CONTROL OF ENTEROTOXIGENIC *ESCHERICHIA COLI* IN EARLY-WEANED PIGS USING THERAPEUTIC ANTIBODY

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By

Augustine Owusu-Asiedu

In Partial Fulfillment of the

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FACULTY OF GRADUATE STUDIES

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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FOREWARD

This thesis was prepared following a manuscript format. There are three manuscripts corresponding to three chapters. Manuscripts one and two have been submitted to The Journal of Animal Science, while manuscript three is yet to be submitted. All manuscripts were formatted to meet the Guidelines for Journal of Animal Science manuscript preparation.

ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) cause diarrheal diseases in both neonatal and post-weaning piglets. Post-weaning diarrhea (PWD) is prominent in the swine industry and it is responsible for high mortality in early-weaned pigs. A major concern facing the swine industry is to identify means for controlling diarrhea and to improve post-weaning pig performance. Various strategies have been used with little success or serious drawbacks. Use of therapeutic antibodies offers an alternative cost-effective approach for the problem. An experimental program was undertaken to compare the efficacy of egg-yolk antibodies (EYA) to spray-dried animal plasma proteins (SDAP) and spray-dried porcine plasma (SDPP), zinc oxide, fumaric acid, and commercial antibiotics in controlling PWD in 10-d old weaned pigs. In trial 1, it was established that SDPP and egg-yolk obtained from hyper-immunized laying hens contained specific anti-ETEC antibodies. However, EYA contained significantly greater concentration of this specific anti-ETEC antibodies compared to SDPP or SDAP. Compared to feeding SDPP, piglets fed plant-based proteins, such as pea protein isolate (PPI), performed poorer and showed a higher incidence of diarrhea. The second study was aimed at comparing 10-d old weaned piglets performance fed PPI-based diets supplemented with either SDPP or EYA and challenged with ETEC (K88). Unlike piglets fed PPI-alone diets, those fed PPI-supplemented with either SDPP or EYA performed better ($P < 0.05$), showed reduced incidence of PWD and mortality. The third study compared the efficacy of EYA, zinc oxide, fumaric acid

and antibiotics in controlling ETEC (K88) in10-d old weaned piglets. Piglets fed PPI-based diets supplemented with these additives performed better and showed lower incidence of scours ($P < 0.05$) compared with piglets fed unsupplemented PPI-based diets. It was concluded that supplementing PPI-based diets with EYA, FA, AB, ZnO or SDPP improved weight gain and reduced incidence of diarrhea and mortality in 10-d old weaned piglets. EYA offers several advantages over the other alternatives as large amounts of specific anti-ETEC antibodies can be produced inexpensively, the product is environmentally friendly, is safe to use and compatible with the current animal production regulations.

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LIST OF ABBREVIATIONS

AA	Amino acid
AB	Antibiotic
ADFI	Average daily feed intake
ADG	Average daily gain
AOAC	Association of Official Analytical Chemists
AuSDPP	Autoclave spray-dried porcine plasma
BW	Bodyweight
CCAC	Canadian Council on Animal Care
CD	Crypt depth
CFU	Colony forming unit
CP	Crude protein
DE	Digestible energy
DM	Dry matter
ELSA	Enzyme-linked immunosorbent assay
EWP	Early-weaned pig
Exp	Experiment
ETEC	Enterotoxigenic *E. coli*
EYA	Egg-yolk antibody
FA	Fumaric acid
FC	Fecal consistency
FCE	Feed conversion efficiency

N	Nitrogen
NSP	Non-starch polysaccharide
PCR	Polymerase chain reaction
PPI	Pea-protein isolate
PUN	Plasma urea nitrogen
PWD	Post-weaning diarrhea
SBM	Soybean meal
SAS	Statistical analysis
SDAP	Spray-dried animal plasma
SDPP	Spray-dried porcine plasma
VH	Villous height
VASS	Visual assessment scoring system
ZnO	Zinc oxide

CHAPTER ONE

GENERAL INTRODUCTION

Increasing emphasis has been placed on the early weaning of piglets in recent years. This trend is compelled by factors such as increasing the number of pigs per sow per year, the need to optimize swine farrowing facilities by pushing more sows through the unit, and to eliminate disease transmission from the sow to the piglets. However, early-weaned pigs suffer a slow-down in growth rate immediately after weaning. Social, environmental and disease stressors are involved, and most importantly diet, clearly has a profound effect. The young pig before weaning receives sow's milk that is not only highly palatable and easily digestible but also contains high levels of immunoglobulins in the colostrum (Deprez et al., 1986). However, after weaning baby pigs are fed dry diets containing complex carbohydrates and proteins from plants that are not readily utilized as a result of a relatively less developed gastrointestinal tract (Cranwell, 1995). Low digestibility of plant-based proteins, a transient hypersensitive response to plant-based proteins (Friesen et al., 1993) and, possibly, the inability to resist enteric diseases, such as post-weaning diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) have been reported to contribute to poor growth or growth retardation in early-weaned pigs (Cranwell, 1995). Weaning as early as three weeks of age has been achieved over the past 20 to 30 years using more complex diets (Okai et al., 1976). However, such diets have only minimized but not prevented the post-weaning lag in growth.

During the past few decades, antibiotics have been the therapy of choice for the control and prevention of ETEC and other infections. Their routine use, however, has contributed to the appearance of multiple drug resistant *E. coli* and other pathogens thereby limiting their use (Hays, 1986). In addition, many of the commonly used antibiotics have been banned in Europe as feed additives (Brufau, 2000) and undoubtedly, this will be extended to North America. Recently spray-dried porcine plasma has been widely used as a feed additive in the diet of early-weaned pigs (Hansen et al., 1993; Kats et al., 1994; Coffey and Cromwell, 1995). This plasma has been shown to be highly effective at enhancing feed intake, weight gain, with improvements being as high as 10 to 100%. The beneficial effects of plasma protein have been attributed to its high content of high quality and readily digestible protein and the presence of specific antibodies. Other procedures that have received considerable attention for the control intestinal disease are diet acidification, use of oligosaccharides and probiotics as competitive inhibitors for binding of the organism to the intestinal receptors, and minerals such as copper and zinc salts as inhibitors of microbial growth. Some of these agents have been shown to be growth enhancers when added to baby pig diets, however, their ability to control intestinal pathogens have only been speculated (Close, 2000). There are also limitations to some of these treatments and often, they are not completely effective when used as the sole means of controlling intestinal diseases. There is also public concern of environmental pollution in the case of mineral salts.

The use of therapeutic antibodies to confer passive immunity appears to be an appealing alternative approach. Numerous experiments have looked at oral administration of antibodies (immunoglobulins) with pre-defined specificity obtained from colostrum (Shimizu et al., 1988), serum (Spier et al., 1989) or monoclonal antibodies (Sherman et al., 1983; Smith and Lida, 1990) to combat intestinal diseases in animals and plasma protein to improve both post-weaning growth and overall health status of early-weaned piglets. Unfortunately, these approaches are either prohibitively expensive, and/or impractical to obtain large amounts of these antibodies (Yolken et al., 1988).

Egg-yolk is a rich source of antibodies (IgY) that are readily extracted and purified (Jensenius et al., 1981; Grossman et al., 1990). It is possible to obtain antigen specific IgY by immunizing chicken with the desired antigen (Bartz et al., 1980; Yolken et al., 1988; Yokoyama et al., 1992). Chicken egg-yolk antibodies have successfully been used as a therapy to control rotavirus infection in mice (Bartz et al., 1980; Yolken et al., 1988), diarrhea caused by *E. coli* K88, K99, 987P in neonatal (Ikemori et al., 1992; Yokoyama et al., 1992; Jaradat, 1999) and weaned piglets (Zuniga et al., 1997; Marquardt et al., 1999). As a result of their availability and ease of production and purification, chicken egg-yolk antibodies seem to be excellent reagents for control of PWD and possibly improve post-weaning pig performance.

It was hypothesized that: 1). Control of intestinal diseases in early-weaned pigs will require new strategies in view of the problems associated with antibiotics and the limited effectiveness of certain treatments such as the use of probiotics

and other additives. This will be achieved, to a considerable degree, by passive immunization through the use of antibodies, especially those produced in the egg-yolk of laying hens. 2). Addition of egg yolk antibody (EYA) to pea protein isolate (PPI) based-diet will reduce transient hypersensitivity and enteric distress that is commonly associated with the use of PPI in a manner similar to that obtained by the use of spray-dried porcine plasma. EYA when added to a diet containing PPI should therefore reduce the post-weaning lag in growth associated with the feeding of plant based-protein diets to early-weaned pigs.

The main objectives of the research described in this thesis were to: 1). Compare the efficacy of controlling *E. coli* pathogeneity in young pigs using egg-yolk antibodies, spray-dried pig (SDPP) and animal plasma (mostly ruminant origin), antibiotics and other treatments such as copper, zinc and organic acid acidifiers. Acidifiers in some cases and in contrast to popular beliefs may enhance rather than reduce *E. coli* pathogeneity. 2). Evaluate the ability of a PPI-based diet supplemented with EYA to replace spray-dried plasma proteins in baby pig nutrition. Spray-dried pig plasma, although widely used in the industry, is expensive, has a variable antibody content and its use is not compatible with control of certain human diseases. 3). Determine if there is a synergistic interaction among the above-indicated treatments. Specific objectives were:

a. Determine if the specific anti-*E. coli* antibodies present in spray-dried porcine plasma (SDPP) is in part responsible for the superior performance of early-weaned pigs when fed SDPP compared with pigs fed other protein sources.

b. Compare performance of 10-d old weaned pig fed diets containing pea protein isolate, pea protein isolate plus egg-yolk antibody, or spray-dried porcine plasma.

c. Determine the effect of the different diets on the health of the piglets as indicated by incidence and degree of scours, mortality and morbidity, shedding of microorganisms, (*E. coli*) and functional characteristics of the gastrointestinal tract (digesta pH, villi height, and crypt depth).

d. Assess the effectiveness of EYA in controlling ETEC relative to conventional additives in early-weaned pigs fed pea protein isolate-based diets.

CHAPTER TWO

LITERATURE REVIEW

Early-weaning and Influence on Pig Performance

Emphasis by the swine industry on increasing the number of pigs weaned per sow per year necessitates a decreased weaning age. This has been the trend in swine production in recent years. However, early-weaned pigs mostly show depressed growth rate in the fortnight after weaning (Leece et al., 1979). Nutritional, physiological, psychological, environmental stresses and changes in immune status are among the factors responsible for this post-weaning 'lag' in growth. In addition, these factors may also predispose piglets to the development of post-weaning diarrhea (PWD).

Young pigs, before weaning, receive sow's milk that is highly palatable, readily digestible and has a high level of immunoglobulins in the colostrum (Deprez et al., 1986). At weaning, the young pig is often fed a dry plant-based diet that has a high content of complex carbohydrates that may not be readily utilized, as this necessitates a dramatic change in endogenous enzyme secretion. A rapid development in the digestive tract has to take place and the combined effects of these changes can result in a temporary reduction in digestive competence, pre-disposing the young animal to malabsorption (Kenworthy and Allen, 1966), which may lead to scouring. Weaning as early as 3 wk of age has been accomplished for a number of years by the use of complex

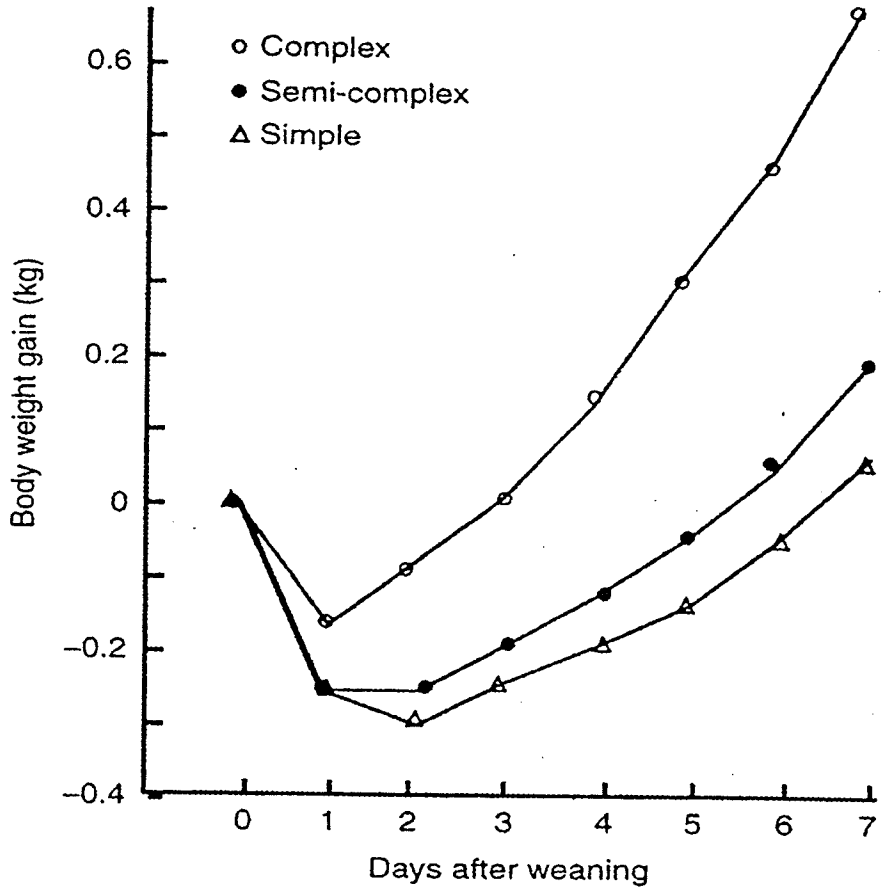

Fig. 1. Post weaning growth rate of pigs weaned at 3 weeks of age, first week. (From Okai et al., 1976).

diets (Okai et al., 1976). Such diets have minimized but have not prevented post-weaning lag at three weeks of age (Fig. 1). Low digestibility of soybean proteins, a transient hypersensitive response to soybean meal and, possibly, the inability to resist enteric diseases may contribute to the poor growth of early-weaned pigs.

Plant Proteins and Hypersensitive Response in Baby Pigs

Early-weaned pigs have been typically characterized by poor growth performance when fed plant based protein. It has been proposed that dietary antigens in plant-based proteins (Li et al., 1990) are responsible for the associated post-weaning diarrhea (Le Guen et al., 1991). The antigenic effects have also been associated with damaged microvilli, villous atrophy, crypt depth hyperplasia, and a reduced crypt/villous ratio in piglets fed diets containing soy proteins and other leguminous plants (Cera et al., 1988; Li et al., 1990, 1991; Dreau et al., 1994; Makinde et al., 1996). Antigenicity refers to the reaction seen at weaning in calves and piglets when protein sources such as purified plant protein are included in the diet (Rooke et al., 1998). Feeding raw pea flour to pre-ruminant calves (Nunes do Prado et al., 1988) and pigs (Le Guen et al., 1991) has been reported to result in the formation of antibodies against pea proteins (legumin and vicilin), along with an increased gut permeability to macromolecules. In normal physiological conditions, the gut-wall prevents the passage of dietary antigens from intestinal lumen into the blood circulation (Walker et al., 1975). Li et al. (1990) also reported that damage to the microvilli,

as evidenced by villous atrophy and crypt hyperplasia, results in an immune response at the intestinal level. Antigenic proteins may cause delayed hypersensitivity or immediate hypersensitivity that may lead to mucosal inflammation. Friesen et al. (1993) reported that glycinin and β-conglycinin present in soy protein are potentially antigenic within the intestinal lumen and suggested that a delayed type hypersensitivity response at the intestinal level takes place.

As indicated above, an immune response to foreign proteins at the intestinal level has been found to damage the microvilli, evidenced by villous atrophy and crypt hyperplasia (Newby et al., 1984; Stokes et al., 1987; Li et al., 1990). Dunsford et al. (1989) observed intestinal damage due to villous atrophy and crypt depth hyperplasia when pigs were no longer offered a milk diet but fed soy protein. In addition, Stokes et al. (1987) reported a decrease in villi height and increase in bacterial infiltration by day 2 post-weaning when a soybean meal diet was fed. The decrease in villi height corresponded to decreases in daily gain and gain : feed ratio, with an increase incidence of scours due to bacterial infiltration. An earlier report by Kenworthy and Allen (1966) indicated that the damage to the intestinal microvilli was associated with decreased enzyme concentration leading to malabsorption of ingested nutrients. Li et al. (1990, 1991) not only reported an increase in serum IgG titres specific to soy protein but also increase in skin fold thickness.

Lalles (1993) reported that although the reaction is transient and variable, it can be very severe and result in decreased performance, health breakdown

and incidence of diarrhea. By feeding antibiotics, the stress load of the young pig is reduced, decreasing the magnitude of delayed-type hypersensitivity response (Yen and Pond, 1987). Newby et al. (1984), therefore, suggested that the young pig must be exposed to the dietary antigen before weaning to initiate the transient hypersensitivity response upon secondary exposure to the dietary antigen post-weaning. Earlier report indicates that young pigs should consume about 600 g of creep feed to develop tolerance to the antigenic properties of dietary protein (English, 1981). Conversely, studies of Giesting and Easter (1986) and Hampson et al. (1988) were in conflict with the above reports. Hansen et al. (1993) suggested that adding SDPP potentially masked the transient hypersensitivity response of pigs fed soybean meal from 0 to 14 d post-weaning.

Diarrheal Disease in Baby Pigs

Diarrheal disease caused by ETEC is by far the most common and well recognized enteric colibacillosis encountered in neonatal and weaned pigs (Morris and Sujka, 1985, Yokoyama et al., 1992, Alexander, 1994, Hampson, 1994). It is also known that colonization of the small intestine of the pig by ETEC adhering to the epithelium accounts for most gastrointestinal disorders in both neonatal and post-weaning piglets (Yokoyama et al., 1992; Alexander, 1994; Hampson, 1994). A survey of pre-weaning disease, showed that diarrhea had the highest morbidity and represented 11% of the pre-weaning mortality, with ETEC being the primary and sole infectious cause (Alexander, 1994). A second disease, referred to as post-weaning *E.coli* diarrhea (PWD), usually starts 3-10

days after weaning (Hampson, 1994). This disease is a major cause of economic losses to the pig industry from both mortality and reduced growth rates, and is the most common cause of post-weaning mortality on many farms, killing approximately 2% of pigs weaned (Hampson, 1994).

Strains of ETEC Associated with PWD

The strains of ETEC that are associated with intestinal colonization are those that express the K88, K99, 987P and F18 fimbrial adhesins (Nakazava et al., 1987; Nagy et al., 1990, 1992; Nagy and Fekete, 1999). These adhesins are located in the rod-like pili (fimbriae) that extend from the *E. coli* and are bound to specific receptors on the intestinal wall, providing a highly specific means of anchoring the *E. coli* to the host animal, a prerequisite for an infectious agent (Erickson et al., 1992). Among the different ETEC strains, those expressing the K88+ fimbrial antigen are the most prevalent form of *E. coli* infection found world-wide wherever pigs are raised in high numbers (Rapaczs and Hasler-Rapacz, 1986). It has been estimated that K88+ ETEC are responsible for 50% of the 10 million piglet deaths each year (Waters and Sellwood, 1982). According to Smith and Linggood (1971), *E. coli* fimbriae are closely associated with the intestinal mucosa, and have specific adhesive factors that prevent them from being dislodged. The K88 fimbriae can exist as antigenic variants, K88ab, K88ac and K88ad, all of which allow adhesion to villous enterocytes throughout the small intestine due to changes that occur in the small intestinal mucosa and associated epithelial receptors in the period immediately following weaning. The newest *E.*

coli pilus which causes diarrhea in young pigs is F18 (Nagy and Fekete, 1999). It differs from the common *E. coli* pili K88, 987P, F41 and K99 in the period of infection. It strikes almost invariably at post-weaning resulting in high mortalities (between 30-40%), diarrhea and decreased performance (Nagy and Fekete, 1999). *E. coli* strains K99 (F5), 987P (F6) and F41 are other fimbriae (pili) that bind to porcine intestinal mucosa but these are not commonly associated with PWD (Nagy et al., 1996).

Diarrhea induced by ETEC in post-weaned pigs is responsible for considerable economic losses in swine production. The existence of F1 to F17 strains, just recently F18 pilus and possibly other strains yet to be identified call for research into more convenient and cost-effective means of treating and preventing *E. coli* infections.

Mechanism of Enterotoxigenic *E. coli* Infection

Adhesion to the gut mucosa is required to overcome the natural clearing mechanisms of the intestine (Kenworthy, 1976). Several factors aid colonization of the intestine by pathogenic *E. coli*. The brush border of the intestinal epithelium of newly weaned pigs may be damaged by components in the feed (Kik, 1991) or by viruses (Lecce and King, 1982) allowing *E. coli* to adhere and colonize the damaged epithelium. In addition, pigs are no longer protected by the milk of the sow after weaning, an important factor that prevents *E. coli* colonization during the suckling period (Deprez et al., 1986). Finally, newly weaned pigs produced less digestive enzymes (Cranwell, 1995). During this

period of temporary enzyme shortage, feed is digested and absorbed poorly, creating a fertile environment for pathogenic *E. coli* to proliferate readily.

Particles laying free in the small intestine lumen are flushed down by the constant cleansing action of secretion in concert with peristalsis (Kenworthy, 1976). High bacterial numbers cannot be maintained if the bacteria do not adhere to the intestinal wall. Interaction between fimbriae and fimbrial receptors on enterocytes bring about adhesion. Four distinct toxins are produced by the causative strains of *E. coli* singly or in variable combinations (Gyles, 1994). The high molecular weight, heat labile toxin, LTI, is a protein of approximately 88 kDa. It is a classical exotoxin consisting of one active or catalytic subunit (subunit A) and five binding subunits, B. Non-antigenic enterotoxins, STI (Sta) and STII (STb), are heat stable small peptides with a molecular weight of 2 kDa. These three enterotoxins act locally on the intestinal mucosa, where they bind to receptors to cause secretory diarrhea (Levinson and Jawetz 1998). As illustrated in figure 2, A subunit exotoxin stimulates adenylate cyclase activity catalyzing addition of ADP-ribose to G protein. As a result, cyclic AMP (cAMP) concentration increases stimulating cAMP-dependent protein kinase. This causes outpouring of fluid, bicarbonate, potassium and chloride from enterocytes into the gut lumen resulting in diarrhea (Levinson and Jawetz, 1998).

