are weaning earlier, i.e. 3 weeks, to increase sow productivity and thus be more efficient. With the advent of intensive confinement systems, facilities and operational costs dramatically increased when compared with open lot systems. In order to offset these costs, producers have to use their facilities to the fullest. Weaning at 3 weeks provides the opportunity to produce more pigs per sow per year then can be produced with late weaning.

Weaning at 3 weeks, however, exposes the pig to many stresses at a time when it is least capable of coping with them. The passive immunity acquired at birth via the colostrum is low at 3 weeks. The active immune system of the pig is not fully developed until 5 weeks of age. The pig is not large and its body reserves of fat are low, therefore it is not capable of regulating its body temperature (Fenten et al., 1985). The stresses surrounding weaning (removal from the sow, mixing with other pigs, being placed in a different environment and offered a new diet) can cause a postweaning lag characterized by little or no weight gain and(or) scouring. Most swine producers accept

this poor postweaning performance which lasts about one week, as a trade off for the benefits of early weaning.

Hypersensitivity and the intestinal immune response

The intestine is a constant interface between the environment and the organism and therefore must be equipped to deal with potentially harmful agents as well as ignore harmless agents (Newby et al., 1983). Upon exposure to a feed antigen, the immune system regards this as a threat and mounts a response. This response entails the production of antibodies specific to that antigen and other less specific defenses that are activated- macrophages, neutrophils, basophils and eosinophils (Mims, 1982). This response is effective in sequestering the feed antigen, but inadvertently causes inflammation and tissue damage in the surrounding area. Also, the system creates memory cells that are able to induce a more rapid, heightened response upon subsequent exposure to the antigen (Kimball, 1983). However, if the immune system is continually exposed to a feed antigen, the system becomes tolerant to that antigen, i.e. the responses are suppressed and no reaction is expressed (Richman et al., 1978).

The intestinal immune system produces an antibody, secretory IgA, whose function is to bind to the antigen in a complex. This local exposure of the gut impairs its capacity to absorb the intact antigen because the complex is bound in the mucosal surface until degraded by proteolytic

enzymes. This protective mechanism is not totally effective; some intact antigens can avoid the immune response and pass through the enterocyte via pinocytosis (Walker and Isselbacher, 1974). The intact protein can then lead to a local and systemic immune response (Bellany and Nielsen, 1974; Kagnoff, 1978). This response causes local intestinal inflammation that leads to a breakdown of the intestinal integrity. This breakdown allows more intact antigens to be absorbed causing further damage until a state of tolerance is achieved (Thomas et al., 1974).

This sequence of events is of importance to the weaning of pigs. At 3 weeks of age, a nutritionally insignificant amount of creep feed is consumed but the amount is important immunologically. This small amount of feed may prime the pigs immune system and lead to a hypersensitivity response when weaned. Creep feed contains proteins which are antigenic, i.e. capable of inducing an immune response. A small amount of antigenic protein can prime the system, i.e. the system reacts severely upon subsequent exposure to the antigen (Kimball, 1983). Priming of the immune system is of concern in early weaning, because the small amount of creep feed consumed prior to weaning may prime the immune system. After weaning, large quantities of these feed antigens are present in the diet, which, when consumed, can lead to a hypersensitive response that can cause malabsorption, intestinal damage and scouring (Kilshaw and Slade, 1980).

With interest in the immune response to feed antigens, a diet that is low in antigenic proteins may have potential in the future. Hydrolyzing the protein with proteases or using low antigenic feedstuffs like rice are methods of reducing the number of antigens (Miller et al., 1984a and Hampson, 1986c). Using these low antigenic diet may eliminate the problem of an immune response to the feed.

Hypersensitivity research

Several researchers have investigated the hypothesis that prior exposure to feed antigens via creep feed can affect subsequent performance postweaning (Porter et al., 1973; Newby et al., 1983; Miller et al., 1984a,b; Hampson, 1986a,b,c). Newby et al. (1983) tested this hypothesis with three treatment groups: i) no creep feed, ii) creep feed from day 7 to weaning at day 21, iii) creep feed for days 7, 8 and 9. Newby et al. (1983) postulated that enough creep feed would be consumed from day 7 to weaning to cause a tolerant state and no scouring would be observed, the group exposed to the creep feed for only 3 days would be in a primed state and scouring would occur, and the abruptly weaned group (no creep feed) would have an incidence of scouring that would be between the two creep-fed groups. This in fact, occur, the tolerant group did not scour, all primed pigs scoured and 33% of the abruptly weaned group scoured. In a later study, it was determined that the severity and duration of diarrhea was greater in the primed

group compared to the other two groups (Miller et al., 1984b). Newby et al. (1980) using mice, showed a delayed hypersensitivity 2 days after feeding 25 mg of ovalbumin per day; tolerance was achieved by day 6 with this feeding regimen. Results from these three studies fit the theory that a transient dietary hypersensitivity is an underlying cause to poor postweaning performance and scouring. Exposure to feed antigens at weaning causes a hypersensitivity reaction 2 to 4 days postweaning, which manifests itself as poor weight gain and scouring. After continual exposure to these feed antigens, tolerance is achieved by one week postweaning. The pig is able to overcome the intestinal damage caused by the hypersensitivity reaction and begins to gain weight and stop scouring.

Miller et al. (1984b), compared pigs fed creep feed from day 7 to 10 with pigs not fed creep feed. They observed that creep-fed pigs shed hemolytic Escherichia coli in their feces and that the onset of diarrhea was earlier than for the non-creep-fed pigs. Pigs were weaned at 21 days of age. Also, the duration and severity of scouring was greater in the exposed pigs. In addition, postweaning scouring was not observed in pigs fed a 25% hydrolyzed casein creep feed from day 7 to 10, but did occur in pigs fed a 25% native casein creep feed for the same period.

Hampson (1986a) also investigated the effect of creep feeding on the incidence of postweaning scouring in early

weaned pigs. He observed scouring in the non-creep-fed pigs (abruptly weaned) but not in the pigs fed creep feed from day 7 to weaning or pigs fed creep feed from day 7 to 10. Scouring in the abruptly weaned pigs was attributed to their gorging themselves. Contrary to results reported by Miller et al. (1984a), Hampson (1986c) observed no difference in the incidence of scouring in pigs exposed to creep feed containing either native or hydrolyzed casein from day 7 to 10.