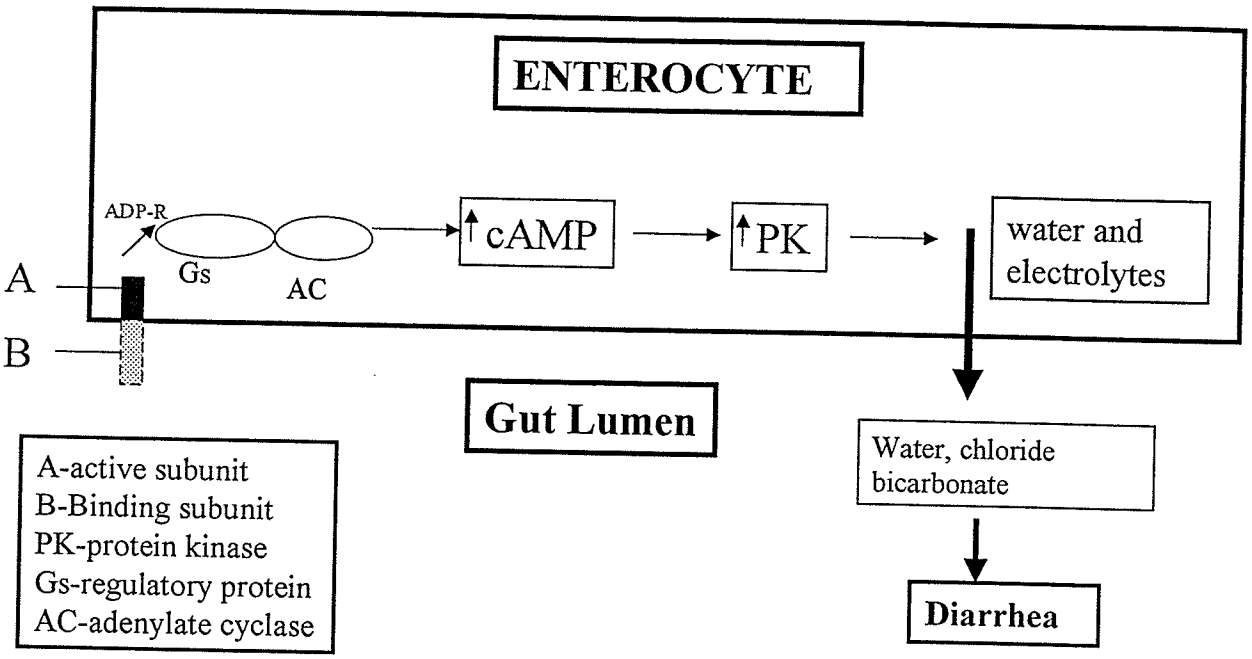

Fig 2. Mechanism by which *E. coli* induce diarrhea

Factors Affecting PWD

Genetic

There is a strong association between colonization of the small intestine of recently weaned pigs by hemolytic ETEC and the occurrence of diarrhea. Hemolytic strains proliferate in the intestinal tracts of healthy pigs after weaning, though in lower numbers than in littermates that develop diarrhea (Svendsen et al., 1977). The absence of appropriate receptors for K88 fimbriae on the brush border of small intestinal enterocytes has been linked to poor colonization in some cases (Nagy and Fekete, 1999). Smith and Halls (1968), however, demonstrated that it was possible to reproduce diarrhea and edema disease in weaned pigs, which lacked the K88 receptor using enterotoxigenic strain O141: K85ac. In their study, the inoculated strain colonized pigs from nine different farms, but the disease was only reproduced in animals from one of the sources, with the inoculated strain present in the feces at about two to three logs higher than it was in the animals from the other piggeries. The increase susceptibility was attributed to the presence of receptors for other adhesins possessed by strains such as F107 (Stamm and Berstchinger, 1992). These and other studies have therefore, demonstrated that there is a genetic predisposition of pigs to colonization by certain strains of *E. coli,* which is determined by the nature of the intestinal receptors.

Loss of Sow's Milk

The occurrence of PWD is most often preceded by the recent withdrawal of the sow's milk which contains a number of specific and non-specific protective factors (Deprez et al., 1986). The withdrawal of a regular supply of this protective material at weaning increases the opportunity for intestinal pathogens, to selectively proliferate over other intestinal flora. Deprez et al. (1986) demonstrated the protective effect of milk in weaned pigs by supplementing their diet with sow's milk, thereby inhibiting the normal post weaning proliferation of hemolytic *E. coli*.

Physiological Factors Influencing PWD

Numerous physiological changes that occur after weaning are exacerbated by management practices such as weaning piglets at an early age. These changes predispose weaned pigs to PWD.

Gastric Acidity

After weaning, the pH of the gastric content increases to non-bactericidal levels. According to Schulman (1973), these changes enable ingested ETEC to survive and gain access to the small intestine. Hampson (1986), on the other hand, stated that weaned pigs tended to have both more acidic gastric contents and fewer viable coliform organisms in the stomach than suckling pigs. Post-weaning diarrhea is mostly initiated from an increase in number of bacteria that colonize the intestine of piglets prior to weaning.

Small Intestinal Structure, Function and PWD

Immediately following weaning there is a series of changes in the small intestinal structure and enterocyte brush border enzyme activities of pigs (Miller et al., 1984). Villi height can be reduced by 25% within 24 h of weaning, while crypt depth normally increases steadily over a 11-d period after weaning. Also, brush border lactase and sucrase activities decline to a minimum by 5 d after weaning, maltases increase, while alkaline phosphatase is largely unaffected (Hampson, 1994). In addition, sodium-dependent alanine transport and capacity for xylose absorption are reduced, resulting in a temporary reduction in intestinal digestive and absorptive function after weaning (Hampson, 1994). Hypersensitivity to dietary antigens (Miller et al., 1984), the action of viruses (Lecce et al., 1978), a loss of appetite or normal adaptive changes that are exaggerated by early weaning (Kelly et al., 1991) could predispose pigs to PWD. Reduction in the digestive and absorptive function of the small intestine encourages the development of osmotic diarrhea. In addition, unabsorbed dietary material might act as a substrate for ETEC in the gastrointestinal tract. Finally, changes in villous enterocytes populations after weaning may expose new receptors to ETEC (Nagy et al., 1992).

Fermentation in the Large Intestine

Baby pigs have small under developed large intestine, which rapidly increase in size following weaning at 3 weeks of age (Hampson, 1986). The large

intestine is the major site for water and electrolyte absorption in the weaned pig (Hamilton and Roe, 1977). Also absorption of volatile fatty acids (VFAs) facilitates this activity (Argenzio, 1982). Newly weaned pigs are predisposed to develop diarrhea because their large intestinal microflora is not completely developed. Hence, fermentation is limited, therefore, water and electrolyte movement associated with absorption of VFAs are impaired (Hampson, 1986). In contrast, Etheridge et al. (1984) disputed this fact and instead suggested that ingestion of poorly digestible diets by weaner pigs result in VFA production, and subsequently, causing an osmotic diarrhea.

Nutritional Factors and PWD

Several aspects of nutrition have been studied in pigs in relation to the development of PWD and the reductions in growth rate.

Dietary Intake and Composition

Different views have been published about the interaction between feed intake, before and after weaning, and the occurrence of PWD. For instance, a study by Miller et al. (1984) indicated that consumption of small amounts of creep feed before weaning may lead to intestinal hypersensitivity reactions to dietary antigens after weaning, which exasperates PWD. Hampson et al. (1988), on the other hand, did not substantiate this observation. Li et al. (1990, 1991) reported that there is good evidence that soybean meal in the diet may be capable of inducing immunological responses that damage the intestine. It has been

generally accepted that intake of creep feed is important in increasing body weight at weaning which, in turn, reduces the risk of developing PWD.

Restriction of feed intake after weaning, alone or in combination with an increase in fibre content of the diet, have been shown to reduce both the proliferation of hemolytic *E. coli* and the occurrence of PWD (Bertschinger et al., 1976). This manipulation, generally, reduces the amount of substrate available in the intestinal tract, which would otherwise be used by enterotoxigenic *E. coli*. Leece et al. (1979) observed that high post-weaning nutrient intake such as obtained by three daily feedings produced more prolonged diarrhea and a greater colonization by hemolytic *E. coli* than did the same diet when fed in 24 equal hourly increments per day. Another study by Hampson et al., (1988) demonstrated a direct positive relationship between the amount of a weaned diet that was consumed, the duration of fecal excretion of hemolytic *E. coli* and the occurrence of PWD. Earlier study by Smith and Hall (1968) also showed that higher concentrations of fishmeal or sugars in the weaner diet predisposed the pig to PWD, again presumably by supplying substrate for bacterial proliferation.

Physical Form of the Diet

Feeding liquid diets to weanling pigs reduced the number of coliform bacteria in the intestinal tract (Decuypere and Van der Heyde, 1972), and prevented both post-weaning growth reduction and PWD (Tzipori et al., 1984). This is more evident for pigs regularly fed milk-based diets, where a direct antibacterial effect of the diet was postulated (Deprez et al., 1986). This

protective effect was associated with other changes in the intestinal tract after weaning. For example, the reduction that normally occurs in height of villi, crypt cell production rates and brush border enzyme activities after weaning are apparently triggered by exposure to a dry pelleted meal at weaning (Hall and Byrne, 1989). This observation is supported by the fact that the extent of these biological changes are reduced if the weaner diet is made up of a liquid sow milk replacer (Hampson, 1986), fed as a liquid slurry (Deprez et al., 1987), or if ewe's milk is fed regularly at 2 h intervals (Pluske et al., 1997). Thus, the occurrence of PWD in susceptible pigs is most likely due to complex series of interactions between morphological and functional changes in the gastrointestinal tract, the amount and the composition of the dietary ingredients, and the extent of proliferation of hemolytic *E. coli*.

Environmental Temperature

Cold temperatures or temperature fluctuations predispose weaned pigs to PWD. English (1981) showed that a sudden drop in temperature could precipitate PWD, even in pigs on an optimal diet. In another study of risk factors associated with the occurrence of PWD, Madec and Josse (1983) reported that large temperature fluctuations greatly increased the risk of developing PWD. In a further study, Wathes et al. (1989) also showed that moderate cold stress (15°C) increased susceptibility to PWD.

The mechanism whereby inadequate thermal environments predispose pigs to PWD has not been well defined. Experiments by Armstrong and Cline

(1977) showed that chilling did cause an increase in the incidence of PWD in one group of pigs, but the number of hemolytic *E. coli* in the intestine was not elevated above the control group. However, in a study by Wathes et al. (1989), chilled pigs with PWD did have elevated numbers of hemolytic *E. coli* in their feces. Chilling has been reported to increase the rate of passage of digesta through the intestinal tract, encourage increased feed intake (Le Dividich et al., 1977), and reduce antibody mediated immune responses (Blecha and Kelley, 1981). Any or all of these effects could interact in the predisposition of pigs to PWD.

Rotavirus Infections and PWD

Rotavirus infection has been implicated in some outbreaks of PWD (Leece and King, 1978). Sequential infection of gnotobiotic piglets with rotavirus and hemolytic *E. coli* have been shown to produce more severe diarrhea than that resulting from infection with each agent alone (Tzipori et al., 1980). Leece and King (1978) suggested that rotavirus at weaning damages the intestine and causes villous atrophy, which provides a favorable environment for subsequent colonization and growth of hemolytic *E. coli*. However, atrophy induced experimentally with transmissible gastroenteritis virus has actually been shown to cause both a marked decrease in intestinal response to *E. coli* heat stable toxins (Whipp et al., 1985), and diminished susceptibility of villous enterocytes to adhesion with K88ac$^+$ *E. coli* (Cox et al., 1988). Thus, with this finding, the potential role of rotaviruses in the etiology of PWD is uncertain, particularly as

rotavirus, with or without hemolytic *E. coli*, can often be recovered from healthy weaned pigs (Hampson, 1994). Moreover, at least one commercially available porcine-origin rotavirus vaccine has been found to be ineffective in controlling PWD (Hoblet et al., 1986). However, this is not conclusive evidence since there are a number of different groups and serotypes of rotavirus, with up to five different types of the virus being found in some piggeries (Fu et al., 1989). Age at weaning may also be important, since piglets weaned at 2 wk of age have a much higher prevalence of rotavirus excretion than those weaned at 4-5 wk (Svensmark, 1983).

Prevention and Control of PWD

Early-weaned piglets frequently suffer from PWD and edema disease. Enterotoxigenic *E. coli* strains play an important role in the pathogenesis of these diseases (Nagy et al., 1990; Nabuurs et al., 1993; Pluske et al., 1997; Nabuurs, 1998). During the first 2 wk after weaning, young pigs have reduced feed intake and increased incidence of PWD which have a negative impact on growth performance. Numerous strategies have been used to improve feed intake and health status of weaned pigs. There are two broad approaches that can be used for the prevention of PWD. The first attempts to minimize factors that predispose pigs to PWD, while the second is more specifically directed at the control of *E. coli*. A major challenge currently facing the swine industry is to identify means for controlloing PWD in the young pigs that are not only cost effective but also suitable for sustainable pork production. Various strategies including use of in-

feed antibiotics, plasma proteins, pro- and prebiotics, zinc and copper salts and recently antibodies and vaccines have been tried with variable results. Furthermore, there are serious safety and environmental concerns regarding the use of some of these products in piglets diets.

Antibiotics in Baby Pig Nutrition

Antibiotics are widely used in swine production to improve production efficiency and to ensure that safe pork is produced. It has been the therapy of choice for the animal industry for the control of animal diseases for more than 50 years. The widespread use of antibiotics has had a recognisable and profound effect on human and animal health. Sub-therapeutic doses are widely used for treatment and prevention of diseases in livestock and poultry production. Approximately, 80 and 75% respectively, of poultry and swine marketed or raised in the USA have been estimated to receive antibiotics at some point in their life (Hays, 1986). Anti-microbial agents in swine feed exhibit their effect through the following mechanisms: a direct suppressive effect on microbes such as pathogenic *E. coli* in the gastrointestinal tract and a nutrient-sparing effect (Dierick et al., 1986; Yen et al., 1987). Nutrient-sparing effects, most likely, are the result of an increase in the amount of nutrients, such as amino acids and carbohydrates, absorbed from the small intestine, as a result of a reduction in the amount of nutrients used by bacteria for growth. Just et al. (1980) observed that the addition of Nebactine to diets for growing pigs increased the apparent ileal amino acid digestibilities of most amino acids except histidine, isoleucine, and

phenylalanine. In a subsequent study with Virginiamycin supplementation, Just et al. (1985) showed that the apparent ileal digestibilities of amino acids improve significantly.

However, the link between performance benefits of antibiotics and an anti-microbial effect have not been convincingly shown. Nevertheless, a number of speculations suggest an indirect involvement of the microflora on the performance of animals as affected by antibiotics (Visek, 1978; Roth-Maier and Kerchgessiner, 1993). The modes of action of antibiotics as growth promoters seem to involve a reduction in bacterial fermentation in the intestinal tract. Ideally the mechanism of action of antibiotics is to disrupt microbial processes or structures that differ from those that are beneficial to the host. Antibiotics may damage pathogens by hampering bacterial cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function or blocking metabolic pathways through inhibition of key enzymes (Levinson and Jawetz, 1998).

Antibiotics Resistance in Farm Animals

The use of antibiotic remains a common practice in US and Canada because of their therapeutic value against disease and their ability to enhance the performance of animals fed sub-therapeutic concentrations (Kiser, 1976). However, health specialists and consumer groups are concerned with increasing resistance of microorganisms to antibiotics as a result of widespread use of drugs in the livestock industry (Hays, 1986; Cassell, 1995). Mathew et al. (1998)

observed that the percentage of *E. coli* resistant to Apramycin, Carbadox, Neomycin, Oxytetracycline and Gentamicin was higher on farms with high use rate than with low use rate in 35-d and 63-d old pigs. These authors further reported that even with low use rate, there has been an increased incidence of *E. coli* resistant to Gentamicin (81.7%) and Neomycin (44.6%) at 7 days of age, whereas the greatest increase in resistance to Oxytetracycline *in E. coli* from low use rate occurred at 63 days of age. Langlois et al. (1986) reported a high persistence of tetracycline resistance following use. Extensive and long-term use of Oxytetracycline and Chlorotetracycline have apparently resulted in a large population of tetracycline-resistant bacteria in swine as well as in other species (Allen et al., 1993). Resistance to Apramycin and Carbadox was found to be higher in *E. coli* isolated from young pigs than in *E. coli* isolates from their respective sows (Mathews et al., 1998). Both of these antibiotics are commonly used to control scours and variety of other problems associated mainly with young pigs. These authors therefore, suggested that transfer of organisms might occur from nurseries to farrowing rooms. Also, a higher percentage of resistant bacteria were observed in young mammals than in adults (Langlois et al., 1986). Langlois et al. (1986) speculated that bacteria from young animals may have a higher percentage of resistance because *E. coli* can easily and rapidly colonize the intestinal tract of younger individuals. This confirms the earlier report by Wierup (1975) that resistance may be greater in younger animals because bacteria in their intestinal tract have increased potential for resistance transfer. Wierup (1975) suggested that large-scale resistance may compromise

performance benefits associated with sub-therapeutic use of antibiotic. Visek (1978) on the other hand, disputed this theory by showing resistance to antibiotics had risen with increased use, whereas associated performance benefits had remained constant over the same number of years.

These observations strongly suggest that antibiotics use is the livestock and poultry industry should be either banned or at least limited and subjected to more severe regulations. Furthermore, many pathogens have developed multiple resistances to several antibiotics. In many cases, there is no effective antibiotic treatment. Moreover, antibiotic residues in meat and meat products may cause allergies in humans. In addition, many antibiotics are now banned for use in Europe (Brufau, 2000) and may be extended to North America in the near future as the resistance factor can be transferred from animal to human pathogens. Last but not the least, treatment of resistance with high doses of newer antibiotics can be expensive or prohibited. It is therefore urgent that alternative strategies be developed to control intestinal pathogens and improve early-weaned piglet performance.

Promicrobial Agents (Probiotics) in Baby Pig Diets

Promicrobial agents are live naturally occurring microbes that are fed directly to animals. Probiotics are thought to affect the host animals beneficially by improving its intestinal microbial balance or serving as a source of enzymes. Probiotics have been widely promoted as an alternative to the use of antibiotics in swine diets (Pollman, 1986; Pollman et al., 1980). Probiotics have the opposite effect to antibiotics on the microorganisms in the digestive tract. Whereas

antibiotics control the microbial population in the intestine by inhibiting or destroying micro-organisms, with probiotics, live bacteria are actually introduced into the intestinal tract for propagation (Pollman, 1986). Many strains of bacteria have been used commercially to produce direct-fed microbials, however, the most commonly used are *Lactobacilli*, *Bacillus*, *Bifidobacteria* and *Streptococci* species. Yeasts, both *Saccharomyces cerevisae* and *Aspergillus* species, have been most commonly included in the diet of monogastric animals to manipulate microbial conditions within the gut.

Both beneficial and potentially harmful bacteria can normally be found in the digestive tract of pigs. Examples of harmful bacteria are *Salmonella*, *E. coli*, *Clostridium perfringens* and *Campylobacter sputurom.* These bacteria will not only produce specific diseases known to be detrimental to the host, but through competition for essential nutrients, they can also decrease animal performance. In contrast to the effects of these disease-causing microorganisms, bacteria such as *Lactobacillus* species and the vitamin B-complex producing bacteria can be beneficial to the host. By encouraging the proliferation of these bacteria in the intestinal tract, it may be possible to improve animal performance (Pollmann, 1986).

Ideally, the beneficial bacteria will competitively exclude the growth of pathogenic bacteria in the intestinal tract, thereby, preventing disease and poor performance. Several authors have assessed the efficacy of probiotics as growth promoters for pigs. Most concluded that when results are averaged over several trials, there is a small improvement in growth rate and in the efficiency of feed

utilization, but that the results are highly variable. For instance, Chesson (1994) reported a 2.5 and 6.8% improvement in growth rate and feed efficiency, respectively. The effects of the probiotics appear to be more consistent and positive in piglets than in growing\finishing animal. However, the variability of results may be partly due to strain differences, dose level, storage condition, diet composition, feeding strategy and interactions with other drugs (Chesson, 1994).

The mode of action of probiotics has not been clearly defined. It has been suggested that probiotics increase the synthesis of lactic acid in the gastrointestinal tract of the pig (White et al., 1969). This increased production of lactic acid is postulated to lower the pH in the intestine thereby preventing the proliferation of harmful bacteria such as *E. coli* (Mitchell and Kenworthy, 1976). The decrease in the number of *E. coli* may also reduce the amount of toxic amines and ammonia produced in the gastrointestinal tract (Hill et al., 1970). In addition, other reports suggest that probiotics may produce an antibiotic-like substance and also stimulate the early development of the immune system of the pig (Shahani et al., 1976).

As indicated above, the value of using probiotics in pig diets has not been proven to be conclusive. For instance Nousianen and Setala (1993) obtained slight improvements in daily gain and feed conversion efficiency as a result of probiotic inclusion in the diet starter pigs which is typical of most of the research conducted with starter pigs. However, this is not always the case and other workers have reported an opposite effect. (Combs and Copelin, 1981; Pollmann et al., 1982). For instance studies involving *Bacillus subtilis* addition to pig or sow

diets failed to consistently improve growth or reproductive performance (Pollmann, 1986). Kornegay et al. (1995) reported no improvement in growth performance of weanling pigs when yeast was added to a corn-soybean meal diet. A recent review by Stavric and Kornegay (1995) also showed inconsistent response to probiotics in young and growing-finishing pigs. Some of the reasons for the variability of results include the fact that the viability of microbial cultures may be dependent on storage method, strain differences, dose level, frequency of feeding, species specificity problems and drug interactions (Pollmann, 1986). The difficulty in maintaining a viable *Lactobacillus* culture in pig feeds may also partially explain the inconsistency in research result (Pollmann and Bandyk, 1984). It is well documented that temperature, change in pH and various antibiotics will decrease the viability of *Lactobacillus* cultures. There is also the speculation that probiotics may actually have some negative effects on pig performance during the growing-finishing phase by competing for nutrients with indigenous organisms of the digestive tract, decreasing carbohydrate utilization and increasing the rate of transit of digesta in the intestine (Polmann, 1986). Thus, although the theoretical concept of probiotics appears promising, the documented evidence of their therapeutic value suggests that the search must continue for a workable alternative to antibiotics.

Non-Starch Polysaccharides (NSP) in Baby Pigs Diets

Plant NSP constitutes up to 90% of the cell wall (Selvendran and Robertson, 1990). Cellulose, hemicelluloses, and pectins are the most abundant

plant cell wall NSP. Other examples of NSP are fructans, glucomannans, galactomannans, mucilages, β-glucans and gums. Unlike starch, which is hydrolysed by pancreatic amylase to glucose, NSP are not hydrolysed by mammalian enzymes, but instead, are fermented by the gastrointestinal tract microflora (Grieshop et al., 2001).

Addition of 5% straw to a piglet starter diet has been found to reduce the transit time of digesta through the gut, increase the proportion of digesta in the hindgut and reduce the percentage of days with diarrhea from 6.0 to 3.5 (Bolduan et al., 1988a). Other sources of digestible fibre, or non-starch polysaccharides, such as sugar beet pulp, have improved overall NSP digestibility of the diet (Longland et al., 1991) and reduced the incidence of post-weaning diarrhea (Goransson et al, 1995). Other authors have observed decreased performance with NSP. For instance, Nunes and Malmlof (1992) demonstrated that feeding 60 g/kg guar gum reduced glucose absorption by 32 and 29% compared with an unsupplemented basal diet or a diet supplemented with 150 g/kg purified cellulose, respectively. Increasing the NSP content of swine diets most often, decreases the energy density and digestibility of the diet. Leclere et al., (1993) observed that addition of 60 g/kg beet pulp or wheat bran decreased the postprandial plasma triacylglycerol response in growing pigs as much as 40%. In another study using 3 wk old weaned pigs, Friere et al. (1998) demonstrated that inclusion of 15% wheat bran in diets reduced total tract apparent digestibility of energy, fat and ADF by 5, 10, and 10% units, respectively. In other studies, DM, nitrogen (Schulze et al., 1994) and amino acid

(Mason et al., 1982) digestibilities decreased when the dietary concentration of NSP was increased.

Oligosaccharides in Baby Pigs Diets

The binding of pathogenic bacteria onto the intestinal mucosa is an essential step in the development of infection and the onset of pathological conditions. In this respect, cell recognition is important and specific carbohydrates and sugars are fundamental to this process. Glycoproteins (lectin/fimbriae) on bacteria cell surface can recognise and combine rapidly and selectively with the sugars on the surface of the gastrointestinal wall. On the other hand, if they attach to a sugar or oligosaccharide, which is not part of the gut wall, but is an indigestible component of the feed, then they pass out with the digesta without causing any digestive problems in the animal. This principle of competitive exclusion is being used in pig nutrition and both mannose and fructose oligosaccharides have been used (Mul and Perry, 1994; Van der Beke, 1997).

Mul and Perry (1994) reported that the inclusion of fructo-ologsaccharides improved the growth rate of weaned piglets (7-18 kg body weight) by 5.1% and reduced the FCR value by 2%. Recently, Van der Beke (1997) fed mannose-oligosaccharide yeast to piglets (7 kg BW) over a 4-wk period and recorded a 7.4% improvement in growth rate, with a 5.2% improvement in feed utilization. This author, interestingly, recorded reduced number of piglets that scoured. Aside from their competitive exclusion properties, mannan-oligosaccharides have

been shown to enhance the immunocompetence of piglets. They increase the release of cytokines, which play an important role in the immune response by co-ordinating the actions of the immune system, and by enhancing immuno-stimulating activity (Spring and Privulescu, 1998). Thus, both piglet health and performance are improved. However, in contrast to these results, Davis et al. (1999) demonstrated improvement in weight gain and feed efficiency but not improvement in immunocompetence in early-weaned pigs provided with mannan-oligosaccharide-containing diet. In fact, the inclusion of higher amounts of viscous and indigestible carbohydrates may actually act as a barrier to diffusion of nutrients within the gut lumen, reducing access of digestive enzymes to substrates, limiting rate and amount of absorption of nutrients and subsequently decreased performance (Campbell and Bedford, 1992; Marquardt, 1997). Also because monogastric animals lack the enzyme, α-galactosidase, required to cleave the α-linked galactose units present in most oligosaccharides, they escape undigested to the lower intestinal tract (Grieshop et al., 2001). In the large intestine, oligosaccharides are subjected to microbial fermentation processes producing VFA and other intestinal gases such as carbon dioxide, hydrogen and methane (Grieshop et al., 2001). Nausea, diarrhea, cramps and discomfort, leading to decrease performance in the animals, are the consequences of the build up of these fermentation by-products. Coon et al. (1990) reported a 20% decrease in metabolizable energy of soybean meal when its oligosaccharides were not extracted.

Herbs and Flavors

Herbs have been widely used as alternative therapies in both human and animal medicine. Certain herbs are thought to contain a sophisticated array of unique organic components that may have specific therapeutic properties. Herbs have been found to enhance anti-microbial activity, anti-viral and anti-oxidative properties and are said to stimulate the endocrine and immune system (Jost, 1996). Furthermore, it has been suggested that they promote higher metabolic activity and, improve the immune status, as well as enhance the welfare of the animal. Their inclusion in the diet has also been shown to stimulate appetite by improving palatability (Jost 1996). This suggests that they have elixir-like activity.

Although a wide range of herbs, spices and oils are available for inclusion in animal feeds, one which has been most widely investigated is garlic. Garlic contains the amino acid L. allin and the enzyme allinase which are thought to be its active ingredients. In humans, garlic has been found to reduce the production of free radical and lipid peroxides, as well as blood lipid content. In pigs, Jost (1996) has shown that the inclusion of 0.05% garlic in the diet maintained the efficiency of feed utilization, reduced the incidence of scouring and number of piglets that died when compared to an anti-microbial growth enhancer. Overall there have been very limited studies on the beneficial effects of using herbs. Additional studies must be carried out to demonstrate their potential benefits.

Trace Minerals as Growth Stimulant in Young Pigs

The role of copper sulfate as a growth-enhancing agent is well established. Customary, 175-ppm copper (Cu) is added to the diets of pigs up to 12 wk of age and 100-ppm thereafter. Similarly, the inclusion of zinc oxide (ZnO) at pharmacological levels in piglet diets is reported to have therapeutic effects in improving performance and, considerably, reduces incidence and intensity of dairrhea/colitis in the post-weaned piglet (Holm, 1988; Kavanagh, 1992; Poulsen, 1995). Zinc oxide also increases growth rates in early-weaned and conventionally weaned pigs, regardless of diarrhea prevalence (Hahn and Baker, 1993; Poulsen, 1995; Carlson et al., 1999).

In Denmark, zinc (Zn) in the form of ZnO, has been used for the prevention of PWD for many years. Poulsen (1989) reported a significant effect of 2500 to 4000 mg Zn as ZnO per kg diet on reducing the severity of PWD. Also, Holm (1989) fed 2400 ppm ZnO during a 14 or 28 d trial and reported a significant reduction on the frequency of PWD. Another study compared 122-176 ppm Olaquindox and 1500–2500 Zn as ZnO with control diet on 6 farms (Holmgren, 1994). The author observed that Zn was as good a prophylactic agent as Olaquindox with no difference in growth promoting effect being seen between the antibiotic and the ZnO treatments. In contrast, higher frequency of PWD was seen in piglets fed the control diet alone. Very recently, Carlson et al. (1999) suggested that nursery pigs need to be fed ZnO supplemented diets for at least 2 wk after weaning for the growth-promoting effects to become apparent.

Aside the problem of environmental pollution, the disadvantage of using ZnO is that feeding grade sources of ZnO are extremely variable. The product can also differ widely in Zn concentration, colour, contaminants, bulk density and relative bioavailability of Zn because of different physicochemical processes employed in the manufacture (Edward and Baker 1999). Maviromichalis et al. (2000) recently compared the growth-promoting effect of high and low available ZnO in young pigs. They observed significant improvements in growth performance by the addition to the diet of high available ZnO during wk 1 of age. However, morphological examination of duodenal, jejunal and ileal intestinal sections at d 21 of the study revealed that neither source of ZnO had effect on crypt depth, villi height or width.

Although the antibacterial properties of ZnO have been established in human medicinal practices (Sordeberg et al., 1990), its mode of action in pigs has not been determined. High concentrations of dietary Zn as indicated, may be beneficial or harmful and may have a direct or indirect effect on the animal. It is well known that adequate dietary Zn is important for normal cell-mediated immunity, phagocyte (Prasad, 1983) and other functions such as control of RNA and DNA (Calson et al., 1999). Excess Zn ingestion, however, could actually be detrimental to immune function and therefore increase sensitivity of animals to disease (Roberts et al., 1999). Carlson et al. (1999) in contrast, suggested that the induction of metallothionein in enterocytes might be responsible for enhanced growth performance from pharmacological doses of Zn. Metallothionein participates in Zn homeostasis at the intestinal and systemic level and its

expression is directly affected by dietary Zn concentration (Cousins, 1985; Menard et al., 1981). Constantly high levels of Zn in the enterocyte due to upregulation of metallothionein may improve intestinal health (Carlson et al., 1999). In contrast, Mavromichalis et al. (2000) did not observe such beneficial effects on intestinal morphology. Thus, suggesting that high levels of dietary ZnO may instead exert either luminal or systemic effects. Huang et al. (1999) reported that high doses of ZnO in pigs was associated with reduced bacterial translocation from the small intestine to the ileal mesenteric lymph node, and with increased stability and homogeneity in coliform populations. Gram-positive bacteria are most susceptible to ZnO, while gram-negative bacteria and streptococci are not usually inhibited by high concentrations of ZnO (Katouli et al., 1999). This, however, need to be verified in diverse environments and management systems. However, recent reports by Mahan et al. (2000) indicated that growth responses to 1500 mg Zn/kg from ZnO was additive with responses to carbadox. Mahan et al. (2000) studied the anti-microbial properties of ZnO in post-weaned pigs exposed either to clean or dirty environmental housing conditions. These authors concluded that ZnO may have a transient advantage only during wk 1 of a 21 d feeding period.

Overall, the research results suggest that ZnO when added to the diet of young pigs improve animal performance and reduces the incidence of diarheal disease in young pigs (Holm, 1988; Kavanagh, 1992; Poulsen 1995). However, the anti-microbial property of ZnO has not been directly tested in animals including pigs challenged with ETEC. Also, excess Zn may exacerbate copper

and iron deficiency in the young pig. Finally, the use of high concentrations of ZnO in diet raises concern about the high rate of its excretion into the environment and the possible problem of Zn toxicity to growing pigs.

Diet Acidification

Organic and inorganic acids are routinely added to baby pig diets. Generally, addition of acid to the diet lowers the pH and buffering capacity of the digesta, increases both gastric proteolysis and nutrient digestibility, promotes beneficial bacteria at the expense of pathogenic microorganisms and finally, decreases the intestinal bacterial growth. Lowering dietary pH by weak organic acids, such as citric, formic, fumaric or propionic acid assists in overcoming problems with post-weaning lag period (Falkowski and Aherne, 1984). Diet acidification improves gastrointestinal health, leading to enhanced growth performance and improved feed utilization efficiency. Diet acidification has been shown to have a pronounced effect during the first few weeks after weaning, a period when the gastrointestinal tract (GIT) of the piglet is less developed and most vulnerable to infection. Several individual or mixed acids have been used with considerable success. In general, organic acids with higher pKa (i.e. the pH at which 50% of the acid is dissociated) values are more effective preservatives and their anti-microbial efficacy is improved with increasing chain length and degree of unsaturation (Foegeding and Busta, 1991).

Acidification and Pig Performance

Numerous experiments have looked at acidification of weanling pig diets as a means to improve the functional capabilities of the gastrointestinal tract, nutrient digestion and subsequently growth efficiency. Kershaw et al. (1966) found that 1% lactic acid addition to the drinking water improved growth rate and feed efficiency, and reduced the *E. coli* count in the duodenum and jejunum of pigs. Kirchgessner and Roth (1982) summarized the results of numerous experiments involving the use of fumaric acid. Improved live weight gain, feed intake and feed conversion efficiency as a result of improved digestibilities of nutrients were observed when weaner pigs were fed diets supplemented with 1.5 – 2.0% fumaric acid. In addition, nitrogen balance was improved by 5 to 7%, while metabolizable energy values of the diets increased by 1.5 to 2.1% (Kirchgessner and Roth, 1980). In a study with pigs weaned at 4 wk of age, and fed diets containing graded levels of fumaric acid, Lewis (1981) observed that weight gain was improved but feed intake or feed conversion efficiency and the incidence of scours was not affected. In another study, using more complex diets, Falkowski and Aherne (1984) reported that weight gain tended to improve by 1 or 2% by addition of 1 or 2% fumaric or citric acid, but the effect was not significant. Compared to negative control diet, Paulick et al. (1996) reported improvement in growth rate as high as 23% when the pig diet was acidified. Recently, Van Kol (2000) observed 1 – 3% and 1 –2% improvement in growth rate and feed utilisation as a result of supplementing baby pigs diet with acid. This author again reported a 28% reduction in mortality in group of pigs fed

acidified diets compared with the control group. Kirchgessiner and Roth (1982) suggested that 10 – 20 g/kg fumaric acid is most efficacious for improving post-weaning performance. In contrast, Giesting and Easter (1985) found no effect on the performance of pigs fed diets containing 10 and 20 g/kg fumaric acid, but gains were significantly improved with 30 and 40 g/kg fumaric acid supplementation. In a recent study with 1, 2 or 3% fumaric acid supplementation, Blank et al. (1999) showed improvement in protein digestion and apparent ileal amino acid digestibilities in weanling pigs. In contrast, adding 1.5 or 3% fumaric acid in diets, for weanling pigs did not increase apparent ileal digestibilities of amino acid (Gabert and Sauer, 1995). Acid additions improved feed intake and daily gains at FCR compared to the control.

Recently Partanen and Mroz (1999) reviewed the effects of dietary organic acids on performance of weaned piglets, and observed that fumaric and citric acid are the most commonly studied organic acids in the weaner pig. As indicated previously, dietary organic acids, in general tend to improve growth performance and the feed:gain ratio of weaned piglets, however, varying response have been reported (Giesting and Easter, 1985; Bolduan et al., 1988a,b; Roth et al., 1996). Possible reasons for the varying results relate to differences in the type and dose of acid used, composition of basal diet, age of animals and existing levels of performance (Ravindran and Kornegay, 1993). Ravindran and Kornegey (1993) suggested that a better performance could be expected under conditions that are sub-optimal for growth of piglets. In contrast, Risley et al. (1993) failed to observe any measurable effect of adding 15 g citric

acid/fumaric acid/kg diet on post-weaned growth performance or scouring in *E. coli* challenged piglets.

Numerous studies have shown that the use of organic acids may reduce coliform burden along the gastrointestinal tract (White et al., 1969; Thomlinson and Lawrence, 1981; Bolduan et al., 1988a,b; Mathew et al., 1991) and reduce scouring and piglets mortality. In a study to test the hypothesis that acidification may reduce the incidence of scours in young pigs, White et al. (1969), showed that acidification provided prophylaxis against scouring. Subsequently, Thromlinson and Lawrence (1981) reported that the multiplication of *E. coli* 0141:K85 (B) was reduced by acidification with a corresponding reduction in piglets mortality. Recently, Callessen et al. (1999) also observed a considerable decrease in frequency of diarrhea compared to that found in piglets fed antibiotic growth enhancers. Low luminal pH and rapid flow of digesta can markedly inhibit growth of microbes along the gastrointestinal tract (Maxwell and Stewart, 1995). Fuller (1977) also showed that acidic conditions in the stomach favor the growth of *Lactobacilli*, which possibly inhibits the colonization and proliferation of *E. coli* by blocking the sites of adhesion or by producing lactic acid and other metabolites which lowers digesta pH and therefore inhibit growth of *E. coli*. Fumaric acid supplementation has been reported to reduce *E. coli* count in the jejunum and the *Lactobacillus-Bifidobacterium* counts in the cecum and colon (Gedek et al., 1992). In contrast, Risley et al. (1992) reported that 15 g citric or fumaric acid/kg diet had no significant effect on *Lactobacilli*, *Clostridia* or *E. coli* numbers in the stomach, jejunum, caecum or colon of piglets. Clark and

Batterham (1989) also observed no appreciable effect of acidification on the incidence of scours. Diet acidification appears to improve baby pig performance depending on the diet composition. However, the direct effect of organic acids on PWD is inconclusive and requires further research. Also, according to Cline (1991), prices of these acids may change dramatically. Finally the current economics does not appear to warrant the use of organic acids in starter diets.

Mode of Action of Organic Acids

Piglets may not be able to maintain appropriate gastric pH during the transition from sows' milk to dry plant-based diets. A deficiency in the early-weaned pigs ability to maintain proper gastric pH necessitates the need for dietary acidification. The pH of the stomach contents is affected by factors such as diet, time after meal at which the pH was measured and sampling site. Stomach pH of mature pigs can reach as low as 2.0 – 3.5 which can be maintained by copious output of hydrochloric acid (HCl) from the parietal cells (Kidder and Manner, 1978). Suckling pigs, in contrast, employed numerous strategies to solve the problem of limited acid secretion. Conversion of lactose in sow's milk to lactic acid by the resident *Lactobacillus* in the stomach of the young pig, consuming frequent relatively small meals (Pond and Manner, 1984), momentally reduces the need for secretion of copious amount of acid and acidifiers in diet (Manners, 1970).

A low gastric pH has a role in preventing the movement of viable bacteria from the stomach into the upper small intestine (Stevens, cited by Easter, 1988).

A rise in gastric pH would, therefore, allow increased proliferation of *E. coli* (Smith and Jones, 1963) associated with scours and increased mortality (Thomlinson and Lawrence 1981). In addition, proliferation of coliforms in the stomach may lead to further dimunition of gastric acid secretion due to the release of a bacterial polysaccharide with an inhibitory effect on acid secretion (Baume et al., 1967). In contrast, acidic gastric conditions have been reported to have pronounced bacteriocidal properties for certain microbes, such as coliforms (Sissons, 1989). Viable microbes entering the digestive tract via the mouth are unable to pass through the acidic conditions of the stomach and successfully colonize the small intestine (Stevens, Cited by Easter, 1988).

Hydrochloric acid is also involved in the activation of pepsinogens. Pepsin has two pH optima one at 2.0 and another at 3.5 while their activities declines at a pH above 3.6 (Rerat, 1981). Therefore pigs with an elevated gastric pH also experience a net reduction in efficiency of protein digestion. In such instance, feed proteins may enter the small intestine essentially intact with an eventual reduction in efficiency of protein digestion (Ravindran and Kornegay, 1993). The end products of pepsin digestion also stimulate the secretion of pancreatic proteolytic enzyme (Rerat, 1981). In addition, acid from the stomach is the primary stimulant for pancreatic secretion of bicarbonate (Kidder and Manner, 1978). Moreover, acid leaving the stomach plays an important role in a feedback mechanism in the regulation of gastric emptying, thus, decreasing the digesta load on the small intestine (Cornelius, 1988). Inefficient digestion may also provide a basis for the initiation of scours in the young pig because of the

provision of abundant undigested substrates in the small intestine to support the proliferation of coliforms (Cranwell and Moughan, 1989). Enterobacteria like *E. coli* grow well on undigested protein (Van Kol, 2000).

Acid appears to influence bacterial growth by first decreasing pH below six which, in general, is less favourable for bacteria growth and secondly, certain acids have a direct action against specific microorganisms. Therefore, a low pH in the stomach of pigs is important for maintaining both optimal digestion of protein and for reducing bacteria growth in the stomach (Ravindran and Kornegay, 1993). In addition, Roth and Kirchgessner (1989), demonstrated that the bactericidal effect of organic acid was not only achieved by its pH lowering effects, but also by a direct effect of the acid anion. Organic acid (RCOOH) can penetrate the cell wall of bacteria. At neutral pH, in the intracellular sphere, the acid dissociates into an anion ($RCOO^-$) and a proton (H^+). To survive, the surplus of H^+ ions needs to be eliminated by the cell. This is a strong energy demanding process, which will often kill the bacteria. In addition, the dissociated anion ($RCOO^-$) of the acid has a disruptive effect on DNA synthesis, a mechanism responsible for cell replication (Van Kol, 2000). The dissociated acid, once inside the cell, also suppresses cell enzymes (decarboxylases and catalases) and nutrient transport.

Therapeutic Antibodies in Baby Pig Nutrition

Numerous approaches have been used to control/or reduce the incidence of diarrhea in early-weaned pigs. However, most of them suffer major setbacks

or are not practical. The use of therapeutic antibodies to confer passive immunity offers an attractive alternative approach.

The use of readily available blood of pigs obtained from slaughter plants is one source of immunoglobulins. The plasma fraction is collected, spray-dried and incorporated into the diet of early-weaned piglets. Numerous studies have shown that using this product result in significant reduction in incidence of diarrhea among piglets fed plasma-containing diets. It was speculated that this beneficial effect was attributable to the presence of antibodies against intestinal pathogens (Marquardt et al., 1997). An earlier study by Elliot (1978) showed that the addition of immunoglobulins obtained from porcine blood to a milk replacer maintained 66% survival rate in piglets compared with piglets fed a plasma-free control diet. In another study, Spier et al. (1989) evaluated the effect of plasma from horses immunized against wild heat-killed *E. coli* in a group of horses with clinical signs of endotoxemia. They observed that the mortality rate was 13% in horses given hyperimmune sera compared to 47% for those receiving the pre-immune sera. These authors again reported that the horses given the hyper-immune serum experienced a shorter recovery period and showed milder symptoms than those receiving the pre-immune serum. In a subsequent study, serum was harvested from pigs vaccinated against whole cell lysates of ETEC, feeding the serum collected protected piglets from diarrhea caused by the same ETEC compared to control piglets given serum of non-immunized pigs (Isaacson, 1994).

The growth promoting effects of porcine serum, as discussed subsequently, have been attributed to the quality of its protein but not to the ability of its antibodies to protect young pigs against pathogenic organisms (Gatnau et al., 1991; Hansen et al., 1993; Rantanen et al., 1994). It is our hypothesis that the beneficial effect of pig plasma is attributable to not only its content of a high quality protein but also the presence of antibodies that protect the pig against infection by intestinal pathogens. Under such conditions, pig plasma must also be considered to be a therapeutic or a prophylactic agent.

Using monoclonal antibodies, some diseases in domestic animals have been treated. An earlier study utilized specific monoclonal antibodies to treat calves infected with K99 diarrhea. In this trial, Sherman et al. (1983) observed a significant reduction in both severity of the diarrhea and mortality (29%) in calves given monoclonal anti-K99 antibodies compared to 82% mortality for the control group. In another study, Smith and Lida (1990) demonstrated that piglets that were fed monoclonal antibodies against *Actinobacillus pleuropneumoniae,* followed by a challenge with the same organism, markedly protected them against pleuropneumoniae; in contrast, those given saline died. In swine infected with pseudorabies virus, Marchioli et al. (1988) used monoclonal antibodies for short-term prophylaxis and therapy. Despite the therapeutic value of monoclonal antibodies, their use is prohibitively expensive and therefore it is not practical to use them for the large-scale treatment of swine diseases such as diarrhea caused by ETEC.

Chicken as a Source of Antibodies

Egg-yolk has been recognized as a rich source of specific antibodies (Gassmann et al., 1990, Jensenius et al., 1981; Marquardt et al., 1997). Chicken egg-yolk antibody IgY has a molecular weight (MW) of 180 kDa with two subunits with the MW of the heavy chain being 67 to 70 kDa and the light chain 22 to 30 kDa (Grossman et al., 1990). Hens produce eggs non-invasively and adjuvant does not cause severe response as occurs in mammals. In addition, the production and maintenance of high levels of specific antibodies over long periods of time is possible in hens. It is now possible to obtain antigen specific antibodies from egg yolk of hyper-immunized hens. These include the potential of producing more specific antibodies against mammalian antigens in birds compared to mammals because of the phylogenic distance between birds and mammals, low cost of production and convenience, and more importantly, compatibility with regulation of modern animal production.

A hen lays 200 - 300 eggs per year and according to Marquardt et al. (1997) one egg yolk contains approximately 150 mg of antibodies, an amount that has the potential to neutralize a large population of micro-organisms and may possibly prevent the post weaning lag in growth as a result of delayed hypersensitivity response associated with the feeding of plant-protein to early weaned pigs. The yolk or purified antibody (IgY) can be prepared by freeze-drying or spray-drying without loss of activity and can be fed directly to the young pig to provide protection against specific pathogens. In addition, the yolk is a source of highly digestible nutrients (Marquardt et al., 1997). These factors

indicate that the egg of a chicken should be a good source of antibodies to control intestinal pathogens in young pigs (Yokoyama et al 1992; Kim et al 1999; Jin et al 1998). Chicken egg-yolk antibodies are also phylogenetically distinct from mammalian antibodies. Therefore, they can be used in a multiple antibody assay with mammalian antibody with minimal cross-reactivity.

Egg-yolk Antibodies as Therapeutic Antibodies

Oral administrations of antibodies with predefined specificity have been reported to enhance immunity (Bartz et al., 1980; Shimizu et al., 1988; Yolken et al., 1988). Bartz et al. (1980) was the first to attempt the use egg-yolk antibodies to protect an animal (infant mice) against a pathogen (murine rotavirus) by feeding EYA raised against this pathogen. These authors reported that treatment of challenged mice with EYA decreased the infection rate from 90% (non-treated) to only 15% for EYA treated mice. Similarly, Yolken et al. (1988) observed that EYA produced against specific rotavirus prevented both virus excretion and virus-induced gastroenteritis, while EYA from non-immunized chicken failed to produce similar result.

Less than a decade ago, Yokoyama et al. (1992) carried out an excellent study on the use of chicken egg-yolk antibodies (EYA) to control ETEC-induced diarrhea. These authors reported complete passive protection of neonatal piglets against fatal enteric colibacillosis. The procedure involved the selection of toxigenic strains of *E. coli* (K88, K99 and 987P), multiplication of the different strains in the laboratory, isolation and purification of the outer rod-like extension

containing the adhesins (the fimbriae), injections of the fimbriae into laying hens, isolation and spray-drying of the antibodies from the egg-yolk after the antibody titre was high and the use of the antibodies to treat young pigs that were infected with the different strains of ETEC. In their study, piglets given chicken egg-yolk antibodies raised against fimbrial adhesions of *E. coli* K88, K99, and 987P at a titer of 625 or 2500 had a 100% survival rate after challenge with the corresponding strain of ETEC. Furthermore, it was reported that the piglets that received the antibodies at a titer of 2500 only had mild diarrhea for a short duration of time. They also demonstrated that fewer piglets in the group receiving the antibodies excreted *E. coli*, which decreased with the amount of antibody given and with duration of the experiment. In contrast, the *E. coli* challenged pigs that did not receive the antibody had high mortalities (often 100%), a high incident of severe diarrhea and they shed the corresponding ETEC for a long period of time.

Similarly, Ikemori et al. (1992) reported significant dramatic protection with a survival rate of 100% in all calves treated with IgY antibodies raised against *E. coli* K99 fimbriae. Moreover, no severe diarrhea and limited mortality occurred among the challenged calves while 100% mortality was seen in the control group. Zuniga et al. (1997) in a recent study using egg-yolk antibodies to control the intestinal colonization by *E. coli* F18 in weaned piglets reported that full protection of piglets was achieved against this strain of *E. coli*, in a dose dependent manner.