Xylose absorption

Xylose absorption is a measure of intestinal damage; a decrease in the amount of xylose absorbed indicates an impairment of the intestinal absorption capability (Helmer and Fouts, 1937). Miller et al. (1984a) measured xylose absorption in four groups of pigs weaned at 21 days: no creep feed, creep feed from day 7 to 10, creep feed from day 7 to weaning, and unweaned pigs. The abruptly weaned pigs (no creep feed) had a higer xylose absorption than the primed pigs (creep feed for 3 days). Xylose absorption of the unweaned group was used as the control. The treatment groups all had a lower xylose absorption than the control (unweaned) pigs. The primed pigs had more intestinal damage presumably due to a hypersensitivity reaction. To the contrary, Hampson (1986a) was unable to observe a difference in xylose absorption in creep-fed pigs (day 7 to weaning), primed pigs (creep feed from day 7 to 10) or abruptly weaned

pigs (no creep feed). There was a reduction in xylose absorption for weaned pigs when compared to unweaned pigs.

Intestinal morphology

The ratio of villus height to crypt depth is an important measure of intestinal integrity. The immature enterocyte is created in the crypt and as it matures, moves up the villus. The enterocyte matures in 4 to 5 days, the normal time it takes to reach the tip of the villus, and remains there until sloughed off and replaced (Etzler, 1979). A depressed villus height to crypt depth ratio indicates an overall immaturity of the gut (Woode et al., 1978). An increase in crypt depth suggests an increase in the number of immature enterocytes produced. Also a decrease in villus height does not allow sufficient time for the enterocyte to mature and therefore it is sloughed off in an immature state. The immature enterocyte is not capable of proper absorption of nutrients; consequently, when the villus height and crypt depth ratio is reduced, the pig is unable to properly absorb the nutrients in the gut. This leads to a malabsorption syndrome causing diarrhea and reduced growth rate.

In order to understand the changes that weaning and prior creep feed consumption had on intestinal morphology, Hampson (1986b) looked at villus height and crypt depth. No difference in villus height and crypt depth in the weaned pigs could be observed regardless of whether creep feed had

or had not been consumed. All weaned pigs showed a marked decrease in the villus height:crypt depth ratio with the unweaned controls exhibiting a gradual increase in crypt depth with little change in villus height.

Kenworthy (1976) also investigated the changes in intestinal morphology that occur after weaning. He observed that the villus height:crypt depth ratio was at its minimal level 7 and 8 days postweaning with subsequent recovery. Hampson (1986b) reported that the villus height:crypt depth ratio was lowest 5 days after weaning. Thus both these studies indicate that shortly after weaning the gastrointestinal tract is in a transition state related to reduced absorptive capabilities.

Hampson and Kidder (1986) measured brush border enzyme activities of sucrase and lactase in early weaned pigs fed creep feed, not fed creep feed and unweaned pigs fed creep feed. Both groups of pigs weaned at 21 days of age showed minimal enzyme activity 4 to 5 days postweaning with sucrase activity increasing afterwards. Unweaned pigs had a continual reduction in lactase activity to day 32. Hampson (1986c) fed two creep diets with native casein or hydrolyzed casein as the protein source; the hydrolyzed casein diet exhibited a protective effect to the pigs by lessening the decrease in sucrase activity.

Escherichia coli

Pathogenic enterotoxin producing Escherichia coli is the organism usually isolated from pigs with postweaning scours (Kenworthy and Crabb, 1963; Kenworthy and Allen, 1966; McAllister et al., 1979). Both pathogenic and non-pathogenic E. coli are a part of the normal gut flora of healthy pigs (Miniats and Roe, 1968). Only when events allow pathogenic E. coli to proliferate and become the dominant organism in the intestinal tract is E. coli able to cause diarrhea.

E. coli has three types of outer antigens, O, K, H, associated with the cell wall, pili and flagella respectively (Kohler, 1972). In order for E. coli to colonize the gut it must be able to adhere to the enterocytes which prevents it from being cleared from the gut via peristalisis. The structure that enables this attachment is the K antigen or pilus (Nagy et al., 1976). The presence of a pilus is one factor that contributes to the pathogenicity of E. coli (Moon et al., 1979; Evans et al., 1986).

The ability of E. coli to produce scours is also dependent on the presence of an enterotoxin. Pathogenic E. coli are able to produce one of two types of toxin, heat stable and heat labile. The heat labile toxin is more pathogenic and also antigenic (Stevens et al., 1972). Enterotoxin causes a great outpouring of fluid into the

lumen of the gut which leads to diarrhea, dehydration and eventual death if the pig does not recover. The mechanism by which this is accomplished has been elucidated. The enterotoxin attaches to receptors on the enterocyte membrane and causes an increased concentration of cyclic adenosine monophosphate (cAMP). This increase in cAMP affect the normal ion concentration and allows more Cl^- ions to be secreted and less Na^+ ions to be absorbed. This results in an osmotic imbalance leading to an outpouring of fluid (Kohler, 1972).

Predisposing factors to postweaning scours

Research has shown that the presence of pathogenic E. coli does not always cause scouring. Several investigators have orally challenged pigs with pathogenic enterotoxin producing strains of E. coli with no subsequent scouring. Therefore, is seems that some predisposing events or factor alters the normal intestinal environment that allows for the colonization and proliferation of the gastrointestinal tract by pathogenic E. coli. The most obvious change surrounding postweaning scouring is weaning itself. The stress of being removed from the sow, and the protective factors in the milk, remixed with different pigs, introduced to a different environment and a new food source are overwhelming to the 21 day old pig. It is reasonable to assume that these stresses can contribute to the low feed intake and hence poor growth of the pig. However, it more difficult to find a

correlation between the stresses of weaning and postweaning scouring. Hamilton and Roe (1977) state that the small intestine of weanling pigs is in a delicate net fluid balance, and any change will upset this balance and result in diarrhea. The stress of changing the diet has been implicated by many researchers to be the cause of scouring (Buxton and Thomlinson, 1961; Richards and Fraser, 1961; Chopra et al., 1964). This could be attributed to poor digestion of the starter diet resulting in a change in the osmotic balance, thus causing scouring. Lecce et al., (1982, 1983) indicated that intestinal damage by rotavirus produces a favorable environment for E. coli colonization and proliferation.

The removal of the pig from the protective effects of sow milk cannot be overlooked. Besides the passive antibodies that are absorbed from colostrum, milk contains several other antibacterial factors (Rutter et al., 1976; Stephens et al., 1979). The most important is secretory IgA. This antibody is constantly bathing the gut during nursing. It functions primarily in two ways, preventing binding to the intestinal lining and binding free antigens into complexes (Ogra, 1976). By binding to receptors that E. coli can utilize, IgA prevents colonization (Porter et al., 1973; Nagy et al., 1979). Free antigen, whether microbial or dietary in nature, can be bound in antibody antigen complexes. This sequesters the potentially harmful

antigens until the proteolytic enzymes can degrade the complexes. The pig can manufacture its own secretory IgA, but this capability is not fully mature until the pig is about 5 weeks old. Milk also contains less specific antimicrobial agents like lactoferrin and lysozyne. Lactoferrin binds iron making it unavailable for use. Because bacteria need iron, this mineral is limiting, therefore microbial numbers are reduced. Lysozyme is an enzyme that breaks down the lipopolysaccride cell walls of bacteria resulting in the lysis of these cells. Removal of this protective substance from the pig can have severe effects on the ability of the pig to cope with newly encountered enteric bacteria as well as feed antigens.

Summary

It can be seen that the postweaning check and scouring in pigs is a complex syndrome. The stresses of weaning all play an important role in the postweaning check. The proliferation of pathogenic E. coli, introduction to a new diet with its associated antigens, and removal of the protection from the sows milk all contribute to the scouring in newly weaned pigs. Hypersensitivity may not be an inclusive answer, but it seems to be a plausible model. While there is some contradictions within the research related to dietary hypersensitivity, the concept has not been totally refuted. Further research and refining of the

hypothesis will be required to understand dietary hypersensitivity as well as its role in postweaning check and scouring.

CHAPTER III

OBJECTIVES

This experiment was conducted to evaluate the effect of creep feeding on postweaning performance and scouring in newly weaned pigs.

The specific objectives were:

1) To observe if creep feed has any effect on the response of the pig to a postweaning challenge of *Escherichia coli*.
2) To determine if creep feed has any effect on intestinal damage as measured by ovalbumin and xylose absorption.

CHAPTER IV

RESPONSE OF EARLY WEANED PIGS TO AN ESCHERICHIA COLI CHALLENGE AND THEIR ABSORPTION OF OVALBUMIN OR XYLOSE AS INFLUENCED BY CREEP FEEDING.

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ABSTRACT

The effect of exposure to creep feed at 10 d of age vs no exposure to creep feed or sow feed on the ability of pigs weaned at 21 d of age to respond to an oral challenge of E. coli or to absorb ovalbumin or xylose was investigated. Eighty pigs (45 exposed, 35 control) were orally challenged 24 h after weaning with $3x10^{11}$ organisms of E. coli (0157:H88AC:H43); control pigs tended ($P<.10$) to scour more than the exposed pigs (46% vs 27%). Ovalbumin absorption was similar for both exposed and control pigs ($P>.10$), but absorption for both treatments increased from d 1 to d 4 ($P<.001$) and decreased to d 13 ($P<.001$). Xylose absorption was less ($P<.02$) at d 1 for exposed pigs compared with control pigs (0.781 vs .825 mmol) with no differences ($P>.20$) occurring thereafter. Lowest xylose absorption for both treatments occurred on d 7. Creep feeding conferred some protection to the E. coli challenge, but had little effect on intestinal absorption of ovalbumin and xylose.

Key words: (Creep Feeding, Egg Albumen, Xylose, Intestinal Absorption, Escherichia Coli)

INTRODUCTION

Swine producers are weaning at 3 weeks of age to increase the number of pigs produced per sow per year; however, weaning at 3 weeks is more stressful than later weaning and can lead to poor postweaning performance and scouring.

Newby et al. (1983) hypothesized that poor postweaning performance and scouring are caused by a transient dietary hypersensitivity. They suggested that consumption of small amounts of creep feed prior to weaning would prime the immune system to the dietary antigens. Subsequent exposure to these dietary antigens after weaning would cause a severe immune reaction resulting in intestinal damage and scouring. However, if large quantities of creep feed were consumed before weaning, the immune system would become tolerant to the feed antigens; thereby reducing postweaning scouring and improving performance. Newby et al. (1983) and Miller et al. (1984a,b) observed severe scouring in primed pigs (exposed to creep feed at 7 to 10 d of age) with no scouring in tolerant pigs (continuously exposed to creep feed from d 7 to weaning at 21 d) with intermediate scouring in pigs with no exposure to creep-fed. To the contrary, Hampson, (1986a,b,c) was unable to observe any differences in scouring or performance of pigs primed to the creep feed,

made tolerant to the creep feed or not exposed to creep feed.

The objective of our research was to investigate the influence of creep feeding on postweaning scouring after an Escherichia coli challenge and to evaluate intestinal damage and malabsorption as measured by the absorption of ovalbumin and xylose.

EXPERIMENTAL PROCEDURES

Gestating sows which had been housed on partially slotted concrete floors in a ventilated confinement building and individually limit-fed 1.8 to 2.7 kg d^{-1} (4 to 6 lb) of a corn-soybean basal diet (appendix table 1) were moved to individual farrowing crates 108 to 111 d post-coitum. The daily intake of the basal diet (equally fed twice daily) was increased to 6.5 to 7.3 kg d^{-1} (14 to 16 lb) after farrowing. Water was available at all times. Farrowing crates had solid flooring in the middle with plastic coated slotted flooring in the front and back of crates. The farrowing room was maintained at a temperature of 18 to 22 C (65 to 70 F) with heat lamps providing supplemental heat in the creep area for the baby pigs.

Nursing pigs were not allowed access to sow feed by removing the litter while the sow was eating. After a 1 h period, any leftover feed was removed or the sow feeder was reversed before releasing the litter. Pigs that were creep-fed were offered a 20% crude protein corn-soybean meal diet

Table 1. Composition of creep feed ration.[a]

Ingredients	Percentage
Ground corn	49.89
Soybean meal (48% CP)	27.73
Dried skim whey	20.00
Defluorinated phosphate	1.03
Limestone	0.75
Salt, plain	0.25
Vitamin-selenium premix[b]	0.25
Trace mineral premix[c]	0.10
	100.00

[a] Calculated to contain 20.0% crude protein, 0.8% Ca and 0.6% P.