The protective effect of EYA was further demonstrated in 21-d old piglets induced with ETEC diarrhea (Kim et al., 1999). Piglets were challenged with a high dose of ETEC (10^{12} colony forming units, CFU). Half of the group, received skim milk plus egg-yolk powder from non-immunized hens, and the other half-received EYA administered 3 times daily for 2 d. These authors reported that the control piglets that were treated with the placebo developed severe diarrhea within 12 h and were dehydrated and lost weight within 48 h, resulting in 30% mortality. In contrast, the piglets treated with EYA showed no signs of diarrhea 24 or 48 h after treatment, had positive weight gain and recorded no mortality. The efficacy of chicken egg-yolk antibody to control diarrhea in baby pigs was recently demonstrated in a study with 3-d old weaned pig (Jaradat, 1999). In this study, piglets were orally infected with *E. coli* K88 and some were orally treated with chicken antibodies, Jaradat (1999) demonstrated that all the piglets challenged with *E. coli* developed diarrhea within 8 h and lost weight after challenge. However, piglets given EYA only had mild compared to the severe diarrhea in the group receiving no antibodies. Recently, Jin et al. (1998) demonstrated that purified antibodies from the yolk of the chicken immunized against the fimbriae of *E coli* (K88) were able to block the binding of ETEC K88 to the mucosal receptor and that the interaction of the antibodies with this strain of ETEC was fairly rapid as maximum protection was provided within 15 min.

It must be mentioned that the presence of egg lysozyme and ovotransferrin may also have antibacterial function, however, such an effect would only be obvious when high dosage of antibody-containing whole egg

powder is used. These component of the egg probably do not contribute significantly to the protection of piglets against ETEC since control diets (contained egg-yolk powder without antibodies) in most of the previous studies, in contrast to diets containing egg-yolk antibodies, did not protect pigs, against ETEC infection.

In summary, chicken EYA have been shown to be highly effective means to control enteric pathogens in both neonatal and weaned piglets. In addition, they are safe (GRAS status), produced non-invasively, sustainable, environmentally friendly and above all will be probably the method of choice for controlling intestinal diseases in animals in the future. More research needs to be carried out to ascertain their efficacy compare to other conventional and non-conventional treatments.

Plasma Proteins in Baby Pigs Diets

Plasma proteins [spray-dried (porcine plasma, SDPP) and (animal plasma protein, SDAP)] are by-products of blood from pork and cattle slaughter plants, respectively, and have been routinely added to the diets of early-weaned pigs in recent times. Numerous studies have shown that these products, at an inclusion rate of 3 to 10%, have consistently and dramatically improved feed intake, weight gain (Coffey and Cromwell, 1995; De Rodas et al., 1995; Jiang et al., 2000) and feed efficiency (Kats et al., 1994; Grinstead et al., 2000), and have reduced incidence and severity of post-weaning diarrhea (Van der Peet-Schwering and Binnendijk, 1995), with its effect being evident during d 0 to 14 post-weaning.

Gatnau and Zimmerman (1990) reported that baby pigs fed porcine plasma had greater ADG than pigs fed a conventional corn-soybean meal diet containing whey. In another study, piglets fed SDPP had increased feed intake and weight gain compared to those fed casein, meat extract or isolated soybean protein (Van der Peet-Schwering and Binnendijk, 1995; Jiang et al., 2000). Similarly, porcine plasma has been reported to be superior to dried skim milk or soybean meal (Hansen et al., 1993). Kats et al. (1994) reported that SDPP and spray-dried blood (SDB) meal were effective protein sources in the diet for the early-weaned pig. These authors reported values of 304 versus 254 g/d for weight gain and 445 versus 372 g/day for feed intake for pigs fed SDPP and fish meal-based diets, respectively. They further concluded that SDPP is an effective protein source for use in the phase 1 (d 0 to 14 post-weaning). Gatnau et al. (1991) and Gatnau and Zimmerman (1992) observed maximum growth performance in weanling pigs fed 6% SDPP. Hansen et al. (1993) and Owen et al. (1993), respectively, reported that pigs fed 13.4 and 10.0% SDPP had a significant improvement in growth and feed intake compared to those fed the control diet. Hansen et al. (1993) also reported an increased ADG when SDPP replaced skim milk in the phase 1 diet. The increase in growth of pigs fed SDPP-based diet was associated with an increased feed intake. This, therefore, supports the earlier finding that pigs had a preference for diets containing SDPP and consumed more feed than pigs fed diets containing skim milk (Ermer et al., 1994). In a preference test with weanling piglets, Ermer et al. (1994) concluded

that the higher ADFI for the feed containing SDPP compared to that containing SDAP could be attributed to its greater palatability.

Numerous studies have compared the composition of a control diet to that of plasma proteins. The response to plasma proteins seems to be partially influenced by the nature of the other dietary proteins in the diet (Table 1). Van Dijk et al. (2001) summarized the results from these studies and concluded that ADG, ADFI and FCE are greater when soy protein was used in the control diet instead of dairy protein. In an experiment to compare porcine to bovine spray-dried plasma, Hansen et al. (1993) observed no response to bovine plasma in the first 2 wk after weaning. In contrast, both SDPP and SDBP improved piglet performance post-weaning, in a direct comparison between SDPP and SDBP in 3 separate experiments. However, the response in ADG to SDPP was greater than that to SDBP (Rantanen et al., 1994; Smith II et al., 1995; Van der Peet-Schwering and Binnendijk, 1997). Gatnau and Zimmerman (1991) reported that differences in nursery environments are responsible for the observed effects of plasma proteins. These authors observed that growth rate and feed intake of individually penned weanling pigs housed in an all-in all-out nursery were not different between pigs fed a 10% SDPP or soybean meal-based diet. However, piglets on the latter diet performed poorly when they were group-penned and housed in a continuous flow nursery. In a subsequent study, Coffey and Cromwell (1995) fed SDPP to weanling pigs housed in an off-site, environmental chamber or a more typical on-farm, conventional nursery. SDPP enhanced piglets performance in the latter treatment and suggested that different inclusion

rates of SDPP in the diet was responsible for the differences in performance enhancement. From the literature, considerable variations between experiments are seen in the response to SDAP or SDPP. Baseline growth and composition of the control diet, as well as health and hygiene status (Coffey and Cromwell, 1995; Bergstorm et al., 1997) are among the factors responsible for these differences. However, these are only speculations based on limited comparisons between SDPP or SDAP and a control protein (Van Dijk et al., 2001).

The biological aspects underlying the superior value of SDAP and SDPP compared to other protein supplements are poorly understood. Different authors have postulated different modes of action for plasma proteins. Feeding plasma proteins has been hypothesized to reduce the incidence and/or severity of PWD. Health parameters, unfortunately, have not been recorded in most published data. Gatnau et al. (1990) and Van der Peet-Schwering and Binnendijk (1995) observed less diarrhea in piglets fed SDPP during the first 2 wk after weaning. In another experiment, piglets fed SDPP were found to require less treatment against gastrointestinal disorders during 2 wk after weaning than piglets fed diet without SDPP (Van der Peet-Schwering and Binnendijk 1997). It has, therefore, been suggested that nursery conditions, including presence of pathogens, play an important role in the magnitude of piglet response to dietary plasma proteins (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995; Angulo and Cubilo, 1998).

Immunoglobulins and complex protein fractions present in SDPP and SDAP are also thought to provide anti-microbial protection (Coffey and Cromwell,

1995; Godfredson-Kisc and Johnson, 1997; Jiang et al., 2000) and might also influence intestinal immune status in the transition to weaning (Jiang et al., 2000). They are thought to contribute to the overall immunocompetence of newborn piglets by binding bacteria thus preventing secretion of enterotoxins (Coffey and Cromwell, 1995) and might therefore, protect against the development of mucosal damage by enteric pathogens by restricting the passage of inert large molecules through the intestinal wall (Van Dijk et al., 2001). Immunoglobulins, more recently, have also been proposed to be responsible for the superior value of SDPP compared to that obtained with other protein sources (Godfredson-Kisic and Johnson, 1997). In other studies, De Rodas et al. (1995) and Gomez et al. (1998) also proposed that blood plasma immunoglobulins, by preventing bacterial damage to the intestinal gut wall, help maintain optimal intestinal function and gastrointestinal growth which, in turn, benefits piglet health and performance. DeGregorio and Barr (1989) found reduced mortality in pigs fed milk replacers containing bovine immunoglobulins. The immunoglobulins would presumably prevent viruses and bacteria from damaging the gut wall resulting in a more functional intestinal wall. Gomez et al. (1998) also showed that immunoglobulins obtained from processed blood and given orally to colostrum-deprived newborn piglets have beneficial effects on health and performance. Dritz et al. (1996) hypothesized that the immunoglobulins fraction of plasma proteins decreases exposure of the immune system to antigens, leading to decreased production of inflammatory cytokines which, in turn, increases feed intake.

In addition to specific and/or non-specific antibodies and immunoglobulins (Coffey and Cromwell, 1995), insulin-like growth factors-I (IGF-I) (De Rodas et al., 1995) and glycoproteins (Sanchez et al., 1993) have also been suggested to be responsible for the superior value of spray-dried plasma proteins. According to Sanchez et al. (1993) and Nollet et al. (1999), the benefits of blood plasma may also be attributed to the presence of oligosaccharide chains of glycoproteins in plasma which also provide binding sites for the fimbrial adhesins of *E. coli.* Using an in vitro study, Sanchez et al. (1993) also demonstrated that the oligosaccharide chains of glycoproteins obtained from plasma could act as a binding site for the fimbrial adhesins of *E. coli.* Attachment of F17 expressing *E. coli* strains to bovine mucus and brush border membranes was prevented by glycoproteins. Bound bacteria are easily flushed down by the constant cleansing action of the secretion in concert with peristalsis, thus maintaining a low intestinal bacterial load. These authors concluded that the inhibition was not due to immunoglobulins present in plasma since neither heat denaturation, proteolytic digestion nor removal of the antibodies from the plasma affected this inhibitory capacity. Cow plasma preparation was used as a source of adhesion blockers in studies of neonatal colostrum-deprived calves infected with F5 and F17 fimbriated *E. coli* strains (Nollet et al., 1999). The results suggested that the presence of glycoproteins in SDAP inhibited the adhesion of *E. coli* to the intestinal receptors and prevented the disease, since the cow plasma preparation lacked specific antibodies against the challenge strains. De Rodas et al. (1995) reported that plasma proteins contain high levels of immunocompetence IGF-I

(0.8 ng/mg), a peptide hormone in the somatotrophic axis that is involved in the regulation of growth by influencing intestinal mucosal function and gastrointestinal growth. However, SDPP-fed piglets showed superior performance but no change in plasma IGF-I concentrations. These authors concluded that the dietary IGF-I might have influenced intestinal mucosal function and gastrointestinal growth. Thus combinations of factors, including specific anti-ETEC immunoglobulins, IGF-1 and glycoproteins have been suggested to be responsible for the superior value of plasma proteins. None of these latter studies involved *in vivo* challenge studies. The effectiveness of each of the factors either alone or in combination, therefore, may be very different than that observed *in vitro*. Further studies must be carried out in the intact animal to clarify this matter

Plasma proteins contain antibodies against the common strains of ETEC but the titres are variable from batch to batch. Also these proteins, being from animals origin, have been banned in Europe because of the BSE scare, hence the future use of these products in North America is uncertain. Lastly, the product is highly expensive. It is therefore highly desirable to devise an alternate strategy to control ETEC in pigs.

Table 1. A summary of experiments evaluating spray-dried porcine/bovine plasma as a protein source for weanling pigs

No.	Experiment	Protein in control diet	Percentage improvement over pigs fed control diet		
			ADG	ADF	FCE
1	Hansen et al. (1990)	Skim milk	+42	+37	-4
2	Gatnau et al. (1991)	Soybean meal	+102	+76	+12
3	Sohn et al. (1991)	Skim milk	+29	+24	+1
4	Gatnau et al. (1991)	Soybean meal	+82	+34	+60
5	Hansen et al (1991)	Skim milk	+15	+28	-10
6	Gatnau et al (1990)	Skim milk	+50	+54	+29
7	Coffey and Cromwell (1995)	Skim milk	+40	+60	+12
8	Van der Peet-Schwering and Binnendijk 1995	Soy protein	+27	+19	-5
9	Grinstead et al. (1998)	Whey protein product	+28	+14	-10.6
10	Jiang et al. 2000	Extruded soy protein	+43	+29	+9

Source: Marquardt et al., 2001; Van Dijk et al., 2001.

Summary of Literature Review

The side effect of early-weaning is the risk of PWD in pigs, which causes retarded growth, increased mortality and extra medication costs. ETEC induced diarrhea in post-weaning pigs are responsible for considerable economic losses in swine production. The existence of F1 to F17 pili strains, now F18 pilus and possibly other strains yet to be identified call for extensive research into more convenient and cost-effective means of treating and preventing infections. Also, digestive disorders and subsequent poor performance can also be a problem in the fattening phase.

A major challenge currently facing the swine industry is to identify means for controlling diarrhea in young pigs that are not only cost-effective but also suitable for sustainable pork production. Thus far, various strategies including use of in-feed antibiotics, diet acidification, spray-dried plasma proteins, pro- and prebiotics, and mineral salts have been tried with inconsistent results. Furthermore, there are serious safety and environmental concerns regarding the use of these products in piglet diets. These concerns include antibiotic resistance associated with use of in-feed antibiotics, effect of spray-dried plasma proteins on human health, relatively high costs, unknown and variable levels of antibodies and possible implications of bovine spongiform encephalopathy (BSE) and environmental implications of excess fecal zinc and copper levels.

Egg-yolk antibodies (EYA) from laying hens hyper-immunized with specific bacterial fimbrial antigens have been proposed as effective means for controlling diarrhea in early-weaned pigs. Because EYA are not affected by the limitations

outlined above, their potential role in the nutrition and management of young pigs is considerable. Moreover, EYA are relatively cheap compared to plasma protein and contain large amounts of specific antibodies. In addition, using EYA may provide long-term and sustainable means of controlling pathogens and also prevent delayed transient hypersensitive response in early-weaned pigs when plant-based proteins are fed. However, to realize the full potential of EYA, a careful and thorough evaluation and demonstration of their advantages over competing alternatives is critical.

CHAPTER THREE

MANUSCRIPT ONE

Response of early-weaned pigs to spray-dried porcine or animal plasma-based diets supplemented with egg-yolk antibodies against enterotoxigenic *Escherichia coli*

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ABSTRACT

Two experiments involving 168 10-d old weaned pigs were conducted to compare growth-promoting properties of dietary spray-dried animal plasma (SDAP), spray-dried porcine plasma (SDPP) and chicken egg-yolk antibodies (EYA) from d 0 to 14 post-weaning. In Exp. 1, 96 pigs (3.2 ± 0.2 kg BW) were used to test the hypothesis that the superior performance of piglets fed SDPP-based diets was partly due to the presence of specific antibodies against enterotoxigenic *Escherichia coli* (ETEC) which can be replaced with EYA. Four experimental diets with six pens per treatment and four pigs per pen in a completely randomized design arranged in a 2 x 2 factorial containing SDPP-EYA (A1), SDPP+EYA (A2), autoclaved AuSDPP-EYA (A3) and AuSDPP+EYA (A4) were used. Autoclaving SDPP at 121°C for 15 minutes completely destroyed anti-K88/F18 antibodies. Feed intake and feed efficiency were similar ($P > 0.05$) among treatments averaging 177.5 g/d and 1.45 respectively. However, pigs fed AuSDPP-EYA diets had poorer ($P < 0.05$) ADG compared to those fed SDPP-EYA and SDPP+EYA. Adding chicken EYA improved ADG by 7-10%. Scours were 4 times higher ($P < 0.05$) for treatment AuSDPP-EYA compared to all other treatments. Plasma urea N (PUN) was higher ($P < 0.05$) in AuSDPP-EYA and AuSDPP+EYA fed pigs. Also twice the number of piglets fed AuSDPP-EYA appeared unhealthy compared to piglets on treatment AuSDPP+EYA. In Exp. 2, 72 10-d old weaned pigs (3.5 kg BW) were used to

compare the effect of EYA supplementation and oral challenge of ETEC strain F18 on performance and visceral organ weights. The experimental diets consisted of SDAP-EYA (B1), SDAP + EYA, (B2), SDPP-EYA (B3) and SDPP + EYA (B4). There were six pens per treatment and three pigs per pen. From d 0 to 7, compared to SDAP-EYA pigs fed SDAP+EYA, SDPP-EYA and SDPP+EYA grew faster and consumed more ($P < 0.05$) feed. PUN was higher ($P < 0.05$) in piglets fed SDAP-EYA diet before and after the oral challenge. FCE, organ weights, villi heights and crypt depths were similar ($P > 0.05$) among treatments. The results indicate that anti-ETEC antibody present in SDPP is one of the factors responsible for its superior nutritional value. Other factor(s) completely lost after autoclaving are involved. SDAP, SDPP and EYA have similar therapeutic and prophylactic value in post-weaned pigs. Pigs given SDPP or SDAP supplemented with EYA had the tendency to perform better than those on SDAP alone.

Key Words: Early-weaned pigs, Egg-yolk antibody, Porcine plasma, Animal plasma, *E. coli*

INTRODUCTION

In recent times, spray-dried porcine plasma (SDPP) and animal plasma protein (SDAP), by-products of blood from pork and cattle slaughter plants respectively, are routinely added to the diets of early-weaned pigs. Numerous studies have shown that these products consistently and dramatically improve

feed intake and weight gain (Coffey and Cromwell, 1995; De Rodas et al., 1995; Jiang et al., 2000), feed efficiency (Kats et al., 1994; Grinstead et al., 2000) and reduce incidence and severity of post-weaning diarrhea (Van der Peet-Schwering and Binnendijk, 1995), with its effect being evident during d 0 to 14 post-weaning. The biological aspects underlying the superior value of SDAP and SDPP are poorly understood. However, it has been suggested that nursery conditions, including presence of pathogens, play an important role in the magnitude of piglet response to dietary SDPP (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995). Immunoglobulins and complex protein fractions present in SDPP and SDAP are also thought to provide anti-microbial protection (Coffey and Cromwell, 1995; Godfredson-Kisc et al., 1997; Jiang et al., 2000) and might also influence intestinal immune status in the transition to weaning (Jiang et al., 2000). The latter might protect against the development of mucosal damage by enteric pathogens thereby restricting passage of inert large molecules through the intestinal wall (Van Dijk et al., 2001).

Adhesion of ETEC to the small intestine epithelium accounts for most gastrointestinal disorders in neonatal and weaning piglets (Yokoyama et al., 1992). Supplementing piglet diets with a source of antibodies against ETEC fimbrial antigens offer a potential prophylactic and therapeutic solution to scours in baby pigs (Yokoyama et al., 1992; Marquardt et al., 1997). For instance, Marquardt et al. (1999) reported that *E. coli* strain K88+ induced diarrhea in 3-d old piglets was cured 24 h after treating with chicken egg-yolk antibodies (EYA) while those treated with egg-yolk powder from conventional laying hens

continued to have severe diarrhea, leading to a 62.5% death. It is not clear whether plasma proteins contain specific antibodies against intestinal pathogens such as ETEC strains. We therefore postulated that plasma proteins may have a therapeutic property likely due to the presence of antibodies against intestinal pathogens such as ETEC strains K88 (F4), K99 (F5), 987P, F41 and F18 which can be replaced by EYA containing specific and of consistent anti-K88, -F18 and -F41 antibodies. Currently, no information, as far as the authors know, has been published on the anti-K88 (F4), K99 (F5), 987P (F6), F41 and F18 antibody titers in SDPP and SDAP.

The objectives of the current study were 1) to determine if SDPP and SDAP contained antibodies against the common ETEC pathogens, 2) to determine titers of each and the amount of titer required in chicken egg-yolk from hens immunized with fimbrial antigens to provide a similar concentration of antibodies, and 3) to determine if the growth stimulating effect of SDAP/SDPP in early-weaned pigs is due to the presence of specific disease preventing antibodies that could be replaced by less expensive and of more consistent composition EYA obtained from laying hens immunized with K88/F18 fimbrial antigen.

MATERIALS AND METHODS

Animal Care and Housing

The experimental protocol was approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of Canadian Council on Animal Care (CCAC, 1993). A total of 168 piglets weaned at 10 d of age (3.5 ± 0.3 kg initial BW) were used in two 14-d experiments. Piglets in each experiment were randomly allotted to each of 4 dietary treatments in a 2 x 2 factorial design. Each treatment was assigned to 6 replicate pens (1.2 x 1.5 m) each with 4 (Exp. 1) or 3 pigs (Exp. 2). Room temperature was maintained at 31 ± 1°C throughout the studies.

Experimental Design, Diets and Feeding

Two experiments were conducted. The objective of the first experiment was to determine if SDPP contained antibodies against the common ETEC pathogens, secondly to determine titers of each and amount of titer required in chicken egg-yolk from hens immunized with fimbrial antigens to provide a similar concentration of antibodies and finally to determine if there is a relationship between the performance of pigs and the corresponding anti-*E. coli* antibody titers. Antibodies in SDPP were to be inactivated hence the heat-treated SDPP. The objective of the second experiment was to determine if the growth-stimulating effect of SDAP/SDPP in early-weaned pigs is due to the presence of specific disease preventing antibodies that could be replaced by less expensive, and of more consistent composition EYA obtained from laying hen immunized with K88/F18 fimbrial antigen and also to compare anti-ETEC antibodies titers in SDAP and SDPP. The experimental diets were SDPP-EYA (A1), SDPP+EYA

(A2), autoclaved SDPP (AuSDPP)-EYA (A3) and AuSDPP+EYA (A4) for Exp. 1 and SDAP-EYA (B1), SDAP+EYA (B2), SDPP-EYA (B3), SDPP+EYA (B4) for Exp. 2. (Table 2). EYA contained 0.3 and 0.2 or 0.2 and 0.3% specific anti-K88 and F18 antibodies in Exp. 1 or 2, respectively (Table 2). Egg-yolk antibodies containing anti-K88 or anti-F18 antibodies were produced using the procedure outlined by Marquardt et al. (1999). SDPP was obtained from Farmland Protein Plant (Maple St., Maquoketa, Iowa, USA) and SDAP from F.N.P. Protein Inc., (Calgary, AB. Canada). The anti-*E. coli* antibody for the different products and diets are outlined in Table 3. Prior to commencement of Exp. 1 a portion of SDPP was autoclaved for 15 min at 121°C and 15-25 psi. These conditions were shown in a preliminary study to deactivate all anti-K88, F18 and F41 antibodies in SDPP. All experimental diets were formulated to exceed the NRC (1998) nutrient requirements for piglets weighing 3.0 to 6.0 kg BW and contained similar CP (26.0%), lysine (1.7%), methionine (0.5%), threonine (1.2%), tryptophan (0.3%) (Table 2). Pigs had unlimited access to feed and water at all times. ADG, ADFI and FCE (F/G) were determined weekly. On d 0, 7 and 14 blood samples (10 ml) were collected from all pigs via jugular vein puncture into vacutainer tubes (Becton Dickinson, Rutherford, NJ), and immediately centrifuged at 2000 x g for 10 min at 5°C to recover plasma. Plasma samples were immediately stored at – 20°C until required for plasma urea analysis.