[b] Supplied per lb of vitamin premix: 800,000 IU vitamin A, 80,000 IU vitamin D_3, 2,000 IU vitamin E, 200 mg vitamin K, 800 mg riboflavin, 4,000 mg pantothenic acid, 4,000 mg niacin, 4,000 ug vitamin B_{12}, 80 g choline, and 80 mg biotin.
Supplied per kg of vitamin premix: 1,763,698 IU vitamin A, 176,370 IU vitamin D_3, 4,409 IU vitamin E, 441 mg vitamin K, 1,764 mg riboflavin, 8,818 mg pantothenic acid, 8,818 mg niacin, 8,818 ug vitamin B_{12}, 176 g choline, 176 mg biotin and 120.3 mg selenium.

[c] Contain 20% Zn, 10% Fe, 5.5% Mn, 1.1% Cu and 0.15% I.

supplemented with 20% whey (table 1) plus 1% chromic oxide as a feed marker at 10 d of age. Consumption of the creep feed was measured until weaning. Also fecal loops were taken twice daily of the creep-fed pigs to detect the presence of the chromic oxide pigment. Color was indicative of positive creep feed consumption.

After weaning, pigs used for the ovalbumin and xylose tests were housed in .9 m x 1.2 m (3 ft x 4 ft) cages with plastic-coated wire floors in a temperature-controlled nursery. Temperature was maintained at 27 to 30 C (80 to 85 F) for the duration of the trial (2 wk). Feed (table 1) and water were supplied ad libitum. Pigs used for the Escherichia coli test were housed in a temperature-controlled isolation trailer in cages with flat expanded metal floors. Temperature was maintained at 24 to 27 C (75 to 80 F) with feed and water being offered ad libitum for the duration of the trial (3 d).

Gilts were vaccinated 4 wk prior to breeding for Leptospirosis (5-way vaccine), parvovirus, Bordatella bronchiseptica, Pasteurella multocida, Erysipelas and Escherichia coli. This was repeated 2 wk prior to breeding for gilts as well as sows with the addition of phenbendazole to the feed for deworming. Piglets were given 2 ml of iron dextran (100 mg of iron per ml) at 3 d of age. At 7 and 21 d of age, piglets were vaccinated for Bordetella

bronchiseptica, Pasteurella multocida and Erysipelas. Prior to weaning, litters were dewormed with phenbendazole.

Four trials were conducted to evaluate the ability of exposed (creep-fed) and control (unexposed to creep feed or sow feed) pigs to respond to an Escherichia coli challenge. In trials 1 and 4, pigs were raised at the Virginia Tech Swine Center, Blacksburg and in trials 2 and 3, pigs were raised at the Virginia Tech Tidewater Agricultural Experiment Station, Suffolk. Sixteen litters in trials 1 and 3 (eight litters in each trial) and 12 litters in trial 4 were randomly assigned to one of two treatments: 1)exposed - creep feed from 10 d of age to weaning, or 2)control - no creep feed or exposure to sow feed. In trial 2, three litters (two exposed, one control) were used. A total of 80 crossbred pigs from the above litters (45 exposed, 35 control) were used in these four trials. After a 24 h fast, a 5 ml suspension of E. coli (0157:K88AC:H43 from Penn State Reference Center) in trypticase soy broth containing approximately 3 x 10^{11} organisms was given orally to each pig (appendix table 2). A five point system was used to subjectively evaluate scouring: 1-hard feces, 2-normal, 3-slightly loose, 4-very loose, and 5-watery. A score of 4 or 5 was considered positive for scouring. All pigs were observed for scouring every 8 h for a total of 48 h.

The absorption of ovalbumin and xylose was measured in four trials with random assignment of treatments to litters as in the E. coli experiment. Pigs from Blacksburg were used in trials 1, 2, and 4 and pigs from Suffolk were used in trial 3. All pigs were weaned at an average of 21 d. Trial 1 consisted of two litters (one exposed, one control), trial 2 consisted of seven litters (four exposed, three control), trial 3 consisted of eight litters (four exposed, four control) and trial 4 consisted of 12 litters (six exposed, six control). A total of 71 crossbred pigs (40 exposed, 31 control) were used for the ovalbumin test and 77 pigs (43 exposed, 34 control) for the xylose test.

A solution of 0.2 g ml^{-1} of ovalbumin (Sigma grade II, #A-5253) was given to each pig at a rate of 0.4 g kg^{-1} of bodyweight on d 1, 4, 7, 10, and 13 postweaning. Prior to drenching the pigs were fasted for 24 h and taken off water for 4 h to insure an empty gastrointestinal tract. Two 5 ml blood samples were taken, one just prior to drenching and the second 2 h after drenching. The blood was centrifuged (2500 x g) and the serum stored at -20 C (-5 F) until assayed. An enzyme-linked immunosorbent assay (ELISA) was used to determine the concentration of ovalbumin (Voller et al., 1980) (appendix table 3).

A solution of 10% w/v of xylose (Adria Laboratory, #NDC 0013-8555-80) was given to each pig at a rate of 1.0 ml kg^{-1} bodyweight on d 1, 4, 7, 10, and 13 postweaning. As in the

ovalbumin test, each pig was fasted for 24 h and taken off water for 4 h to insure an empty gastrointestinal tract. Two 5 ml blood samples were taken, one just prior to drenching and the second 1 h later. The blood was centrifuged (2500 x g) and the plasma stored at -20 C (-5 F) until assayed. A colorimetric assay was used to determine the xylose concentration (Trinder, 1975) (appendix table 4).

Data was analyzed using the Statistical Analysis System (SAS) (1985). A Chi-square test was used to analyze the E. coli challenge data. All trials were analyzed together; testing of individual trials was not statistically valid due to small sample size. An analysis of variance (ANOVA) was also conducted with individual pig scouring scores as the experimental unit; adjustments for weaning weight and average creep feed consumption were made. Data for the ovalbumin and xylose absorption tests were analyzed by an ANOVA using individual pig values as the experimental unit. Adjustments for weaning weight, total weight gain from d 1 to d 13 postweaning and average creep feed consumption were made. Time (days) and treatment (creep feed) and the two-way interaction were included in the model for analysis of variance of ovalbumin and xylose absorption.