Table 2. Composition of experimental diets

	Experiment 1[a]				Experiment 2[b]			
	A1	A2	A3	A4	B1	B2	B3	B4
Ingredients (%)								
Wheat	12.0	12.0	12.0	12.0	14.0	14.0	14.0	14.0
Oat groats	24.0	24.0	24.0	24.0	15.0	15.0	15.0	15.0
Whey	10.0	10.0	10.0	10.0	13.0	13.0	13.0	13.0
Corn	-	-	-	-	4.5	4.5	4.5	4.5
Soybean meal	13.0	13.0	13.0	13.0	14.0	14.0	14.0	14.0
Fish meal	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
SDPP[c]	10.0	10.0	-	-	-	-	10.0	10.0
AuSDPP[c]	-	-	10.0	10.0	-	-	-	-
SDAP	-	-	-	-	10.0	10.0	-	-
Vegetable Oil	7.0	7.0	7.0	7.0	6.0	6.0	6.0	6.0
Sucrose	10.5	10.5	10.5	10.5	10.0	10.0	10.0	10.0
Premix[d]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Lysine	0.2	0.2	0.4	0.4	0.2	0.2	0.2	0.2
Methionine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Threonine	-	-	0.1	0.1	0.1	0.1	0.1	0.1
Egg-yolk antibody	-	0.5	-	0.5	-	0.5	-	0.5
Egg-yolk powder	0.5	-	0.5	-	0.5	-	0.5	-
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Analyzed Composition (%)								
DE (kcal/kg)	3412.2	3412.2	3412.2	3412.2	3426.0	3426.0	3426.0	3426.0
Crude protein	26.0	26.0	26.0	26.0	26.0	26.0	26.0	26.0
Lysine	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Threonine	1.2	1.2	1.1	1.2	1.2	1.2	1.3	1.1
Methionine	0.4	0.5	0.5	0.5	0.7	0.6	0.6	0.6
Tryptophan	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3

[a]A1 = SDPP (spray-dried porcine plasma) – EYA (egg-yolk antibody); A2 = SDPP + EYA; A3 = AuSDPP (autoclaved SDPP) – EYA; A4 = AuSDPP + EYA.

[b]B1=SDAP (spray-dried animal plasma)-EYA; B2= SDAP + EYA; B3= SDPP-EYA; B4= SDPP+EYA.

[c]The analyzed means and composition for SDPP and AuSDPP respectively were (g/kg plasma) aspartic acid, 7.4 and 7.2; threonine, 5.0 and 4.6; serine, 4.8 and 4.6; glutamic acid 11.4 and 10.9; proline, 4.9 and 4.9; glycine, 2.8 and 2.7; alanine, 4.1 and 4.1; cysteine, 2.6 and 2.0; valine, 4.5 and 4.1; methionine, 0.9 and 0.9; isoleucine, 2.5 and 2.3; leucine, 7.8 and 7.4; tyrosine, 4.0 and 3.8; phenylalanine, 4.5 and 4.1; histidine, 1.6 and 1.6; lysine, 6.7 and 6.0; arginine, 4.0 and 4.3. The available lysine as determined by the method of Kakade and Liener (1969) was 6.3 and 5.7 mg/kg plasma respectively for SDPP and AuSDPP.

[d]Premix provided per kg of diet: 9 000 IU vitamin A, 1 500 IU vitamin D3, 18 mg vitamin E, 1.5 mg vitamin K, 250 mg choline, 30 mg niacin, 27.5 mg calcium pentothenate, 9.4 mg B2, 1 mg B6, 25 mcg B12, 50 mcg biotin, 0.5 mg folic acid, 5.75 g calcium, 2.6 g phosphate, 3.5 g sodium chloride, 27.5 mg manganese, 105 mg iron, 125 mg copper, 0.6 mg iodine.

Bacteria Culture, Oral Challenge and Health Status

In Exp. 2, all pigs were orally challenged with F18, a local strain of ETEC, obtained from the Animal Health Center, Veterinary Services Branch, Manitoba Department of Agriculture, Winnipeg, MB, Canada. Primary cultures of the ETEC strain were grown overnight in tryptic soya broth (TSB, CASO-Bouillon, Mikrobiologie Darmstadt, Germany) at 37°C using 1% inoculum from stocks stored at –20°C in 30% glycerol. The F18 *E. coli* strain was prepared as described by Yokoyama et al. (1992). Briefly, ETEC F18 was grown overnight in blood agar plate (Atlas Laboratories Co. Ltd. Winnipeg, MB. Canada) at 37°C using 1% inoculum from stock. Cells were washed twice with 2 mL sterilized saline solution (0.9%, pH 7.2), and then the suspension [10^{12} colony forming unit (CFU) mL^{-1}] was used for oral challenge. On d 7, each pig received 6 mL of bacterial suspension contained in a syringe attached to polyethylene tube held in the oral cavity. Severity of diarrhea was characterized using fecal consistency (FC) score described by Marquardt et al. (1999). FC scouring (0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea) and Visual Assessment Scoring System (VASS), (1, poor; 2, good and 3, better) performed independently by 4 trained individual with no prior knowledge of the treatments allocation were used to assess the health status of pigs.

Detection of Antibodies Titre

Enzyme-linked immunosorbent assay (ELISA) with purified fimbrial antigen was used to determine anti-K88, K99, 987P, F18 and F41 antibody titers

in SDAP SDPP, AuSDPP, EYA and all experimental diets using the procedure of Kim et al. (1999). Wells of Microtest III flexible assay plates (Falcon 3911, Immunol 4, Dynatec Laboratories, Chantilly, Va, USA) were coated with 100μg of the fimbrial antigen suspended in 20 mL phosphate buffer saline (PBS, pH 7.2) at 37°C for 2 h. The plates were washed 3 times with PBS and Tween 20 (0.5%) (PBS-T) and then blocked with 5% w/v skim milk in PBS at 37°C for 2 h, followed by washing with PBS-T as above. The plates were then inoculated with dilutions of samples (100mg/mL) and kept for 2 h at 37°C. After washing with PBS-T, the plates were incubated with 100 μL of alkaline phosphatase conjugated secondary antibody anti-chicken IgY, anti-calf IgG or anti-swine serum (Jackson ImmunoResearch Laboratory Inc., diluted 1:3000) depending on the source of antibody and incubated for 2 h at 37°C. The plates were washed 3 times with PBS-T and 100 μL of enzyme substrate (10% diethanolamine with 0.5mM $MgCl_2$, pH 9.8) were added to each well. The plate was incubated at room temperature for 20-30 min. The optical density of the wells was read at 405 nm with a micro-plate reader (Bio-Rad, Model 3550, Richmond, CA, 94804 USA). The titer was the dilution of antibody required to give one-half of the maximum absorbency reading. Assays at different times were corrected using standard samples containing known K88, K99, F18, 987P or F41 antibody titers.

Chemical Analyses

All analyses were done in duplicate. When necessary samples of SDAP, SDPP, AuSDPP and diets were ground through a 1-mm screen (Cyclotec 1093,

sample mill, tecator, Hoganas, Sweden), prior to analysis. Samples were dried in a convection oven at 105°C for 16 h for DM determination, while CP (N x 6.25) content was determined using Leco NS 2000 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI., USA). A 100-mg sample was prepared for acid hydrolysis according to AOAC (1984) and as modified by Mills et al. (1989) for AA analysis. The method involved digestion in 4 mL of 6 *N* HCl in vacuo for 24 h at 110°C followed by neutralization with 4 mL w/v NaOH and allowed to cool to room temperature. The mixture was then made to 50 mL volume with sodium citrate buffer (pH 2.2). Methionine and cysteine were analyzed as methionine sulfone and cysteic acid, respectively, after oxidation with performic acid. Amino acids were then analyzed using LK 4151 Alpha analyzer (LKB Biochrom, Cambridge, UK). Available lysine was determined using the method of Kakade and Liener (1969). Plasma samples were analyzed for urea nitrogen concentrations according to Crocker (1967), using a standard kit (Procedure No. 535, Sigma Diagnostic, St. Louis, MO., USA).

Histological Measurement

On d 14, of Exp. 2, 4 pigs (5.0 ± 0.5 kg BW) per treatment were held under general anesthesia and killed by an intra cardiac injection of sodium pentobarbital (50 mg/kg BW). Stomach, spleen, small intestine and liver were removed, flushed with ice-cold phenylmethyl sulfonyl fluoride (PMSF) saline (0.9% saline, pH, 7.4 2L + 2-mL of 100 mM PMSF) and the weights and the length of the small intestine were also determined. A 10-cm segment of the small

intestine, taken 100 cm from the gastric pylorus junction was removed and stored in 10% formalin to fix the villous and the crypt for subsequent histological measurement. Six cross-sections were obtained from each formalin-fixed sample and processed for histological examination using the standard Hematoxylin and Eosin (H&E) method. The measurement of villous height (VH), and crypt depth (CD) was made on 10 well-oriented villi per specimen using a Zeiss photomicroscope equipped with a Sony 3 chip CCD colour camera. The images were captured using Empix's Northern Eclipse Image Processing Software (Empix Imaging, Inc., Mississauga, ON., Canada). The height of the villous was measured from the tip to the crypt-villous junction and the depth of the crypt from the crypt-villous junction to the base.

Statistical Analysis

Villi height (VH) and crypt depth (CD) were determined by averaging the individual measurements in similarly treated pigs. Mean VH and CD were obtained by averaging 6 measurements from each of 4 pigs. All data (ADG, ADFI, FCE, VH and CD) were analyzed as a completely randomized design in a 2 x 2 factorial arrangement. For ADFI and FCE a pen was considered the experimental unit. Treatment means were compared using Fisher's protected least significant difference procedure (Cody and Smith, 1991). Statistical significant was accepted at $P < 0.05$. All statistical analyses were performed using the GLM procedure of SAS (1988).

RESULTS AND DISCUSSION

Nutrients Composition and Antibody Titres

Amino acid composition of SDPP and AuSDPP were similar and within the range of literature values (Van der Peet and Binnendijk, 1997; NRC. 1998). Thus indicating that heating did not greatly alter the composition of SDPP (Table 2, footnote). Because available lysine in SDPP was reduced from 6.3 to 5.7% following autoclaving, AuSDPP diets were fortified with synthetic lysine and threonine to account for any destruction of amino acids. Methionine was added to all diets since all diets were limiting in this amino acid.

The antibody titers were monitored in the different egg-yolk preparations, animal plasma products and diets (Table 3). Typical antibody titer curves for raw and autoclaved SDPP (Fig 3), SDPP and SDAP (Fig 4), for the diets in Exp. 1 (Fig 5 and 6) and Exp. 2 (Fig 7) indicate that there are considerable differences in antibody titers among the different preparations. In addition, the assays were specific for the source of antibody. That is, the assay for egg-yolk anti-K88 antibodies is specific for the egg-yolk antibodies that do not cross-react with the anti-K88 antibodies from SDAP or SDPP and vice versa. Also, there is no cross-reactivity among the different fimbrial antigens (i.e., K88, K99, F18, F41 and 987P).

Data in Table 3 demonstrate that the antibody for anti-K88 antibody in egg-yolk is much higher than that of SDPP (33 fold) and that of SDAP (1200 fold). A similar pattern is seen for anti-F18 antibodies. SDAP, however, has higher anti-K99, 987P and F41 antibody titers. No information as far as we know,

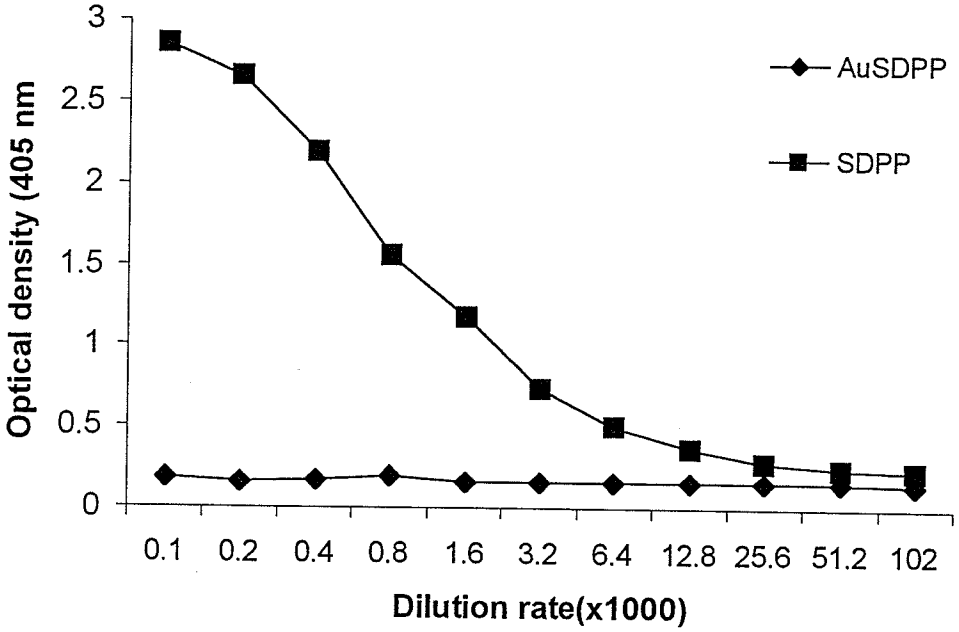

Figure 3. Typical anti-K88 antibody titer levels in spray-dried porcine plasma (SDPP) and autoclaved SDPP (AuSDPP), as determined by ELISA using alkaline phosphate-conjugated affinipure goat anti-swine IgG.

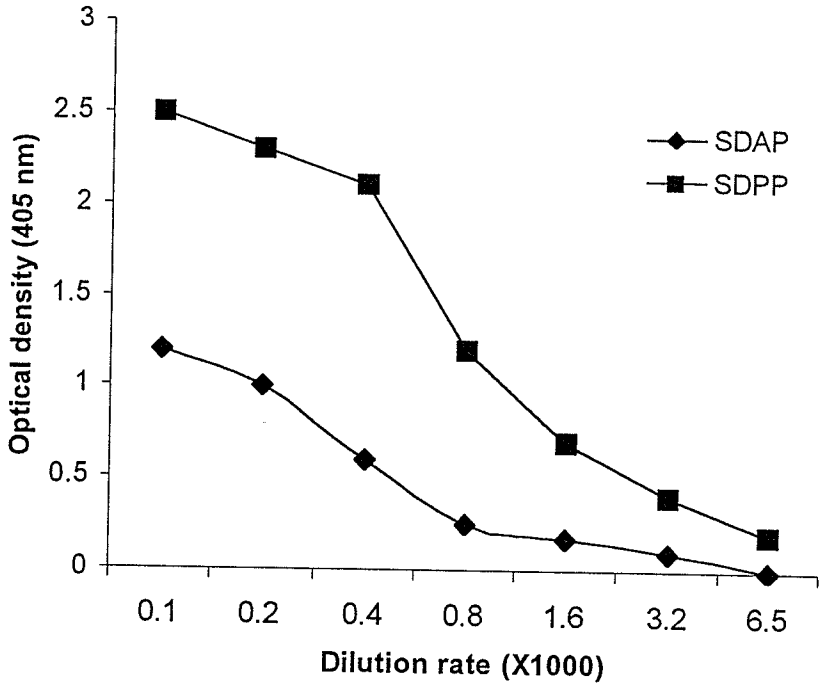

Figure 4. Typical anti-K88 antibody titer levels in spray-dried porcine plasma (SDPP) and spray-dried animal plasma (SDAP) as determined by ELISA using alkaline phosphate-conjugated affinipure goat anti-swine IgG.

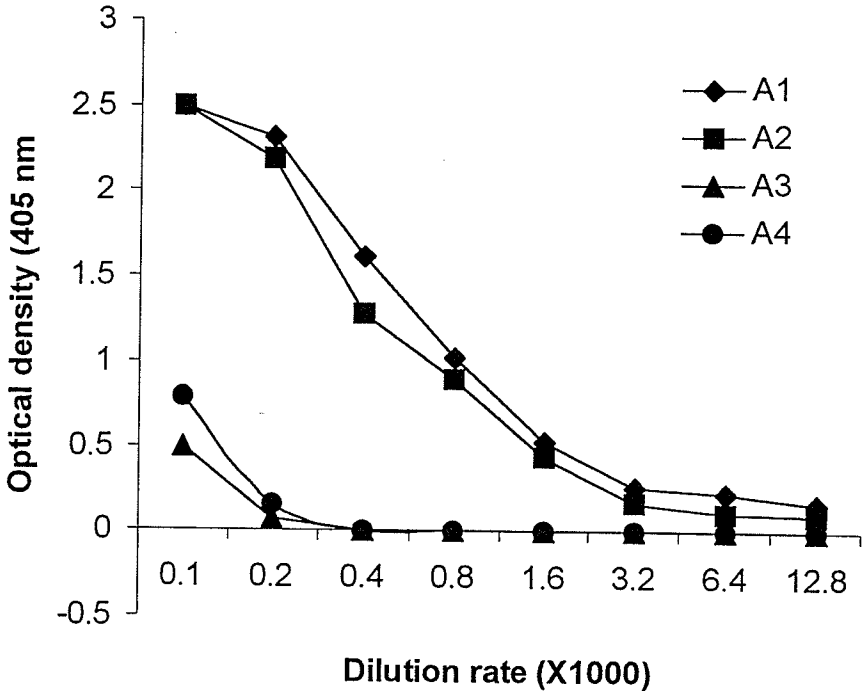

Figure 5. Anti-K88 antibody titer levels in Experiment 1 diets (A1, SDPP-EYA; A2, SDPP+EYA; A3, AuSDPP-EYA; A4, AuSDPP+EYA) as determined by ELISA using alkaline phosphate-conjugated affinipure goat anti-swine IgG.

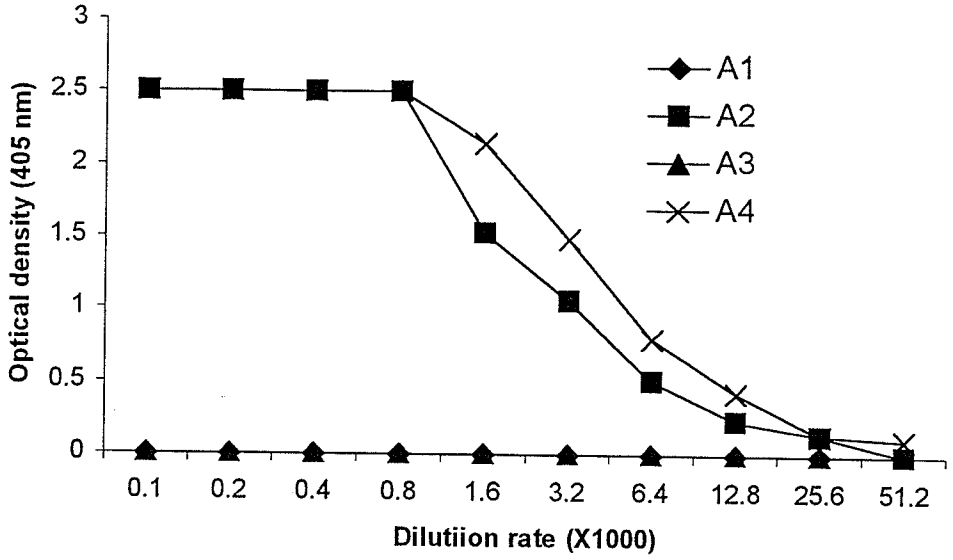

Figure 6. Typical anti-K88 antibody titer levels in Experiment 1 diets A1, SDPP-EYA; A2, SDPP+EYA; A3, AuSDPP-EYA; A4, AuSDPP+EYA) as determined by ELISA using alkaline phosphate-conjugated affinipure rabbit anti-chicken IgY.

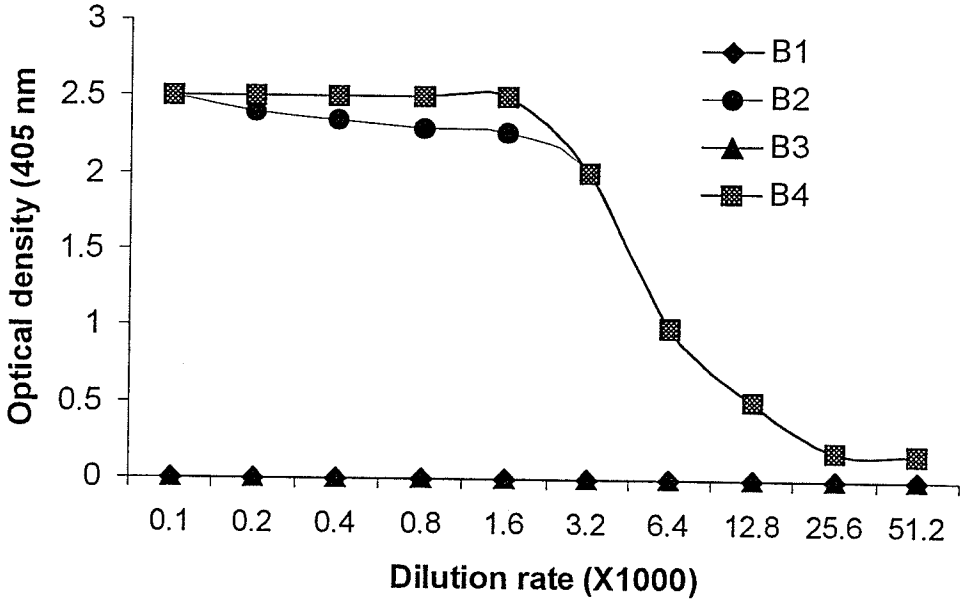

Figure 7. Typical anti-F18 antibody titer levels in Experiment 2 diets B1, SDAP-EYA; B2, SDAP+EYA; B3, SDPP-EYA; B4, SDPP+EYA) as determined by ELISA using alkaline phosphate-conjugated affinipure rabbit anti-chicken IgY.

Table 3. Comparative antibody titer of egg-yolk antibody (EYA), spray-dried animal plasma (SDAP), spray-dried porcine plasma (SDPP) and experimental diets

Parameters	Titer[a]				
	Anti-K88	Anti-F18	Anti-K99	Anti-987P	Anti-F41
Egg-yolk, non-immunized hens	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Egg-yolk, immunized with K88	6.0×10^5 (33)[b]	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Egg-yolk, immunized with F18	$< 10^2$	4.5×10^5 (30)[b]	$< 10^2$	$< 10^2$	$< 10^2$
Spray-dried porcine plasma	1.8×10^4 (1.0)	1.5×10^4 (1.0)	$< 10^2$	$< 10^2$	$< 10^2$
Autoclaved (Au) SDPP	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Spray-dried animal plasma	0.5×10^3 (0.06)[b]	$< 10^2$	4.0×10^4	2.5×10^3	2.0×10^3
Experimental Diet					
Experiment 1					
SDPP - EYA (A1)	1.8×10^3	1.5×10^3	$< 10^2$	$< 10^2$	$< 10^2$
SDPP + EYA (A2)[c,d]	3.7×10^3	3.1×10^3	$< 10^2$	$< 10^2$	$< 10^2$
AuSDPP-EYA (A3)	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
AuSDPP+ EYA (A4)[d]	1.7×10^3	1.35×10^3	$< 10^2$	$< 10^2$	$< 10^2$
Experiment 2					
SDAP-EYA (B1)	$< 10^2$	$< 10^2$	1.2×10^3	$< 10^2$	$< 10^2$
SDAP+EYA (B2)[d]	1.2×10^3	1.35×10^3	1.4×10^3	$< 10^2$	$< 10^2$
SDPP-EYA (B3)	1.8×10^3	1.5×10^3	$< 10^2$	$< 10^2$	$< 10^2$
SDPP+EYA (B4)[c,d]	3.0×10^3	2.9×10^3	$< 10^2$	$< 10^2$	$< 10^2$

[a]Titer, is defined as the dilution of antibody preparation that gives 50% of maximal absorption in the ELISA assay.
[b]Values in brackets represent the concentration of the specific antibody relative to that represent in SDPP
[c]Values are sum of antibody titers in both SDPP and EYA.
[d]The amount of anti-K88 and anti-F18 EYA incorporated in A2 were 0.3 and 0.2% of the diet. Corresponding values for B2 and B4 were 0.2 and 0.3%.

has been published, on the anti-K88, -K99, -F41 and -F18. Autoclaving SDPP at 121°C for 15 min eliminated the entire antibody titers specific to anti-K88 antibodies (Fig 3, Table 3). Previous studies have demonstrated that the *E. coli* expressing the K88 (F4) are mainly responsible for pre- and post-weaning diarrhea and F18, in post-weaning pig (Alexander, 1994). Diarrhea caused by the other strains of *E. coli* is rare. Therefore, antibody titer of SDPP and SDAP is a reflection of the corresponding exposure of pigs or other species such as ruminants to the different strains of *E. coli*. For example, SDPP had high anti-K88 and -F18 antibody titers and very low titers for the other antibodies (anti-K99, -987P and -F41). In contrast to SDPP, SDAP was considerably lower in anti-K88 but higher in anti-K99, -987P and –F41 antibody titers (Table 3). The reason for this is that *E. coli* K88 or F18 do not colonize ruminants, the source of SDAP, whereas *E. coli* K99 and the other 2 strains are able to colonize ruminants. As a result ruminants produce antibodies against the later organism but not the former two. The antibody titer is therefore a reflection of the source of the plasma protein. These observations and the knowledge that K88 and F18 ETEC are the two strains of *E. coli* that are the major cause of pathogenecity in pigs (Alexander, 1994), suggest that anti-K88 and –F18 antibodies are the antibodies that would be of major benefit in the nutrition and management of young pigs.