RESULTS AND DISCUSSION

Creep-fed pigs used in the E. coli challenge test consumed an average of 30 g, 25 g, 28 g and 50 g per pig (SE ± 25 g) for trials 1, 2, 3 and 4, respectively. Consumption

of creep feed for pigs assigned to the ovalbumin and xylose absorption tests averaged of 32 g, 20 g, 15 g, and 15 g per pig (SE ± 28 g) for trials 1, 2, 3 and 4, respectively. These levels are consistent with reported values for creep feed consumption in early weaned pigs (Okai et al., 1976; Hampson, 1986b; Hampson and Kidder, 1986), but they are well below the 400 g per pig reported by Miller et al. (1984a) to be necessary to overcome the hypersensitivity reaction. Therefore, it may be assumed that our pigs had the potential for a hypersensitivity reaction.

The E. coli. challenge was used to assess intestinal damage caused by the hypersensitive reaction. E. coli in itself, however, is unable to cause scouring, but requires that some predisposing factor like weaning, rotavirus infection or hypersensitivity (Sojka et al., 1960; Richards and Fraser, 1961; Tzipori et al., 1980; Lecce et al., 1983; Newby et al., 1983; Miller et al., 1984a,b). Pigs suffering from intestinal damage presumably would be more susceptible to an E. coli infection and scouring. In trial 1, 40% of both exposed and control pigs scoured, (figure 1, appendix table 5). In trial 2, 42% of exposed pigs scoured, whereas, all control pigs scoured (100%). In trial 3, 25% of exposed pigs scoured with 38% of control pigs scouring. In trial 4, 47% of exposed pigs scoured, and 53% of control pigs scoured. Over all trials, 46% of control pigs scoured and 27% of exposed pigs scoured (Chi-square test; P=.13). Using

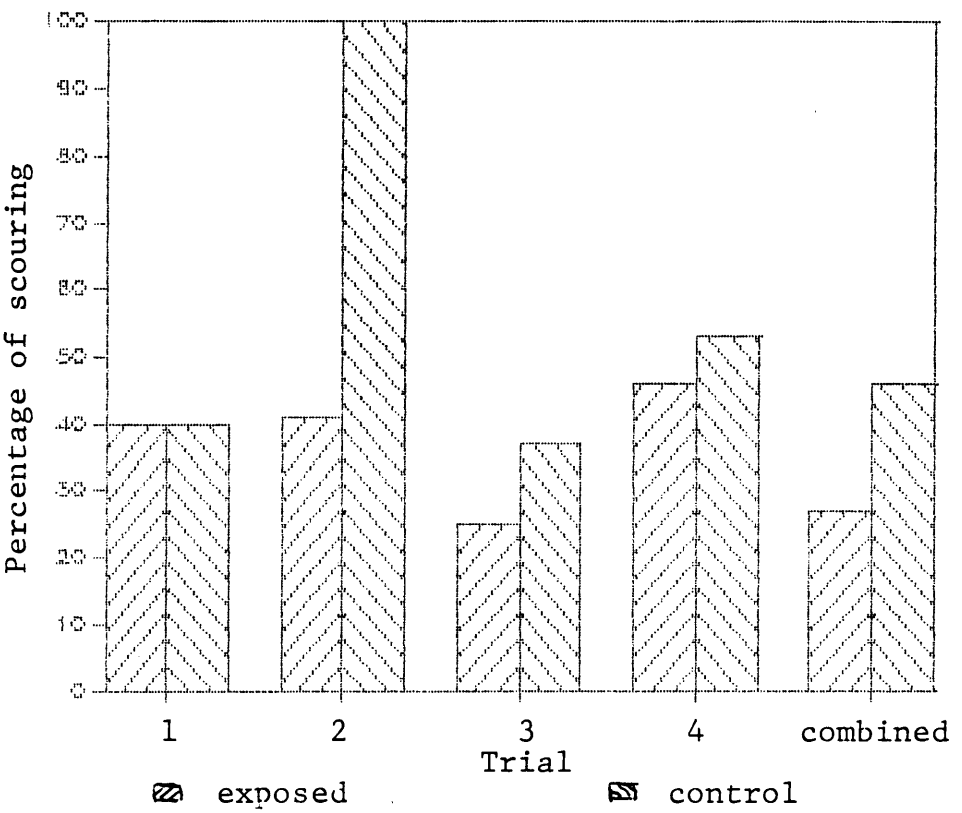

Figure 1. Percentage of exposed and control pigs scouring after an oral challenge of E. coli in trials 1, 2, 3, 4 and combined.

an analysis of variance, creep-fed pigs scoured less ($P<.09$) than control pigs when adjustments for weaning weight and average creep feed consumption were made. Thus, there seems to be a trend for creep feeding to confer some protection against an E. coli challenge, presumably due to less intestinal damage in creep-fed pigs.

It was assumed that if the creep-fed pigs were suffering from a hypersensitivity reaction, they would have more intestinal damage due to their low creep feed consumption and would then succumb to the E. coli challenge. However, an opposite trend was observed. It may be possible that the duration of exposure to creep feed is more important than is the amount consumed. Newby et al. (1983) and Miller et al. (1984 a,b) observed a higher degree of scouring in pigs exposed to creep feed for only three days and observed less severe scouring for pigs continuously exposed to creep feed. Also several researchers have reported that the presence of pathogenic E. coli does not always result in scouring (Kenworthy and Crabb, 1963; Smith and Jones, 1963; Kenworthy and Allen., 1966; Hampson et al., 1985). Finally, susceptibility to an E. coli infection resides in the pig with much individual pig variation (Kenworthy and Allen, 1966). These factors may have played a role in the unexpected results in our study.

Ovalbumin (protein) absorption is a measure of intestinal damage (Thomas et al., 1974; Walker and

Isselbacher, 1974; Kilshaw and Sissions, 1979). The more protein detected in the blood, the more severe the intestinal damage. During normal protein digestion, the protein is broken down into small peptides and free amino acids. These are then absorbed across the gut wall. However, if the intestinal tract is impaired in some manner, protein may not be degraded and larger peptides are absorbed across the gut wall.