The anti-K88 and –F18 antibody titers in diets SDPP-EYA (A1), SDPP+EYA (A2) and AuSDPP+EYA (A4) were of a similar order of magnitude and much greater than that of diet A3, which contained AuSDPP. These comparisons should therefore, provide a basis for establishing the importance of

Table 4. Performance of 10-d old weaned pigs fed autoclaved (AuSDPP) and raw (SDPP)-spray-dried porcine plasma supplemented with egg-yolk antibody (EYA), Exp. 1

	Dietary Treatments [a,b]				
Parameter	A1	A2	A3	A4	SEM
Initial weight (kg)	3.2	3.2	3.2	3.2	0.60
Final weight (kg)	5.0	5.2	4.6	4.9	0.98
ADG (g/d)[c]					
Day 0-7	66.3	80.5	60.7	72.3	4.50
Day 7-14	188.2[a]	202.2[a]	128.7[c]	158.4[ab]	8.16
Day 0-14	127.4[a]	139.8[a]	102.7[b]	121.3[ab]	5.56
ADFI (g/d)					
Day 0-7	100.2	119.0	89.6	104.3	13.8
Day 7-14	280.9[a]	294.3[a]	186.6[b]	232.2[ab]	22.0
Day 0-14	185.2	197.5	152.0	177.5	13.5
FCE (F/G)					
Day 0 – 7	1.52	1.48	1.47	1.45	0.05
Day 7 – 14	1.48	1.48	1.50	1.45	0.05
Day 0 – 14	1.45	1.42	1.48	1.47	0.04
PUN ml/dL					
Day 0	6.5	5.9	6.0	6.6	1.13
Day 7	14.2	13.7	14.5	13.8	1.64
Day 14	19.1[b]	18.9[b]	24.5[a]	22.2[a]	0.91

For ADG, there were 6 pens/treatment each with 4 pigs/pen. ADFI and FCE were calculated using pen values.
[ab]Means in the same row lacking a common superscript letter differ ($P < 0.05$).
A1, SDPP – EYA; A2, SDPP + EYA; A3, AuSDPP – EYA; A4, AuSDPP + EYA.

anti-K88 and anti-F18 antibodies in the diet of young pigs. Likewise, in Exp. 2, diet B1 which contained SDAP had very low concentrations of anti-K88 and –F18 antibodies and higher amount of anti-K99 antibodies, whereas the other three diets (B2, B3 and B4) contained substantial concentrations of the anti-K88 and F18 antibodies.

Experiment 1

Body weight, ADG, ADFI and FCE are shown in Table 4. At the end of the 14-d experimental period, BW was similar among treatments, ranging from 4.6 to 5.2 kg. During d 0 to 7, diet influenced (P=0.014) ADG with piglets fed SDPP+EYA (A2) diet having the highest (80.5 g/d) ADG while those fed AuSDPP–EYA (A3) had the lowest (60.7 g/d). From d 7 to 14 as well as over the entire 14-d experimental period, there were differences (*P= 0.001*) in ADG among reatments. SDPP-EYA (A1) and SDPP+EYA (A2)-fed piglets grew faster than AuSDPP-EYA (A3) piglets (*P < 0.05*) but their FCE was similar to AuSDPP+EYA (A4) piglets (*P > 0.05*). The rate of growth observed in the current study are in close agreement with previous observations made in 14 – 21 d old weaned pigs fed diets containing 3 to 10% SDPP for 14 d (Kats et al., 1994; De Rodas et al., 1995; Angulo and Cubilo, 1998; Grinstead et al., 2000). These data suggest that autoclaving SDPP reduces pig performance. Supplementing SDPP or AuSDPP diets with EYA numerically improved ADG, with a greater benefit seen in AuSDPP-based diet than in SDPP diet (Table 4). This observation

supports previous observation suggesting that weaner diets with EYA improve growth performance (Marquardt et al., 1997; 1999).

The ADFI was not affected (*P=0.318*) by dietary treatments (Table 4) during day 0 to 7. However, ADFI was lower (*P=0.014*) for piglets fed AuSDPP-EYA (A3)-based diet compared to SDPP (A1 and A2) or EYA (A4) supplemented fed pigs on d 7 to 14. Also, on d 0 to 14, piglets fed AuSDPP-EYA (A3)-based diet tended ((*P=0.091*) to consume less feed than SDPP-EYA (A1), SDPP+EYA (A2) and AuSDPP+EYA (A4) fed pigs. Feeding EYA numerically (16.1%) increase ADFI in AuSDPP+EYA (A4) compared to piglets fed AuSDPP-EYA (A3) during wk 1. Compared to SDPP-EYA (A1) and SDPP+EYA (A2), pigs fed AuSDPP-EYA (A3) and AuSDPP+EYA (A4) diets had 21.7% lower (*P > 0.05*) ADFI during the entire experimental period. By adding EYA to AuSDPP-based diet numerically (16.8%) improved (*P > 0.05*) feed intake, but not to similar level as in SDPP (Table 4). Increased in feed consumption associated with feeding SDPP has been attributed to greater palatability (Van Dijk et al., 2001). The current data suggest that in addition to destroying antibodies in SDPP, heating may have also destroyed other factors that influence feed intake. This suggests that specific antibodies and other factors that are lost during heating may be involved in superior feeding value of SDPP. Slight reductions in ADFI with corresponding depression in growth rate for AuSDPP-EYA (A3) and AuSDPP+EYA (A4) compared to SDPP-EYA (A1) and SDPP+EYA (A2)-fed pigs suggest a reduction in palatability probably due to destruction of flavor compounds as a result of heating. Supplementing EYA has been shown to

Table 5. Performance of 10-d old weaned pigs when challenged with F18 ETEC and fed spray-dried animal (SDAP) or porcine (SDPP) plasma supplemented with egg-yolk antibody (EYA), Exp. 2.

	Dietary Treatments[a,b]					
Parameter	B1	B2	B3	B4	SEM	P-Level
Initial weight (kg)	3.5	3.4	3.4	3.5	0.60	0.912
Final weight (kg)	5.5	5.9	5.9	6.0	0.87	0.645
ADG (g/d)						
Day 0-7	74[b]	84[ab]	91[a]	97[a]	13.8	0.036
Day 7-14	226	260	261	264	32.8	0.145
Day 0-14	146	175	176	180	30.6	0.223
ADFI (g/d)						
Day 0-7	107	122	136	143	22.6	0.06
Day 7-14	303	340	349	355	68.8	0.510
Day 0-14	192	230	235	237	40.2	0.180
FCE (F/G)						
Day 0 – 7	1.5	1.4	1.5	1.5	0.15	0.955
Day 7 – 14	1.3	1.3	1.3	1.3	0.18	0.978
Day 0 – 14	1.3	1.3	1.3	1.3	0.14	0.978

For ADG, there were 6 pens/treatment each with 3 pigs/pen. ADFI and FCE were calculated using pen
[ab]Means in the same row lacking a common superscript letter differ ($P < 0.05$).
SEM, Standard error of the mean.
B1, SDAP – EYA; B2, SDAP + EYA; B3, SDPP – EYA; B4, SDPP + EYA.

Table 6. Plasma urea nitrogen (PUN), Villi height, crypt depth and organ weight of 10-d old weaned pigs fed spray-dried animal (SDAP) and porcine (SDPP) plasma supplemented with egg-yolk antibody (EYA)

	Dietary Treatments				
Parameter	B1	B2	B3	B4	SEM
PUN, mL/dL (n=18)					
Day 0	6.3	6.1	5.9	6.2	0.49
Day 7	15.8[ab]	13.1[b]	16.7[a]	15.0[ab]	1.52
Day 14	19.5	17.1	18.4	16.1	2.60
Organ weight, g/kg BW (n=4)					
Spleen	3.0	3.2	2.9	2.9	0.69
Liver	30.2	29.9	31.5	30.4	0.97
Small Intestine	33.2	31.8	29.9	32.0	0.87
Stomach	12.1	11.0	10.9	12.0	0.44
Small Intestine histology, μm (n=4)					
Villous height	661.3	689.8	692.1	613.2	36.4
Crypt depth	343.6	336.7	345.3	366.2	26.3

[ab]Means in the same row lacking a common superscript letter differ ($P < 0.05$).
SEM, Standard error of the mean.
B1, SDAP – EYA; B2, SDAP + EYA; B3, SDPP – EYA; B4, SDPP + EYA.

improve feed intake in immunologically challenged pigs (Marquardt et al., 1999). Piglets in the present study were not immunologically challenged and were kept in a disease-free environment, which may explain why EYA supplementation did not improve feed intake in AuSDPP-fed pigs. Antibiotics have been reported to have little or no effect on growth in animals kept under clean or germ-free conditions (Roura et al., 1992). Coffey and Cromwell (1995) on the other hand, stated that the feed intake response to SDPP is independent of inflammatory challenge and likely the concentrations of inflammatory cytokines.

Overall dietary treatments did not affect ($P > 0.05$) FCE during the entire experimental period (Table 4). PUN was similar ($P > 0.05$) among treatments on d 0 and 7 (Table 4). However, on d 14, PUN levels were higher ($P < 0.05$) in AuSDPP-EYA and AuSDPP+EYA fed pigs than in those fed SDPP and SDPP+EYA (Table 4). The higher PUN levels in AuSDPP-fed pigs indicate evidence of body protein catabolism for energy or glucose and inefficient utilization of dietary protein for body protein synthesis (Coma et al., 1995).

During the entire study period, incidences of severe scours were seen in 75% of AuSDPP-EYA compared to 30% of AuSDPP+EYA-fed piglets. In contrast, only 12.5% of SDPP-EYA and SDPP+EYA-fed piglets had mild scours. Adding EYA to AuSDPP was able to reduce the incidence and severity of scours. This also agrees with the observation that compared to placebo, feeding EYA led to a transient diarrhea and 100% survival rate (Marquardt et al., 1999). The current observation suggests that both SDPP and EYA are able to reduce enteric

distress often seen in piglets within 2 wk post-weaning. It further suggests that SDPP contain specific anti-ETEC antibodies that could be replaced by EYA. This also confirms the observation by Van der Peet-Schwering and Binnendijk (1995) that piglets given feeds with SDPP require less treatment against gastrointestinal disorders during the first 2 wk post-weaning.

Body conformation and visual assessment scoring system (VASS) at the end of the 14-d experimental period indicated that 92% of pigs fed AuSDPP-EYA compared to 45% in AuSDPP+EYA (data not shown), were classified as unhealthy and had a VASS score of (mean ± SD) 1.4 ± 0.2 and 2 ± 0.1, respectively. In contrast, all pigs fed SDPP-EYA and SDPP+EYA diets appeared healthy and had a VASS score of 2.6 ± 0.2 and 2.9 ± 0.2 respectively. Thus, SDPP contain specific anti-ETEC antibodies that are probably involved in the prevention of enteric distress resulting in slight improvement in feed consumption, daily weight gain and overall health status of early-weaned piglets. However, in the process of eliminating these antibodies, other compounds were destroyed, which may have led to reduction in feed intake, growth depression and increased muscle protein degradation as demonstrated by higher PUN levels.

Experiment 2

Initial and final BW of piglets in Exp. 2 were similar among treatments (Table 5). Piglets fed SDPP-based diets with or without EYA supplementation grew faster ($P < 0.05$) during week 1 than those fed SDAP-based diet ($P > 0.05$)

without EYA. Adding EYA to SDAP and SDPP-based diets numerically improved (*P > 0.05*) ADG by 13.5% and 6.6%, respectively. ADG from d 7 to 14 and 0 to 14 were not affected by dietary treatment (*P > 0.05*), although EYA addition improved ADG by 15% and 20%, respectively, during these periods (Table 5). Although growth rate of SDPP-fed piglets in the current study was numerically higher than that for SDAP-fed piglets, this observation is in agreement with that of Kats et al. (1994) who observed similar growth rate in piglets fed SDPP or spray-dried bovine plasma-containing diets

There were no dietary effects on ADFI during any of the periods but SDPP fed piglets tended (*P = 0.06*) during wk 1 to consume more feed than their SDAP-fed counterparts. In general, EYA supplementation improved ADFI by an average 15% in SDAP-based diets compared to SDPP-based diets (Table 5). ADFI for wk 2 (7 to 14) and overall (d 0 to 14) were not affected (*P > 0.05*) by dietary treatments (Table 5). ADFI was slightly lower (*P > 0.05*) during d 7 to 14 (period of ETEC challenged) for pigs receiving diet SDAP-EYA compared to SDAP+EYA, SDPP-EYA or SDPP+EYA. Adding EYA to SDAP numerically improved (*P > 0.05*) overall ADFI by 19.7%. The FCE was similar among dietary treatments (*P > 0.05*, Table 5). In this experiment, feed intake and growth rates were slightly lower (*P > 0.05*) for SDAP-EYA compared to all other groups for the period of oral challenged. In a recent study to evaluate the effect of whey protein product and SDAP or SBM and dried skim milk on growth performance, Grinstead et al. (2000) observed that adding SDAP significantly increased ADG and ADFI of piglets during d 0 to 7 post-weaning. These authors reported a 2.5%

improvement in ADG over the SBM-control diet, but no other treatment effect was observed during 0 to 14 d period. Thus, from the result of Grinstead et al. (2000) and the present results, it can be speculated that feeding SDPP and SDAP have a protective effect in early-weaned pigs. Klasing et al. (1987) indicated that immune challenge leads to decrease feed intake as well as partitioning nutrients away from growth. In the current study, feed intake, growth rate and feed utilization were similar for all treatment groups before and after ETEC challenge suggesting similar nutritional and therapeutic value of SDAP and SDPP. Dietary treatment and ETEC challenge did not influence ($P > 0.05$) PUN levels, visceral organ weights, villous height and crypt depth (Table 6).

The incidence of growth retardation and diarrhea during the first 5-10 d post-weaning is a major problem in the management of baby pigs. Colonization of the small intestine with enterotoxigenic strains of *E. coli* in this period results in a severe secretory diarrhea and besides mortality and the requirement for anti-microbial medication, the associated growth checks can result in overall increase in time taken to reach market weight. No mortality was recorded in this study. DeGregorio and Barr (1989) found reduced mortality for pigs consuming milk replacers containing bovine immunoglobulins. From d 0 to 7 none of the piglets showed any signs of scours. However 100, 33, 33 and 33% of pigs fed SDAP-EYA and SDAP+EYA, SDPP-EYA and SDPP+EYA, respectively had transient diarrhea 4-6 h after oral challenge with ETEC (F18) that lasted about 6 h.

The second experiment was to compare piglets fed 2 different sources of spray-dried plasma, SDPP (from pigs) and SDAP from other animals, mainly

cattle followed by oral challenge with ETEC strain F18. In Exp. 1, we observed that in the process of eliminating anti-ETEC antibodies in SDPP, other factors were destroyed resulting in depressed performance. The second study was therefore geared towards using SDAP with relatively less specific anti-ETEC (K88/F18) antibodies compared to SDPP (Table 3). SDAP has essentially only one anti-*E. coli* antibody (anti-K99) which is not an important intestinal pathogen in pigs (Marquardt et al., 1999). The oral ETEC challenge provided direct evidence of the protective ability against *E. coli*-induced diarrhea by SDPP, SDAP and EYA. This is because in Exp. 1, lack of immunological challenge was attributed to similarities in performance of SDPP, AuSDPP and EYA supplemented diets. *E. coli* diarrhea or scours or post-weaning diarrhea is commonly used indicators of intestinal disorder of post weaned pigs. In a recent study, Marquardt et al. (1999) observed that *E. coli*-induced diarrhea in 3-d old piglets was cured within 24 h after treating with EYA while those treated with egg-yolk powder with no antibodies continued to have diarrhea resulting in 62.5% death. The transient diarrhea and the subsequent cure observed in the current study for all groups of pigs may be the positive effect of specific anti-F18 and K88 antibodies present in SDAP, SDPP and EYA. Specific antibodies raised against ETEC fimbrial antigens and administered orally to piglets offer a potential prophylactic and therapeutic means for controlling enteric disease young piglets (Yokoyama et al., 1992; Marquardt et al., 1997). These antibodies might have prevented colonization of the small intestine of the pigs by ETEC adhering to the epithelium that accounts for most of the gastrointestinal disorders in post-

weaning piglets (Marquardt et al., 1997; 1999). In addition to specific antibodies and immunoglobulins (Coffey and Cromwell, 1995), insulin-like growth factors (IGF-I) (De Rodas et al., 1995) and glycoproteins (Sanchez et al., 1993) have been suggested to be responsible for the superior value of spray-dried plasma proteins. Immunoglobulins are important components of both SDPP and SDAP and are thought to contribute to the overall immunocompetence of newborn piglets by binding bacteria thus preventing secretion of enterotoxins (Coffey and Cromwell, 1995). This may explain the transient and mild scours observed in all treatment groups in the current study. By preventing bacterial damage of the intestinal gut wall, blood plasma immunoglobulins help maintained optimal intestinal function and gastrointestinal growth which, in turn, benefits piglets health and performance (De Rodas et al., 1995; Gomez, et al., 1998). According to Sanchez et al. (1993) and Nollet et al. (1999), the benefits of blood plasma may also be attributed to the presence of oligosaccharide chains of glycoproteins in plasma which also provide binding sites for the fimbrial adhesins of *E. coli.* Bound bacteria are easily flushed down by the constant cleansing action of the secretion in concert with peristalsis, thus maintaining a low intestinal bacterial load. The presence of glycoproteins, immunoglobulins and IGF-I in blood plasma could therefore explain the current observations.

The results of these studies demonstrate that SDPP and SDAP have very different concentrations of different anti-*E. coli* antibodies. Also, specific antiK88 and F18 antibody titers of SDPP and SDAP are considerably lower than those obtained from eggs of hens immunized against specific fimbrial antigens (K88

and F18). Nearly all antibody activity in SDPP was lost after heating. This was corrected for by the addition of specific anti-K88 and -F18 antibodies, which also enhanced performance of pigs. These data further suggest that the type and amount of antibody present in animal plasma products (SDPP vs. SDAP) may affect performance and that the benefit of these antibodies can probably be met with adding other sources, such as those from egg-yolks.

Implication

The results of the present study demonstrate that spray-dried plasma proteins and egg-yolk antibody have important therapeutic and prophylactic value in baby pig diet by way of controlling and preventing enterotoxigenic *E. coli* infection. In addition, supplementing EYA to diets for early-weaned pigs have the potential to improve weight gain, feed intake and the efficiency of dietary protein and amino acids utilization for body protein deposition.

CHAPTER FOUR

MANUSCRIPT TWO

Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg-yolk antibody

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ABSTRACT

In practical swine production, enterotoxigenic *E. coli* (ETEC) infection and resulting scours is a major problem for young pigs especially where purified plant proteins are fed. The effect of supplementing a pea protein isolate (PPI)-based diet with egg-yolk antibodies (EYA) obtained from laying hens immunized against ETEC K88 antigen on piglet performance, incidence of scours and gut histology was studied in a 14-d experiment. Ninety-six 10-d old weaned pigs were assigned to 5 dietary treatments in a completely randomized design to give 6 replicate pens per treatment with 3 pigs per pen except for PPI-EYA diet which had 4 pigs per pen. The treatments were PPI-EYA (D1), PPI + EYA (D2), SDPP-EYA (D3), SDPP + EYA (D4) and PPI + SDPP (D5). All diets were formulated to similar nutrient levels and provided *ad libitum*. Blood from all pigs was taken on d 0, 7 and 14 for determining plasma urea N (PUN). On d 7, pigs were orally challenged with $6x10^{12}$ CFU mL^{-1} of ETEC K88. Piglets were weighed on d 7 and 14. On d 7, 8 and 14, 4 pigs per treatment were sacrificed to study the histology of the small intestine. Weekly feed intake, BW changes, and feed conversion efficiency were determined. The number of piglets with scours and scour scores were also recorded. Fecal swabs from 10 pigs per treatment were taken for polymerase chain reaction (PCR) test to detect K88 *E. coli*. Feed efficiency over the 14 d period ranged from 1.3 to 1.4 and was not affected ($P > 0.05$) by dietary treatment. Mean ADFI was lower ($P < 0.05$) in piglets fed PPI-EYA (64.3 g/d) compared to PPI + EYA (94.8 g/d) or SDPP (102 g/d) during wk 1. Piglets fed PPI-EYA tended to have a lower ($P = 0.06$) overall ADG (84 g/d) compared to

those fed PPI + EYA (123 g/d) or SDPP (127 g/d)-based diets. Scours appeared in all groups of pigs 6h after ETEC K88 oral challenge. However, piglets fed EYA or SDPP-containing diets recovered after 10 to 72 h post-challenge, whereas those fed PPI continued to have severe diarrhea resulting in 33% mortality. PCR results showed that a greater percentage of piglets fed PPI compared to those fed EYA-containing diets continued to shed ETEC K88 at the end of the 14-d experimental period. Piglets fed PPI-EYA in general had shorter villi ($P < 0.05$), higher intestinal pH ($P < 0.05$), and higher PUN ($P < 0.05$) than those fed the EYA-containing diets. It is concluded that specific EYA and SDPP can provide passive control of ETEC (K88) infection and potentially improve feed intake and weight gain in early-weaned pigs fed PPI.

Key words: Pea protein isolates, Porcine plasma, Early-weaned pig, Egg-yolk antibodies, *E. coli,* Scours

INTRODUCTION

Increasing emphasis has been placed on early weaning of piglets in recent years. However, early-weaned pigs (EWP) are unable to effectively utilize dry complex carbohydrate and plant protein-based diets because of their relatively less developed gastrointestinal tracts (Cranwell, 1995). Factors responsible for poor growth in EWP may be a transient hypersensitive response to plant-based proteins (Friesen et al., 1993) and possibly, their weak resistance to enteric diseases (Cranwell, 1995). The addition of spray-dried porcine plasma

(SDPP) to the diets of EWP has been shown to consistently improve feed intake and weight gain (Hansen et al., 1993; Sohn et al., 1991 and Coffey and Cromwell, 1995), and to reduce incidences of scours (Van der Peet-Schwering and Binnendijk, 1995) with most of the effect being evident during d 0 to 14 post-weaning. However SDPP is expensive and the use of animal plasma has been banned in Europe and may be extended to North America because of the scare from bovine spongiform encephalopathy (BSE).

A current interest in swine nutrition is to identify inexpensive protein sources that could be used in place of SDPP in diets for EWP. Processed plant protein sources such as pea protein isolate (PPI), could potentially be used as an alternative to SDPP. Unfortunately, PPI does not provide any specific anti-enterotoxigenic *Escherichia coli* (ETEC) antibodies, which are thought to be largely responsible for the superior feeding value of SDPP in piglet diets (Godfredson-Kisc and Johnson, 1997; Owusu-Asiedu et al., 2000). Chicken egg-yolk antibody (EYA) from hyper-immunized laying hens containing specific anti-ETEC antibodies has been shown to reduce incidence of diarrhea, increase feed intake, weight gains and reduce mortality when fed to EWP (Yokoyama et al 1992; Kim et al., 1999; Marquardt et al., 1999). Furthermore, we have shown that the feeding value of SDPP in diets for EWP was partly due to its specific anti-ETEC antibodies (Owusu-Asiedu et al., 2000). We, therefore, postulate that weight loss and reduced feed intake associated with the feeding of plant based protein (PPI) to EWP can be prevented by addition of EYA to the diet and that this diet will support similar performance of piglets as achieved with SDPP. The

objective of the current research was to evaluate the use of PPI supplemented with EYA as an alternative to SDPP in diets for EWP.