Absorption of ovalbumin was variable with coefficients of variation of 51.0, 58.5, 63.7, 74.9 and 101.0% for days 1, 4, 7, 10 and 13 postweaning, respectively. Across trials and days, there was a trend for exposed pigs to absorb less ($P<.10$) ovalbumin than control pigs (figure 2, appendix table 6). Unadjusted ovalbumin absorption was not different ($P>.10$) between treatments when compared on a day by day basis. However, when ovalbumin absorption values were adjusted for weaning weight and average creep feed consumption, absorption was less ($P<.07$) on d 10 and d 13 postweaning with no differences ($P>.20$) in absorption on d 1, d 4 or d 7 postweaning. This suggest that creep-fed pigs are able to recover from intestinal damage more readily than non creep-fed pigs.

Absorption increased ($P<.001$) for both exposed and control pigs from d 1 to d 4 postweaning followed by a linear decline thereafter to d 13 ($P<.001$). Total weight gain from d 1 to d 13 postweaning had no effect ($P>.30$) on

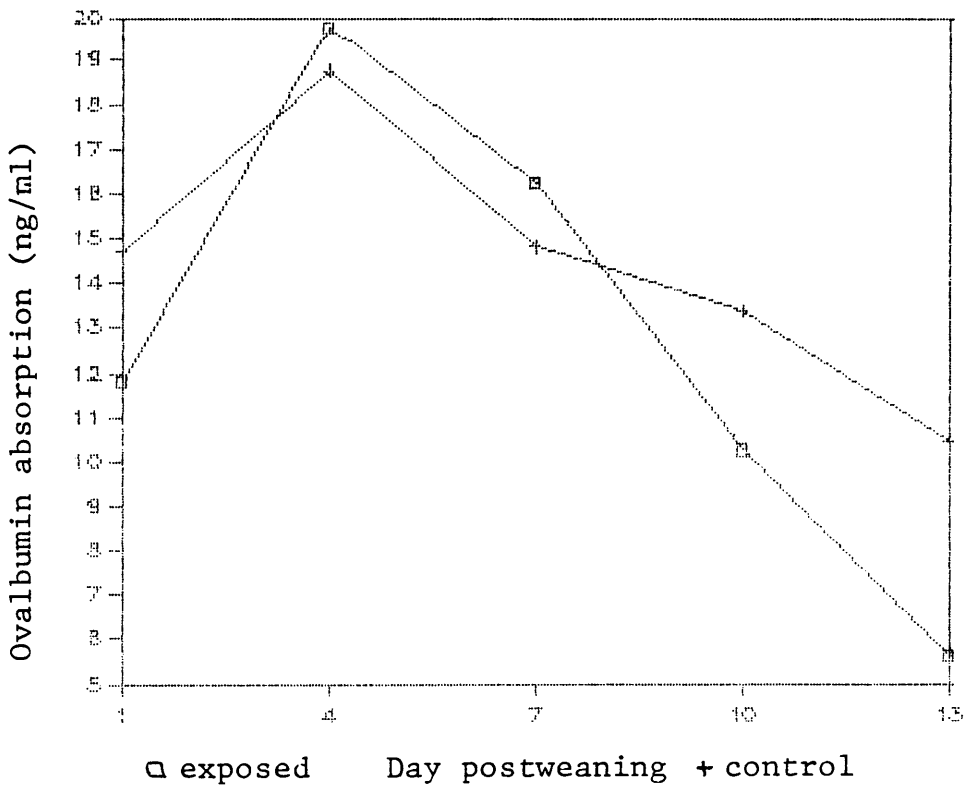

Figure 2. Least square means of postweaning ovalbumin absorption (ng/ml) for trials 1 to 4.

ovalbumin absorption. The high level of ovalbumin absorbed on d 4 postweaning indicates that at this time the most severe intestinal damage was present, with recovery thereafter. These results suggest that there was little difference in the ability of exposed pigs or control pigs to absorb ovalbumin, indicating that both groups had similar patterns of intestinal damage.

Xylose absorption is also a measure of intestinal damage (Helmer and Fouts, 1937; Woode et al., 1978; Hall and Parsons, 1983; Larkin and Hannan 1984; Hampson and Kidder, 1986). Xylose is actively absorbed across the gut wall by a transport system. During intestinal damage, this transport system may be impaired and less xylose can be taken up. Therefore, the lower the amount of xylose absorbed, the more severe the intestinal damage.

Absorption of xylose was not as variable as the absorption of ovalbumin; coefficients of variation of 34.4, 32.8, 43.4, 47.8 and 54.5% were observed for days 1, 4, 7, 10 and 13 postweaning, respectively. Across trials and days, no differences ($P>.20$) in absorption of xylose was observed. The pattern of xylose absorption was similar ($P>.74$) for exposed and control pigs (figure 3, appendix table 7). Exposed pigs absorbed less ($P<.02$) xylose than control pigs on d 1 postweaning with no differences ($P>.20$), observed thereafter. Xylose absorption of exposed pigs increased slightly ($P>.30$) from d 1 to d 4 postweaning with