MATERIALS AND METHODS

Animal Care, Housing and Experimental Design

The experimental protocol was approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of Canadian Council on Animal Care (CCAC, 1993). A total of 96 piglets weaned at 10 d of age (3.5 ± 0.3 kg initial BW) were used in a 14-d trial. Piglets were randomly allotted to each of 5 dietary treatments in a completely randomized design. Each treatment was assigned to 6 replicates pens (1.2 x 1.5 m) each with 3 pigs, except PPI-EYA diet that had 4 pigs per pen. Room temperature was maintained at 31 ± 1°C throughout the study.

Feed, Feeding and Experimental Procedure

PPI and SDPP were obtained from Parrheim Foods (Portage La Prairie, MB.) and Farmlands Proteins Plant (Maquoketa, Iowa MO.), respectively. EYA was produced in our laboratory as described previously (Marquardt et al., 1999). The experimental diets were PPI – EYA (D1), PPI + EYA (D2), SDPP – EYA (D3), SDPP + EYA (D4) and PPI + SDPP (D5, a 1:1 mixture of diets 1 and 3). The EYA contained 0.3 and 0.2% egg-yolk powder each containing specific anti- K88

Table 7. Composition of experimental diets

	Dietary treatments[a]				
Ingredients (%)	D1	D2	D3	D4	D5
Wheat	14.0	14.0	14.0	14.0	14.0
Oat groats	14.0	14.0	14.0	14.0	14.0
Whey	13.0	13.0	13.0	13.0	13.0
Corn	5.0	5.0	5.0	5.0	5.0
SBM	16.0	16.0	16.0	16.0	16.0
Fish meal	8.0	8.0	8.0	8.0	8.0
SDPP[b]	-	-	10.0	10.0	5.0
PPI[b]	10.0	10.0	-	-	5.0
Vegetable Oil	4.6	4.6	4.7	4.7	4.6
Sucrose	9.0	9.0	9.0	9.0	9.0
Premix[c]	5.0	5.0	5.0	5.0	5.0
Lysine	0.3	0.3	0.3	0.3	0.3
Methionine	0.3	0.3	0.2	0.2	0.3
Threonine	0.3	0.3	0.3	0.3	0.3
Egg-Yolk Antibody	-	0.5	-	0.5	-
Egg-yolk powder	0.5	-	0.5	-	0.5
Total	100.0	100.0	100.0	100.0	100.0
Nutrient Content (%)					
Digestible energy (kcal/kg)[d]	3490	3490	3491	3491	3493
CP	26.4	26.5	26.2	26.4	26.5
Lysine	1.6	1.6	1.6	1.6	1.6
Threonine	1.2	1.2	1.3	1.3	1.3
Methionine	0.7	0.7	0.6	0.6	0.7
Tryptophan[d]	0.4	0.4	0.4	0.4	0.4

[a]D1, PPI – EYA; D2, PPI + EYA; D3, SDPP – EYA; D4, SDPP + EYA; D5, SDPP + PPI

[b]The analyzed composition of SDPP and PPI respectively were (g/kg) CP, 79.0 and 78.4; aspartic acid, 9.1 and 9.2; threonine, 4.4 and 3.0; serine, 4.2 and 4.5; glutamic acid 10.9 and 13.2; proline, 3.5 and 3.4; glycine, 2.8 and 3.1; alanine, 4.2 and 3.4; cysteine, 2.3 and 0.6; valine, 3.6 and 3.1; methionine, 0.9 and 0.9; isoleucine, 2.8 and 1.9; leucine, 6.8 and 6.5; tyrosine, 3.8 and 2.9; phenylalanine, 4.0 and 3.9; histidine, 2.6 and 2.0; lysine, 6.1 and 5.8; arginine, 4.2 and 6.3.

[c]Premix provided per kg of diet: 9 000 IU vitamin A, 1 500 IU vitamin D3, 18 mg vitamin E, 1.5 mg vitamin K, 250 mg choline, 30 mg niacin, 27.5 mg calcium pentothenate, 9.4 mg B2, 1 mg B6, 25 mcg B12, 50 mcg biotin, 0.5 mg folic acid, 5.75 g calcium, 2.6 g phosphate, 3.5 g sodium chloride, 27.5 mg manganese, 105 mg iron, 125 mg copper, 0.6 mg iodine.

[d]Calculated composition.

and -F18 antibodies, respectively. All experimental diets were formulated to exceed NRC (1998) nutrient requirements for piglets weighing 3.0 to 6.0 kg BW and contained similar CP (26.5%), lysine (1.6%), methionine (0.7%), and threonine (1.2), (Table 7). Pigs had unlimited access to feed and water at all times. ADG, ADFI and FCE (F/G) were determined. On d 7, 8 and 14 blood samples (10 mL) were collected from all pigs via jugular vein puncture into vacutainer tubes (Becton Dickinson, Rutherford, NJ), and immediately centrifuged at 2000 x *g* for 10 min at 5°C to recover plasma, which were immediately stored at –20 °C until analyzed for PUN.

Bacteria Culture, Oral Challenge and Health Status

All pigs were orally challenged with a local strain of ETEC expressing the K88 (F4) fimbriae, obtained from the Animal Health Center, Veterinary Services Branch, Manitoba Department of Agriculture, Winnipeg, MB, Canada. The K88 strain of *E. coli* used, is one of the most common causes of diarrheal disease in EWP (Nagy and Fekete, 1999). Primary cultures of the ETEC strain were grown overnight in tryptic soya broth (TSB, CASO-Bouillon, Mikrobiologie Darmstadt, Germany) at 37°C using 1% inoculum from stocks stored at –20°C in 30% glycerol. The K88 *E. coli* strain was prepared as described by Marquardt et al. (1999). Briefly, ETEC K88 was grown overnight in blood agar plate (Atlas Laboratories Co. Ltd. Winnipeg, MB. Canada) at 37°C using 1% inoculum from stock. Cells were washed twice with 2-mL sterilized saline solution (0.9%, pH

7.2), and then the suspension (10^{12} colony forming unit [CFU] mL^{-1}) used for oral challenge. On d 7, each pig received 6 mL of bacterial suspension contained in a syringe attached to polyethylene tube held in the oral cavity. Severity of diarrhea was characterized using fecal consistency (FC) score described by Marquardt et al. (1999). FC scoring (0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea), performed by 2 individually trained personnel with no prior knowledge of dietary treatment allocation, were used to ascertain the health status of pigs.

Detection of Antibodies Titer

Enzyme-linked immunosorbent assay (ELISA) with purified fimbrial antigen was used to determine anti-k88, -K99, -987P, -F41 and -F18 antibody titers in SDPP, PPI, EYA and all experimental diets using the procedure of Kim et al. (1999). Wells of Microtest III flexible assay plates (Falcon 3911, Immunol 4, Dynatec Laboratories, Chantilly, Va, USA) were coated with 100μg of the fimbrial antigen suspended in 20 mL phosphate buffer saline (PBS, pH 7.2) at 37°C for 2 h. The plates were washed 3 times with PBS and Tween 20 (0.5%) (PBS-T) and then blocked with 5% w/v skim milk in PBS at 37°C for 2 h, followed by washing with PBS-T as above. The plates were then inoculated with dilutions of samples (100 mg/mL) and incubated for 2 h at 37°C. After washing with PBS-T, the plates were incubated with 100 μL of alkaline phosphatase conjugated secondary antibody anti-chicken IgY, or anti-swine serum (Jackson ImmunoResearch Laboratory Inc., diluted 1:3000) depending on the sample and incubated for 2 h at 37°C. The plates were washed 3 times with PBS-T and 100 μL of enzyme

substrate (10% diethanolamine with 0.5mM $MgCl_2$, pH 9.8) were added to each well, and, Incubated at room temperature for 20-30 min. The optical density of the wells was read at 405 nm with a micro-plate reader (Bio-Rad, Model 3550, Richmond, CA, USA, 94804). The titer was the dilution of antibody required to give one-half of the maximum absorbency reading. Assays at different times were corrected using standard samples containing known K88, K99, F18, 987P or F41 antibody titers.

Chemical Analyses

All analyses were done in duplicate. When necessary samples of SDPP, PPI and experimental diets were ground through a 1-mm screen (Cyclotec 1093, sample mill, Tecator, Hoganas, Sweden), prior to analysis. Samples were dried in a convection oven at 105°C for 16 h for DM determination, while CP (N x 6.25) content was determined using Leco NS 2000 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI., USA). A 100-mg sample was prepared for acid hydrolysis according to AOAC (1984) and analysed for AA as modified by Mills et al. (1989). The method involved digestion in 4 mL of 6*N* HCl in vacuo for 24 h at 110°C followed by neutralization with 4 mL w/v NaOH and cooling to room temperature. The mixture was then made to 50 mL volume with sodium citrate buffer (pH 2.2). Methionine and cysteine were analyzed as methionine sulfone and cysteic acid, respectively, after oxidation with performic acid. AA were then analyzed using a LK 4151 Alpha analyzer (LKB Biochrom, Cambridge, UK).

Plasma samples were analyzed for urea nitrogen concentrations according to Crocker (1967), using a standard kit (Procedure No. 535, Sigma Diagnostic, St. Louis, MO., USA).

Polymerase chain reaction (PCR)

Fecal swab samples for microbial analysis were collected in duplicate from 10 pigs randomly selected per treatment using Culture Swab Transport System (Difco) on 6, 24 and 48 h, as well as 7 d post-ETEC challenge. Samples were plated onto TSB and the individual colonies used for the PCR-based method for detection and differentiation of K88 adhesive *E. coli*. The PCR-technique was based on the procedure described by Sambrook et al. (1989). The sense and anti-sense primers that encoded the specific K88 fimbrial gene were used. PCR was performed following a standard procedure in a thermocycler with the following program; 30 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, and an extension step at 72°C for 5 min at the end of the cycle. The product of the PCR reaction was then electrophoresed on a 0.8% agarose gel and recovered with glass beads (Bio 101. Inc, Canada). The resultant PCR product corresponded in size (2.6 kDa) to structural subunit of the K88 operon that was selected. A product was deemed positive when it produced a distinctive band consistent with its expected migration on the agarose gel as determined by comparison with the DNA fragment standards.

Histological and Other Measurements

On day 7, 8 and 14, 4 pigs per treatment were sacrificed to determine the effect of dietary treatments and oral ETEC challenge on weights and morphology of the gastrointestinal tract. Pigs were held under general anaesthesia and killed by an intra-cardiac injection of sodium pentobarbital (50 mg/kg BW). Stomach, spleen, small intestine and liver were removed from the animals and 20 mL of digesta each from the stomach and the small intestine was obtained for pH measurement. The organs or sections were flushed with ice-cold phenylmethyl sulfonyl fluoride (PMSF) saline (2 L of 0.9% saline, pH, 7.4 + 2-mL of 100 mM PMSF). The pH of the digesta was determined by inserting a combination electrode directly into aqueous suspension. The weights and length (small intestine) of these organs were determined. Segments (10 cm) of 3 sections of the small intestine were removed 20 and 150 cm from the pylorus junction, and 40 cm from the ileo-caecal junction to represent the duodenal, jejunal and ileal regions. The sections were stored in 10% formalin to fix the villous and the crypt for subsequent histological measurement. Six cross-sections were obtained from each formalin-fixed sample and processed for histological examination using the standard Hematoxylin and Eosin (H&E) method. The measurement of villous height (VH), and crypt depth (CD) was made on 10 well-oriented villi per specimen using a Zeiss photomicroscope equipped with a Sony 3 chip CCD colour camera. The images were captured using Empix's Northern Eclipse Image Processing Software (Empix Imaging, Inc., Mississauga, ON., Canada). The

height of the villous was measured from the tip to the crypt-villous junction and the depth of the crypt from the crypt-villous junction to the base.

Statistical analysis

VH and CD were determined by averaging the individual measurements in similarly treated pigs. Mean VH and CD were obtained by averaging the measurements from 4 pigs and 6 replicates per pig. All data (ADG, ADFI, FCE, VH and CD) were analyzed as a complete randomized design. For ADFI and FCE the pen was considered the experimental unit. Treatment means were compared using Duncans Multiple range tests (Cody and Smith, 1991). Statistical significant was accepted at $P < 0.05$. All statistical analyses were performed using the GLM procedures of SAS (1988).

RESULTS AND DISCUSSION

Crude protein and AA compositions were similar in PPI and SDPP (Table 7). Also, analysed and calculated nutrient composition indicated that all of the experimental diets had similar CP, AA and digestible gross energy levels (Table 7). An analysis of EYA, SDPP, PPI and experimental diets demonstrated that all preparations contained their own unique combination of specific anti-ETEC (anti-K88 and –F18) antibodies and in very different amounts (Table 8). SDPP contained good titers of anti-K88 (18×10^3) and anti-F18 (15×10^3). The presence of antibodies in SDPP have been attributed to exposure of pigs to

Table 8. Comparative antibody titer of egg-yolk antibody (EYA), spray-dried porcine plasma (SDPP) and experimental diets.

Parameters	Titer[a]				
	Anti-K88	Anti-F18	Anti-K99	Anti-987P	Anti-F41
Egg-yolk, non-immunized hens	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Egg-yolk, immunized with K88	6.0×10^5	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Egg-yolk, immunized with F18	$< 10^2$	4.5×10^5	$< 10^2$	$< 10^2$	$< 10^2$
Spray-dried porcine plasma	1.8×10^4	1.5×10^4	$< 10^2$	$< 10^2$	$< 10^2$
Pea protein isolate	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Experimental diets[b]					
PPI - EYA (D1)	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
PPI + EYA (D2)	1.8×10^3	0.9×10^3	$< 10^2$	$< 10^2$	$< 10^2$
SDPP-EYA (D3)	1.8×10^3	1.5×10^3	$< 10^2$	$< 10^2$	$< 10^2$
SDPP + EYA (D4)[c]	2.7×10^3	2.4×10^3	$<10^2$	$< 10^2$	$< 10^2$
SDPP + PPI (D5)	0.9×10^3	0.75×10^3	$< 10^2$	$< 10^2$	$< 10^2$

[a]Titer, is defined as the dilution of antibody preparation that gives 50% of maximal absorption in the ELISA assay
[b]Each diet contained 10% PPI or 10% SDPP except for D5 which contained 5% PPI plus 5% SDPP. All diets contained egg-yolk powder (0.5%) some without antibody (-EYA) and some with antibody (+ EYA)
[c]Values are mean of antibody titers in both SDPP and EYA.

ETEC that express the K88 and F18 fimbrial antigens which are also the prime cause of diarrheal disease (Alexander, 1994). In contrast to SDPP, EYA from hens immunized with either the K88 or F18 fimbrial antigens had much higher titers of the corresponding specific anti-K88 and –F18 antibodies with the values being 6.0 X 10^5 and 4.5 X 10^5, respectively (Table 8). The amount of EYA required to protect pigs against ETEC-K88 or ETEC-F18 should therefore be 38 or 30-fold less than that for SDPP. As a result, the amount of egg-yolk that would have to be added to the diet to provide the same anti-K88 antibody titers as present in a diet containing 10% SDPP would be only 0.3%. The other component used to replace SDPP in the diet was PPI, which was prepared by wet milling of peas. PPI contained a high concentration of protein with the balance of essential AA being similar to that of SDPP (Table 7). PPI however, was devoid of both anti-K88 and –F18 antibodies (Table 8). Therefore, the addition of EYA or SDPP to PPI diets resulted in addition of specific anti-K88 and anti-F18 ETEC antibodies (immunoglobulins).

Performance data for the pre-challenge (d 0 to 7), post-challenge (7 to 14) and the entire experimental period are shown in Table 9. Initial and final BW was similar among treatments, ranging from 3.7 to 3.8 and 5.1 to 5.4 kg, respectively. During the pre-challenge period (wk 1), ADFI and ADG were lower ($P < 0.05$) for PPI-EYA (D1)-fed piglets compared with all treatment groups (D2 to D5). Adding EYA or SDPP to PPI or feeding SDPP alone improved ADG ($P < 0.05$) during this phase of growth (Table 9). The ADFI and ADG for the post-challenge period (wk 2) were similar ($P > 0.05$) for all treatment groups. However, the piglets fed

Table 9. Performance of 10-d old weaned piglet fed spray-dried porcine plasma (SDPP) or pea protein isolate (PPI) supplemented with egg-yolk antibody (EYA)

Parameter	Dietary Treatment[a]					SEM
	D1	D2	D3	D4	D5	
Initial Weight (kg)	3.8	3.7	3.7	3.7	3.7	0.36
Final Weight (kg)	5.1	5.4	5.5	5.5	5.6	0.42
Feed Intake (g/d)						
Wk 1	64.3^{y}	94.8^{x}	102.0^{x}	103.3^{x}	109.1^{x}	6.75
Wk 2	158.7	210.1	223.2	220.4	235.3	27.61
Overall	113.7^{y}	156.6^{xy}	166.5^{x}	173.6^{x}	172.7^{x}	14.11
Weight gain (g/d)						
wk1	46.1^{y}	72.3^{x}	77.9^{x}	78.4^{x}	83.0^{x}	7.45
wk2	121.8	168.5	169.8	174.1	177.9	30.92
Overall	84.5	123.4	127.4	129.9	132.3	16.71
Feed conversion efficiency (Feed/gain)						
wk 1	1.42	1.30	1.32	1.32	1.32	0.29
wk 2	1.32	1.32	1.30	1.28	1.32	0.074
Overall	1.36	1.30	1.31	1.34	1.31	0.03
Scour Records						
Scours[b]	23/24	16/18	14/18	15/18	16/18	0.27
Scour Score[b]	2.8^{y}	1.4^{x}	1.4^{x}	1.2^{x}	1.8^{x}	0.31
Dead pigs[c]	8.0	2.0	0	1.0	2.0	0.25
% Dead[c]	33.3	11.1	0	5.5	11.1	-
Polymerase Chain Reaction to detect *E. coli*						
4 h after inoculation	75%	75%	75%	75%	75%	-
48 h after inoculation	100%	75%	50%	75%	75%	-
7 d after inoculation	100%	25%	50%	50%	50%	-

[a]D1, PPI – EYA; D2, PPI + EYA; D3, SDPP – EYA; D4, SDPP + EYA; D5, SDPP + PPI.
[b]Day 7 of experiment and 48 h after E. coli challenge
[c]Day 14 of experiment
[xy]Means within a row, lacking a common superscript differ ($P < 0.05$).

PPI-EYA (D1)-based diet had numerically lower ($P > 0.05$) ADG and ADFI (37 and 32%, respectively), during the post-challenge period when fed the diet without EYA (D1) compared to those fed the diet with EYA (D2). As shown in Table 9, PPI-EYA (D1)-fed piglets compared to all other treatments (D2 to D5), tended to have reduced ADFI ($P = 0.08$) and ADG ($P = 0.06$), for the entire 14-d experimental period. FCE for the pre-, post-challenge and the entire experimental period were similar ($P > 0.05$) for all dietary treatments (Table 9). Feeding PPI-EYA (D1) compared with SDPP resulted in a 46 and 50% decline in ADFI and ADG, respectively, from d 0 to 14. This agrees with the report of Friesen et al. (1993) that ADG and ADFI of 21-d old weaned pigs were reduced when they were fed SBM-based diets compared to those fed milk-based diets containing SDPP. By feeding a blend of PPI and SDPP (D5, 1:1) compared to feeding PPI-EYA (D1), ADFI and ADG improved by 52 and 56%, respectively. These results also agree with our recent observation that 10-d old weaned piglets fed diet with no specific anti-ETEC antibody performed poorer than those fed EYA and SDPP containing diets, each of which contained the specific anti-ETEC antibodies (Owusu-Asiedu et al., 2000). Similarly, Grinstead et al. (2000) reported that adding SDPP to SBM or whey-based diets increased feed consumption and weight gains more than each protein alone. Kats et al. (1994) also observed increases of 46% in ADFI and 55% in ADG from d 0 to 14 for pigs fed diets with 8% compared to 0% spray-dried animal plasma. Hansen et al. (1993), suggested that adding SDPP potentially masked transient hypersensitivity response for pigs fed higher levels of SBM from d 0 to 14 post-weaning.

The concentration of specific ETEC antibodies provided by diet D5 (PPI + SDPP) contained one-third the antibody concentration of D4 (SDPP + EYA) (Table 7), yet performance values were similar (Table 9). Also, diet D2 (PPI + EYA), D3 (SDPP - EYA) and D4 (SDPP + EYA) had approximately 2 to 3 times greater specific ETEC antibody concentrations than those of diets D5 (SDPP + PPI) yet performance values and other parameters were also similar (Tables 9, 10 and 11). These data therefore suggest that an excess concentration of specific immunoglobulins in the diet does not produce enhanced performance but that this level may approach a limiting value, at least under the conditions of the current experiment when the concentration of antibodies are similar to those present in the diet containing 5% SDPP.

In previous studies, ADG and ADFI were often dramatically improved when SDPP was added to the diet of early-weaned pigs while the FCE either was not improved or only marginally improved (Hansen et al., 1993; Sohn et al., 1991; Coffey and Cromwell, 1995). In a related study, Ermer et al. (1994) carried out a preference test in which weanling pigs could chose between diets containing either SDPP or dried skim milk. They observed that ADFI was higher for the feed containing SDPP and suggested that the higher intake was associated with a greater palatability. Similar results were also observed in the current study with D1 (PPI – EYA) and D2 (PPI + EYA). The current observations would therefore suggest that EYA, in contrast to egg-yolk without the two antibodies, when added to a PPI diet, as did SDPP, had an appetite enhancing effect and that this effect is attributable to the presence of the specific antibodies

which protect the piglets from the pathogenic effects of K88 and F18 strains *E. coli*. This protection presumably enabled the antibody-fed piglets to consume more feed and therefore have an enhanced growth rate.

No scours were recorded during wk 1 and all pigs appeared healthy. However, 3 d after the oral challenge PPI-EYA-fed piglets had severe diarrhea with a scour score of 2.8 that lasted for more than 7 d, resulting in 33% mortality. On the other hand, all piglets in the other 4 treatments (PPI + EYA, SDPP – EYA, SDPP + EYA and PPI + SDPP) had only mild diarrhea and mild scour scores (between 1.2 and 1.8) after which the pigs recovered after 3 d. Fecal swabs taken at 6 h post-challenged, showed that 75% of all treatment groups gave positive identification of ETEC-K88 (Table 9). However, 24 h after ETEC challenge, 100% of PPI-EYA (D1) compared to 50% of PPI+EYA (D2)-fed pigs continued to shed *E. coli* strain K88. Although most piglets on EYA or SDPP supplemented diets showed no signs of scours on d 7 post-challenge, 50% of these pigs compared to 100% of those fed PPI-EYA (D1) continued to shed ETEC-K88. The observation further demonstrates that fewer piglets in the groups receiving EYA or SDPP excreted *E. coli* and that the excretion decreased with duration of the experiment. The result also showed that antibodies prepared from the yolk of eggs from hens immunized with fimbrial antigen of *E. coli* are protective in piglets challenged with homologous ETEC strain. Severity of diarrhea and incidence of mortality was reduced in all cases by adding EYA or SDPP to PPI-based diets. The current data also support earlier reports that the feeding of plasma proteins (Gatnau and Zimmerman,

1990 and Van der Peet-Schwering and Binnendijk, 1995) and EYA (Marquardt et al., 1999; Owusu-Asiedu et al., 2000) during the first 14-d after weaning reduces the incidence of diarrhea and the number of piglets requiring treatment against gastrointestinal tract infections.