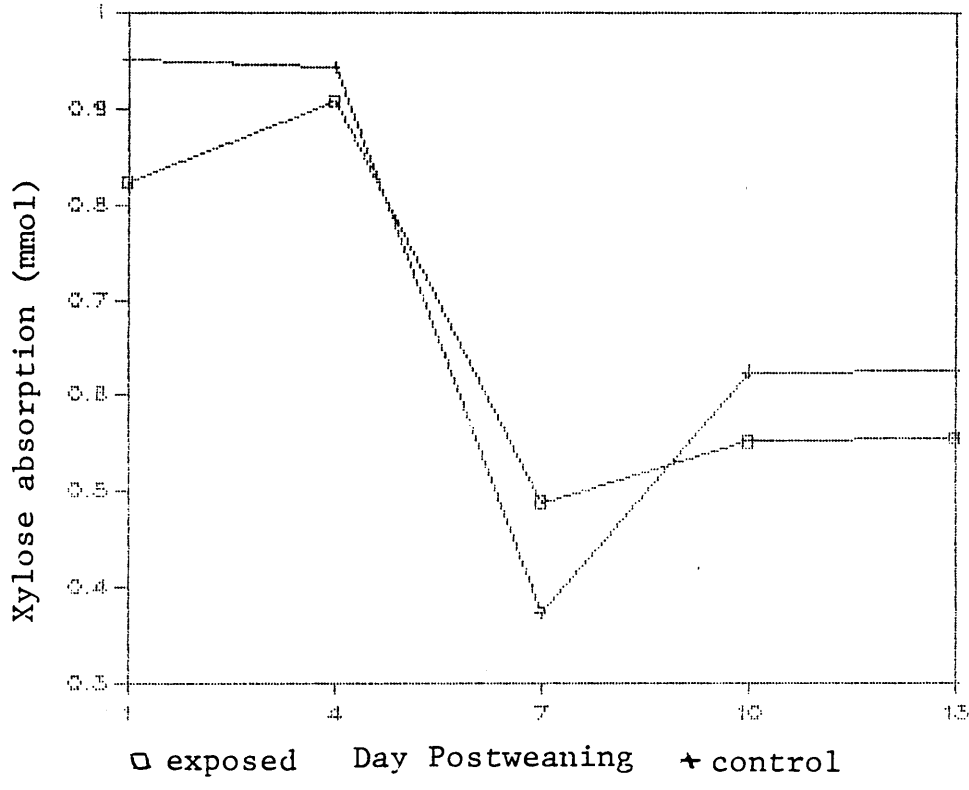

Figure 3. Least square means of postweaning xylose absorption (mmol) for trials 1 to 4.

little change for control pigs, then absorption decreased ($P<.001$) for both groups to its lowest point on d 7 postweaning with the lowest level observed for control pigs. Thereafter, xylose absorption increased ($P<.10$) for both groups to d 10 postweaning and then increased slightly for control pigs and remained about the same for exposed pigs on d 13 postweaning. Adjusting for weaning weight and average creep feed consumption had no effect ($P>.10$) on the pattern of xylose absorption. Total weight gain from d 1 to d 13 postweaning had no effect ($P>.20$) on xylose absorption.

These results suggest that there was no difference in the ability of exposed or control pigs to absorb xylose, indicating that both groups had similar patterns of intestinal damage. The low level of xylose absorbed on d 7 postweaning for both treatments appears to be the time when the most intestinal damage is present. This observed pattern of xylose absorption from d 1 to d 13 postweaning is similar to other findings (Miller et al., 1984b; Hampson and Kidder, 1986).

It is interesting that the occurrence of intestinal damage as assessed by the ovalbumin and xylose absorption tests coincide with the reported time intervals of postweaning scouring (Smith and Jones, 1963; Stevens, 1963a,b; Kenworthy, 1976; Tzipori, 1980; Lecce et al., 1983; Newby et al., 1983; Miller et al., 1984b; Hampson, 1986a,b).

Therefore, it seems that postweaning scouring may be associated with some intestinal damage.

In summary, the results of the presented study fail to support the hypothesis that a transient dietary hypersensitivity is the cause of the postweaning check and scouring observed in early weaned pigs, but suggest that intestinal damage may play a role. It seems creep feeding can confer some protection to a postweaning challenge of *E. coli*, but has little effect on the pattern of absorption of ovalbumin or xylose in early weaned pigs.

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Appendix Table 1. Composition of sow ration.[a]

Ingredients	Percentage
Ground corn	81.64
Soybean meal (44% CP)	15.60
Defluorinated phosphate	1.57
Limestone	0.64
Salt, plain	0.25
Vitamin-selenium premix[b]	0.25
Trace mineral premix[c]	0.05
	100.00

[a] Calculated to contain 14.0% crude protein, 0.8% Ca and 0.6% P.

[b] Supplied per lb of vitamin premix: 800,000 IU vitamin A, 80,000 IU vitamin D_3, 2,000 IU vitamin E, 200 mg vitamin K, 800 mg riboflavin, 4,000 mg pantothenic acid, 4,000 mg niacin, 4,000 ug vitamin B_{12}, 80 g choline, 80 mg biotin and 18.2 mg selenium. Supplied per kg of vitamin premix: 1,763,698 IU vitamin A, 176,370 IU vitamin D_3, 4,409 IU vitamin E, 441 mg vitamin K, 1,764 mg riboflavin, 8,818 pantothenic acid, 8,818 mg niacin, 8,818 ug vitamin B_{12}, 176 g choline, 176 mg biotin, and 40.1 mg selenium.

[c] Contained 20% Zn, 10% Fe, 5.5% Mn, 1.1% Cu and 0.15% I.

Appendix Table 2. Protocol for Escherichia coli challenge.

Stock Culture Protocol

Culture: 0157:K88AC:H43 from reference center at Penn State

1. From culture, streak for isolated colonies onto a TSA plate. Pick 10 appropriate colonies and streak a lawn onto 10 TSA plates. Check for confluent growth.

2. Add 2ml of TSB + 15% glycerol to each plate and make a suspension of cells. Transfer suspension to cryro-tubes and store in liquid nitrogen.

Oral Challenge Protocol

1. From stock culture vial, streak one TSA plate for isolated colonies and check for purity.

2. Add 0.1ml from stock culture suspension to each of ten TSA plates and spread over plate. (0.25ml on large plates)

3. Add 2ml glycerol of TSB to each plate and transfer cell suspension to a large TSA plate and spread suspension.

4. Add 2ml + 15% glycerol of TSB to each plate and collect cells. Aliquot into cryotubes and freeze. Check for purity and do a plate count.

5. Orally dose each pig with a 5ml suspension by diluting suspension to 5ml in TSB.

6. To check for number of organisms:

   a) take 0.1ml of 10ml suspension and make five serial dilutions: 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8}, 10^{-10}.

   b) take 1.0ml of serial dilution 10^{-8} and 10^{-10} and plate out onto TSA plates.

   c) use dilution that has between 30 and 300 colonies.

Appendix Table 3. Enzyme-linked immunosorbent assay for the detection of ovalbumin in swine serum.

Reagents

1. Chicken egg albumin (Grade VI) Sigma

2. Rabbit anti-chicken egg albumin (Cooper Biomedical #0204-0762)

3. Rabbit anti-chicken egg albumin IgG peroxidase conjugate (Cooper Biomedical #3204-0762)

4. Coating Buffer
   a) mix 1M $NaHCO_3$ (5.1 ml), 1M Na_2CO_3 (1.6 ml), 10% (w/v) NaN_3 (0.2 ml).
   b) Add distilled H_2O to 100 ml and verify pH is 9.6.
   c) Store at 0-4°C.

5. PBS/Tween
   a) Add 8.0 gm NaCl, 0.2 gm KH_2PO_4, 1.15 gm Na_2HPO_4, 0.2 gm KCl and 0.5 ml Tween 20 to ~ 950 ml distilled H_2O.
   b) When dissolved, add distilled water to 1 liter and verify pH is 7.3-7.4.

6. Substrate
   a) add 10mg O-phenylenediamine (Sigma #P-9029) to 1ml Absolute Methanol.
   b) add 100ml of distilled H_2O and then 0.1ml of H_2O_2

7. 8N H_2SO_4

8. Dynatech Immulon II plates

Procedures

1. Coat plate with 10ug/ml of Rabbit anti-chicken egg albumin diluted in coating buffer. Add 100ul of coating buffer to column 1. Add 100ul of diluted IgG to columns 2-12. Incubate 30 min at 37°C and store overnight at 0-4°C.

2. Discard antibody in wells. Wash the plates by adding PBS/Tween to all the wells. Leave PBS/Tween in the wells 2 min before discarding. Repeat this step 2 additional times.

3. Add 100ul PBS/Tween to column 1. Add 100ul of twofold serial dilutions [5ng/ml to 0.625ng/ml] of chicken egg albumin standard antigen diluted in PBS/Tween to plate. Add 100ul of twofold serial dilutions of unknown antigen samples diluted in PBS/Tween to plate. All samples are run in duplicate. Incubate plate for 30 min with shaking at room temperature.

4. Wash plate as in step 2 above.

5. Dilute rabbit anti-chicken egg albumin IgG peroxidase conjugate 1:1,000 in PBS/Tween. Add 100ul of PBS/Tween to column 1. Add 100ul of conjugate dilution to remainder of wells in plate. Incubate the plate for 30 min with shaking at room temperature.

6. Wash plate as in step 2 above.

7. Add 100ul of Substrate to all wells in plate. Incubate plate 30 min in the dark at room temperature.

8. Stop reaction by adding 25ul of sulfuric acid to all wells in plate.

9. Read plate at Mode 1 and Filter 4 using a Titertek Multiskan. Blank plate against column 1.

10. Construct a linear regression using standard antigen absorbance values. Calculate concentration of unknown antigen samples using the standard curve.

Appendix Table 4. Assay for determination of xylose in swine plasma.

Reagents

1. Concentrated hydrochloric acid ~11.5M

2. Zinc sulphate ($ZnSO_4*7H_2O$) solution 10% m/V

3. Sodium hydroxide solution 0.5M

4. Stock xylose solution, 33.33 mmole/liter in 0.2% benzoic acid solution. Dissolve 0.5g of xylose in sufficient 0.2% m/V benzoic acid solution to make 100ml of solution.

5. Standard xylose solution. Dilute 4ml of stock solution in 0.2% benzoic acid solution to a final volume of 100ml.

6. Color Reagent, just before use, dissolve 0.5g of phloroglucinol in 100 ml of glacial acetic acid.

Procedure

1. Add to standard (S) and test (T) tubes 1.2ml of water and 1.4ml into blank tube.

2. To S tube add 0.2ml of standard xylose solution 1.333 mmol/liter and 0.2ml of heparinized plasma to T tube.

3. To all tubes add 0.3ml of 10% m/V zinc sulphate solution, mix and add 0.3ml of 0.5M sodium hydroxide solution.

4. Mix and centrifuge at 2,500 rpm for 15 min.

5. Transfer 0.5ml of supernatant fluid to labelled tubes.

6. Add 0.5ml of concentrated HCl, mix and add 5ml of color reagent.

7. Mix well and place in boiling water bath for exactly five minutes, and then cool rapidly.

8. Set spectrophotometer to zero absorbance with B tube and read absorbances of S and T tubes, using 554nm and cuvettes of 10mm light path.

9. Determine plasma xylose concentration:

$$\text{plasma xylose, mmol/liter} = \frac{\text{Absorbance T}}{\text{Absorbance S}} \times 1.333$$

Appendix Table 5. Number and percentage of exposed and control pigs scouring in trials 1, 2, 3, 4 and combined.

Trial	Treatment	Scouring Total	Percentage
1	Exposed	4/10	40
	Control	2/5	40
2[a]	Exposed	5/12	41.7
	Control	7/7	100
3	Exposed	2/8	25
	Control	3/8	37.5
4	Exposed	7/15	46.7
	Control	8/15	53.3
Combined[b c]	Exposed	18/45	27
	Control	20/35	46

[a] Treatments differ (Chi-square test P<.01)
[b] Treatments differ (Chi-square test P<.10)
[c] Treatments differ (ANOVA test P<.10)

Appendix Table 6. Absorption of ovalbumin (ng/ml) in exposed and control pigs during trials 1, 2, 3, 4 and combined.

Trial	Treatment	Absorption Postweaning (ng/ml) Day 1	4	7	10	13
1	Exposed	5.4	13.3	20.8	7.1	1.8
	Control	12.7	16.0	12.0	6.2	0.2
2	Exposed	19.2	26.4	18.0	16.7	10.8
	Control	21.1	25.8	28.2	32.4	26.0
3	Exposed	9.6	13.4	7.3	8.2	5.9
	Control	12.1	16.4	7.5	7.8	10.1
4	Exposed	12.9	26.0	18.7	9.0	3.9
	Control	13.0	16.8	11.6	7.1	5.4
All[a b]	Exposed	11.8	19.8	16.2	10.3	5.6
	Control	14.7	18.8	14.8	13.4	10.4

[a] Across all trials and days, exposed means less than control means ($P<.10$)

[b] Across all trials and treatments; day 1 < day 4, day 4> day 7 ($P<.001$); day 7 > day 10, day 10 > day 13 ($P<.05$)

Appendix Table 7. Absorption of xylose (mmol) in exposed and control pigs during trials 1, 2, 3, 4 and combined.

		Absorption postweaning (mmol)				
Trial	Treatment	Day 1	4	7	10	13
1	Exposed	.82	1.03	.42	.39	.55
	Control	1.68	1.02	.22	.74	.66
2	Exposed	1.11	1.02	.49	.70	.34
	Control	.99	1.30	.47	.73	.51
3	Exposed	.63	.71	.48	.69	.92
	Control	.35	.67	.37	.68	.85
4	Exposed	.74	.80	.55	.43	.42
	Control	.79	.91	.44	.35	.48
All[a]	Exposed	.82[b]	.91	.49	.55	.55
	Control	.95	.94	.37	.62	.63

[a] Across all trials and treatments, day 4 > day 7 ($P<.001$)
[b] Across all trials, exposed means less than control means for day 1 ($P<.02$)

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