The protective effect of EYA obtained from hens immunized with antigens from a local strain of ETEC (K88) have been evaluated in a number of studies (Yokoyama et al., 1992; Kim et al., 1999; Marquardt et al., 1999). In a study with 21-d old weaned pigs fed either EYA or egg-yolk powder (EYP) without antibody and challenged with a high dose of ETEC (10^{12} CFU ml^{-1}), Kim et al. (1999) observed that control pigs that received egg-yolk powder developed severe diarrhea within 12 h, were dehydrated and lost BW within 48 h, resulting in 30% mortality. In contrast, the pigs treated with EYA showed no signs of diarrhea 24 or 48 h after treatment, had positive weight gain and no mortality. These authors concluded that EYA provided a 100% protection against deleterious effects of ETEC. In the current study, piglets fed PPI + EYA (D2) or SDPP (D3 to D5) had only mild and transient diarrhea, while those on PPI-alone (D1) diet had severe diarrhea and continued to shed *E. coli* (K88) 7 d after ETEC challenge. This observation is likely due to the binding of the K88 fimbrial by the specific anti-ETEC antibodies in EYA or SDPP, thus, preventing the adhesion of K88 fimbrial to its receptor in the small intestine. Specific anti-ETEC antibodies were absent in the PPI-EYA (D1) diet, which implies that *E. coli* continued to multiply after adhesion and colonization of the small intestine, thus resulting in the severe diarrhea observed in piglets fed this diet (Nagy and

Fekete, 1999). The current data agrees with the finding of Jin et al. (1998) that purified antibodies from the yolk of the chicken immunized against the fimbriae of ETEC K88 were able to block the binding of *E. coli* K88 to the mucosal receptor and that the interaction of antibodies with this strain of *E. coli* was fairly rapid as maximum protection was provided within 15 min. The better performance of pigs fed D2 (PPI + EYA) compared to D1 (PPI – EYA), as indicated earlier, can be attributed mainly or solely to the anti-K88 and anti-F18 antibodies since the only difference between the two diets was that D1 (PPI – EYA) did not contain the above specific antibodies while D2 (PPI + EYA) contained these antibodies.

Visceral organ weights and digesta pH are shown in Table 9. Liver weights were influenced by dietary treatment, with piglets fed PPI – EYA (D1), PPI + EYA (D2) and SDPP + EYA (D4) for 14 d having the smallest ($P < 0.05$) relative liver weight compared to those fed SDPP – EYA (D3) or SDPP + PPI (D5), (Table 10). Piglets fed the PPI-EYA (D1) diet for 14 days had higher ($P < 0.05$) digesta pH than those on the other dietary treatments (D2 to D5). It has been proposed that colonization of the small intestine by ETEC adhering to the epithelium accounts for most of the GIT disorders in post-weaned pigs (Nagy and Fekete, 1999). Higher pH in the small intestine is necessary for ETEC adhesion. The higher digesta pH may also explain the severity of diarrhea observed in this group. Higher gastric pH as a result of inefficient digestion is speculated to provide an optimal environment for the ETEC to colonize the

Table 10. Effect of dietary treatments and *E. coli* challenge on liver, spleen, small intestine, digesta pH and plasma urea nitrogen (PUN) of 10-d old weaned pig

Parameter	Dietary Treatment[a]					SEM[b]
	D1	D2	D3	D4	D5	
Liver wt (g/kg BW)						
7-d on treatment	27.9^{y}	28.5^{y}	33.7^{x}	31.1^{xy}	30.0^{y}	0.87
24-h after *E. coli* infection	32.5^{x}	32.3^{x}	26.4^{y}	31.8^{x}	27.3^{y}	0.64
14-d on treatment	29.1^{y}	30.6^{y}	33.9^{x}	28.7^{y}	35.4^{x}	0.45
Spleen weight (g/kg BW)						
7-d on treatment	2.8	2.6	3.0	2.6	2.5	0.44
24-h after *E. coli* infection	2.4^{z}	4.5^{x}	4.6^{x}	3.4^{xy}	2.9^{z}	0.29
14-d on treatment	3.0	2.9	3.0	3.1	2.9	0.31
Small Intestine weight (g/kg BW)						
7-d on treatment	28.4	32.4	28.6	27.7	27.1	0.99
24-h after *E. coli* infection	26.9	29.2	23.4	27.8	26.9	0.99
14-d on treatment	39.5^{x}	35.6^{y}	38.5^{x}	33.6^{z}	33.8^{z}	0.32
Stomach wt. (g/kg BW)						
7-d on treatment	7.1	8.1	8.7	7.9	8.3	0.36
24-h after *E. coli* infection	8.1	8.5	7.5	8.2	8.6	0.46
14-d on treatment	10.3	10.1	9.7	9.9	10.0	0.16
Stomach digesta pH						
7-d Pre-challenge	2.6	3.6	3.5	3.0	3.1	0.18
24-h Post-challenge	4.2	2.4	3.1	2.7	3.6	0.42
14-d Post-challenge	3.5^{x}	3.3^{y}	3.2^{y}	3.2^{y}	3.2^{y}	0.02
Digesta pH						
7-d Pre-chllenge	6.4^{x}	6.1^{x}	5.5^{y}	6.3^{x}	6.2^{x}	0.11
24-h Post-challenge	7.4^{x}	7.1^{xy}	7.2^{xy}	7.3^{xy}	7.1^{y}	0.04
14-d Post-challenge	6.6^{x}	5.9^{y}	6.0^{y}	6.1^{y}	6.2^{y}	0.01
PUN (mg/dL)						
7-d Pre-challenge	16.1^{x}	14.5^{x}	14.3^{x}	15.4^{x}	11.7^{y}	0.68
24h Post-challenge	19.4^{x}	16.7^{xyz}	13.5^{z}	14.8^{yz}	17.2^{xy}	1.13
7-d Post-challenge	25.7^{x}	14.6^{y}	12.3^{y}	13.5^{y}	14.3^{y}	1.10

[a]D1, PPI – EYA; D2, PPI + EYA; D3, SDPP – EYA; D4, SDPP + EYA; D5, SDPP + PPI.
[b]SEM, standard error of the mean.
[xyz]Means within a row, lacking a common superscript differ ($P < 0.05$).
Number of observation=4

surface of the villi, resulting in the initiation of scours in young pigs, particularly after weaning (Smith and Jones 1963). In contrast, low luminal pH favors the growth of lactobacilli in the stomach, which possibly inhibits the colonization and proliferation of *E. coli* by blocking the sites of adhesion (Fuller, 1977).

Plasma urea N level of piglets fed a combination of PPI and SDPP was lower ($P < 0.05$) compared to piglets on the other treatment groups during the pre-challenge period (Table 10). However, on d 14 (post-challenge period), PUN levels were higher ($P < 0.05$) in PPI-EYA (D1)-fed piglets compared to those fed the other four diets (Table 10). Infectious diseases or inflammation markedly reduce feed intake and cause a redistribution of nutrients away from growth processes to support the immune system. In such instances, AA are liberated from muscle breakdown and can be utilized for the synthesis of acute phase proteins in the liver and as an energy source (Wannemacher, 1977). Furthermore, Van Heugten et al. (1994) showed that the efficiency of protein utilization during an inflammation response is decreased in 21-d old weaned pig injected with lipopolysaccharide. Thus, the higher PUN level in the PPI-alone fed pigs after ETEC challenge is an indication that infection in this treatment group activated the immune system, and might have led to increased body protein-breakdown as well as a reduced efficiency of dietary protein utilization for body protein accretion (Coma et al., 1995).

Table 11 shows the histology of the gastrointestinal tract mucosa before and after an ETEC challenge. Compared to D2 (PPI + EYA), D3 (SDPP – EYA)

Table 11. Effect of dietary treatments and *E. coli* challenge on villi height and crypt depth (μm) of 10-d old weaned pig

Parameter	Dietary Treatments[a]					
	D1	D2	D3	D4	D5	SEM[b]
Day 7 (Before *E. coli* challenge)						
Villi Height						
Duodenum	499^{z}	602^{y}	605^{y}	758^{x}	565^{yz}	17
Jejunum	579	609	635	759	604	26
Ileum	415	459	499	488	467	26
Crypt Depth						
Duodenum	279	268	256	332	269	16
Jejunum	205	223	204	180	185	13
Ileum	220	233	200	186	182	14
Day 8 (24-h after *E. coli* challenge)						
Villi Height						
Duodenum	474^{y}	669^{x}	650^{x}	688^{x}	573^{xy}	30
Jejunum	374^{z}	504^{xy}	550^{x}	576^{x}	516^{xy}	20
Ileum	325	378	404	538	434	45
Crypt Depth						
Duodenum	317	314	293	276	301	28
Jejunum	272	270	250	241	212	27
Ileum	289	190	190	163	172	24
Day 14 (7-d after *E. coli* challenge)						
Villi Height						
Duodenum	499^{z}	701^{x}	622^{y}	749^{x}	565^{y}	13
Jejunum	556	651	699	669	622	38
Ileum	341	398	546	510	413	34
Crypt Depth						
Duodenum	341	274	254	241	250	16
Jejunum	305	295	279	268	269	19
Ileum	257	220	197	174	185	21

[a]D1, PPI-EYA; D2, PPI + EYA; D3, SDPP-EYA; D4, SDPP + EYA; D5, SDPP + PPI.
[b]SEM; standard error of the mean.
[c]Number of observations = 4
[xyz]Means within a row, lacking a common superscript differ ($P < 0.05$).

and D4 (SDPP + EYA) piglets fed D1 (PPI – EYA) and D5 (SDPP + EYA) had shorter ($P < 0.05$) villi in the duodenum of the gastrointestinal tract immediately before (d 7) and after (d 8) the *E. coli* challenge. However on d 14, D1 (PPI – EYA) fed piglets had the shortest ($P < 0.05$) villi compared to all other treatments (D2 to D5), (Table 11). However, crypt depths were similar ($P > 0.05$) and were not influenced by dietary treatment or oral ETEC challenge (Table 11). Gut morphology has been examined in several weaning studies and reduced villi height has been linked to post-weaning growth lag (Cera et al., 1988) and diarrhea (Hornich et al., 1973) in weaned pigs. It has been proposed that dietary antigens in PPI (Li et al., 1990) are responsible for the associated post-weaning diarrhea (Le Guen and et al., 1991). The antigenic effects have also been associated with damaged microvilli, villous atrophy, crypt depth hyperplasia, and a reduced crypt/villous ratio in piglets fed diets containing soy proteins and other leguminous plants (Cera et al., 1988; Li et al., 1990, 1991; Dreau et al., 1994; Makinde et al., 1996). Antigenicity is the reaction seen at weaning in calves and piglets when protein sources such as purified plant protein are included in the diet (Rooke et al., 1998). Feeding raw pea flour to pre-ruminant calves (Nunes do Prado et al., 1988) and pigs (Le Guen et al., 1991) has been reported to result in formation of antibodies against pea proteins (legumin and vicilin), along with an increased gut permeability to macromolecules. In normal physiological conditions, the gut-wall prevents the passage of dietary antigens from intestinal lumen into the blood circulation (Walker et al., 1975). Li et al. (1990) also reported that damage to the microvilli evidence by villous atrophy and crypt

hyperplasia result in immune response at the intestinal level. Antigenic proteins may cause delayed hypersensitivity or immediate hypersensitivity that may lead to mucosal inflammation. Lalles (1993) reported that although the reaction is transient and variable, it can be very severe and result in decreased performance, health breakdown and incidence of diarrhea. The observed reduction in villi height seen in the current study was also associated with a corresponding decrease in ADG and ADFI, and increased incidence of scours in the PPI-EYA (D1)-fed pigs. Pigs fed diets containing SDPP or EYA had longer villi, less severe diarrhea and grew faster than did PPI-EYA (D1)-fed pigs, suggesting that piglets in these treatments, that is, only the pigs that received the antibodies, experienced minimum post-weaning stress. Intestinal damage as a result of *E. coli* infection is therefore a possible cause of the observed villous atrophy in the diet without antibody (D1, PPI-EYA) but not the other diets that contained anti-K88 *E. coli* antibodies. Presumably pigs from the former but not the latter groups would have absorbed the large PPI molecules which would have induced an immune (antigenic) response. On the basis of our results and that reported in the literature, it is concluded that the antigenic effect observed with legume diets is not caused by the legume protein per se, but is attributable to the lack of antibodies against intestinal pathogens, especially *E. coli* K88 (and probably F18), which result in colonization of the gut and destruction of the villi. This results in malabsorption of foreign proteins, which are antigenic. Therefore, antibodies in the diet prevent damage of the villi by pathogens, as a result, normal nutrient absorption is maintained without absorption of intact or partially

digested protein. This means that any proteins, vegetable or animal, would induce an antigenic response if villi are damaged by infection and the proteins are absorbed. This hypothesis, however, need to be tested.

It may, therefore, be concluded that a diet containing PPI + EYA when fed to pigs will yield performance values greatly superior to those containing PPI – EYA (D1). The only apparent difference between the two diets was the content of anti-K88 and anti-F18 antibodies. In addition, the performances of pigs fed diets containing SDPP were similar to those fed PPI + EYA (D2). Therefore, it should be possible to replace SDPP in the diet with a high quality plant-protein supplement such as PPI and a source of anti-K88 and possibly anti-F18 antibodies such as present in egg-yolk powder from appropriately hyper-immunized hens. Furthermore, diets containing antibodies from either SDPP or EYA yielded ADG and ADFI values that were, respectively, 51, 46 and 46, 38% greater than those obtained without antibodies. However, neither antibodies containing diets (SDPP or EYA) improved the FCE. These data therefore, suggest that indirectly the antibodies had an appetite stimulating effect. This effect may be attributed to the ability of the antibodies to prevent intestinal infection by pathogens such as *E. coli* which, in turn, not only protect the integrity of the absorptive surface of the gastrointestinal tract but also the overall health of the animal including appetite. Lastly, the antigenic effect of PPI or that of any foreign protein may be a consequence of prior inflammation of the intestinal membrane, which in turn would permit the malabsorption of intact or partially hydrolyzed protein resulting in a subsequent immune and associated allergic

response. Presumably this malabsorption of protein would not occur in piglets with an intact, undamaged gastrointestinal tract mucosa.

IMPLICATION

The current results suggest that feeding early-weaned pigs diets containing SDPP, PPI plus EYA or a combination of PPI and SDPP during d 0 to 14 post-weaning prevents gastrointestinal disorders associated with *E. coli* infection. Supplementing weanling pig diets with EYA or SDPP will allow utilization of processed plant proteins such as pea protein isolate in diets for early-weaned pigs. This will not only reduce the cost of feeding early-weaned pigs, but also offers a means for managing post-weaning diarrhea, which is a major problem in the management of early-weaned pigs.

CHAPTER FIVE

MANUSCRIPT THREE

Response of early-weaned pigs to an enterotoxigenic *Escherichia coli* (K88) challenge when fed diets containing spray-dried porcine plasma or pea protein isolate plus egg-yolk antibody, zinc oxide, fumaric acid or antibiotics

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ABSTRACT

A major challenge currently facing the swine industry is post-weaning *E. coli* diarrhea in early-weaned pigs (EWP) and to identify means for its control that are not only cost-effective but also suitable for sustainable pork production. The effect of feeding diets containing either spray-dried porcine plasma (SDPP) or pea protein-isolate (PPI) supplemented with either egg-yolk antibodies (EYA) obtained from laying hens immunized with enterotoxigenic *E. coli* (K88 and F18) antigens, zinc oxide (ZnO), fumaric acid (FA) or antibiotics (carbadox, AB) on piglet performance, incidence of scours and gut morphology was studied in a 14-d experiment. Ninety 10-d old weaned pigs were assigned to 6 dietary treatments in a completely randomized design to give 5 replicate pens per treatment with 3 pigs per pen. The treatments were SDPP+EYP, PPI+EYP, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB. All diets were formulated to similar nutrient levels and were available at all times. On day 0, 7 and 14, blood samples were taken for all pigs for determination of plasma urea N (PUN). All pigs were orally challenged with 6 x 10^{12} colony forming units of ETEC K88 on day 7. Piglets were weighed on 0, 7 and 14 d. On day 14, 3 pigs per treatment were sacrificed to study the histology of the small intestine. Weekly feed intake, BW changes and feed conversion efficiency were determined. The number of piglets with scours and scour scores were also recorded. Fecal swabs were taken from all pigs before and after oral ETEC challenge for polymerase chain reaction (PCR) test to detect K88 *E. coli.* Feed efficiency was similar *($P > 0.05$)* for all treatments. Piglets fed PPI-EYA had lower *($P < 0.05$)* ADFI during week 2 (137 g/d) and had lower *($P < 0.05$)* ADG

from day 0 to 14 (97 g/d), respectively, compared to those fed SDPP-EYA (276 and156 g/d), PPI+EYA (245 and 151.2 g/d), PPI+ZnO (264 and 158.8 g/d), PPI+FA (259 and 155.5 g/d) and PPI+AB (255 and 152.9 g/d)-based diets. Scours appeared in all groups of pigs 8 h after ETEC K88 oral challenge, however, most of the piglets fed SDPP-EYA or PPI-supplemented with EYA, ZnO, FA or AB recovered 3 to 5 d post-challenge, whereas those fed PPI-EYA continued to have severe diarrhea resulting in 40% mortality vs 13% or less in the other groups. The PCR results showed that 81% of piglets fed PPI-EYA continued to shed *E. coli* K88 7 d after oral ETEC chalenge. Piglets fed PPI-EYA in general had shorter villi *($P < 0.05$)*, reduced villi:crypt ratio *($P < 0.05$)*, higher intestinal pH *($P < 0.05$)* and higher PUN *($P < 0.05$)* than those fed SDPP-EYA or PPI supplemented with EYA, ZnO, FA and AB diets. However, all pigs fed SDPP or PPI-supplemented diets had similar performance *($P > 0.05$)* for all measured parameters. It was concluded that SDPP, EYA, ZnO, FA and AB provided passive control of ETEC (K88) infection and potentially improved EWP performance.

Keywords: Early-weaned pigs, Post-weaning diarrhea, Antibodies, *E. coli*, Fumaric acid, Zinc oxide, Antibiotics.

INTRODUCTION

Economic losses in the swine industry associated with intestinal diseases are extremely high. Results of a recent survey by Alexander et al. (1994) show that diarrhea represents 11% of all post-weaning piglet mortality and enterotoxigenic *Escherichia coli* (ETEC) diarrhea is the most common enteric disease seen in neonatal and weaned piglets. Furthermore, ETEC is reported to be responsible for 50% of the 10 million piglets that die annually across the world (Gyles, 1994). ETEC strains are associated with intestinal colonization in piglets include those that express the K88 and F18 fimbrial adhesions. *E. coli* K88 is mainly responsible for diarrhea problems in weaned pigs, although *E. coli* F18 also has more recently been shown to be a problem (Nagy and Fekete, 1999).

A major challenge currently facing the swine industry is to identify means for controlling diarrhea in young pigs that are not only cost-effective but also suitable for sustainable pork production. Thus far, various strategies including use of in-feed antibiotics (NRC, 1979; Hays, 1986), spray-dried plasma proteins (Coffey and Cromwell, 1995; Godfredson-Kisk et al., 1997; Gomez et al., 1998), pro- and prebiotics (Mitchell and Kenworthy, 1976; Nousiainen and Setala, 1993), organic acids (Ravindran and Kornegay, 1993; Paulick et al., 1996) and zinc and copper salts (Poulsen, 1995; Carlson et al., 1999; Maviromichalis et al., 2000) have been tried with mixed results. Furthermore, there are serious safety and environmental concerns regarding the use of some of these products in piglet diets. These concerns include antibiotic resistance associated with use of in-feed antibiotics (Mathew et al., 1998), effect of plasma proteins on human

health and toxicity and environmental implications of excess zinc and copper salts.

Egg-yolk antibodies (EYA) from laying hens hyper-immunized with specific bacterial fimbrial antigens have been proposed as an effective means for controlling diarrhea in early-weaned pigs (Yokoyama et al 1992; Marquardt et al., 1999). In addition, EYA are also not affected by the limitations outlined above. Moreover, EYA are relatively inexpensive compared to plasma protein and can contain large amounts of specific anti-ETEC antibodies if obtained from hens hyper-immunized with the appropriate antigen (Marquardt et al., 1999; Owusu-Asiedu et al., 2000). Therefore, the potential benefits of using EYA in the nutrition and management of EWP are considerable. However, to realize the full potential of EYA, a careful and thorough evaluation and demonstration of their advantages over competing alternatives is critical. The objectives of the current study were to assess the effectiveness of EYA in controlling ETEC relative to conventional additives in early-weaned pigs fed pea protein isolate-based diets and to assess the effect of dietary treatments and enterotoxigenic *E. coli* challenge on morphology of the gastrointestinal tract.

MATERIALS AND METHODS

Animal Care, Housing and Experimental Design

The experimental protocol was approved by the University of Manitoba Animal Care Committee and pigs were cared for according to the guidelines of Canadian Council on Animal Care (CCAC, 1993). A total of 90 piglets weaned at 10 d of

age (3.8 ± 0.1 kg initial BW) and balanced for initial BW were used in a 14-d trial. Piglets were randomly allotted to each of 6 dietary treatments in a completely randomized design. Each treatment was assigned to 5 replicates pens (1.2 x 1.5 m) each with 3 pigs per pen. Room temperature was maintained at 31 ± 1°C throughout the study.

Diets, Feeding and Experimental Procedure

PPI and SDPP were obtained from Parrheim Foods (Portage La Prairie, MB.) and Farmlands Proteins Plant (Maquoketa, Iowa MO.), respectively. EYA was produced in our laboratory as described previously (Marquardt et al., 1999). The relative concentration of antibodies in the different preparations (SDPP and EYA) and the diets were similar to our earlier report (Owusu-Asiedu et al., 2002) and as outlined in Table 12. The experimental diets included SDPP as a positive control (diet E1, SDPP-EYA), and five PPI-based diets, which were E2, PPI without EYA (PPI-EYA); E3, PPI with EYA (PPI+EYA); E4, PPI supplemented with ZnO (PPI+ZnO); E5, PPI with fumaric acid (PPI+FA) and E6, PPI supplemented with a carbadox (PPI+AB). The EYA contained 0.3 and 0.2% egg-yolk powder each containing specific anti-K88 and -F18 antibodies, respectively. Egg-yolk powder from hens not immunized with K88 and F18 fimbrial antigens (referred to as EYP) was added to all diets except diet E3 which contained EYA. The ZnO, FA and AB were added at 2000 mg/kg, 20 g/kg and 55 mg carbadox/kg diet, respectively as described in the literature (Blanks et al., 1999; Hill et al., 2001).

Table 12. Composition of experimental diets

Ingredients (%)	Dietary Treatments[a]					
	SDPP+ EYP	PPI+EYP	PPI+EYA	PPI+ZnO	PPI+FA	PPI+AB
Wheat	14.0	14.0	14.0	14.0	14.0	14.0
Oat groats	14.0	14.0	14.0	14.0	14.0	14.0
Whey	13.0	13.0	13.0	13.0	13.0	13.0
Corn	5.0	5.0	5.0	5.0	5.0	5.0
SBM	16.0	16.0	16.0	16.0	16.0	16.0
Fish meal	8.0	8.0	8.0	8.0	8.0	8.0
SDPP[a]	10.0	-	-	-	-	-
PPI[a]	-	10.0	10.0	10.0	10.0	10.0
Vegetable Oil	4.7	4.6	4.6	4.6	4.6	4.6
Sucrose	9.0	9.0	9.0	8.6	8.0	8.75
Premix[b]	5.0	5.0	5.0	5.0	5.0	5.0
Lysine	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.2	0.3	0.3	0.3	0.3	0.3
Threonine	0.3	0.3	0.3	0.3	0.3	0.3
EYA[a]	-	-	0.5	-	-	-
Egg-yolk powder[c]	0.5	0.5	-	0.5	0.5	0.5
ZnO	-	-	-	0.4	-	-
Fumaric Acid	-	-	-	-	2.0	-
Antibiotics[d]	-	-	-	-	-	0.25
Calculated (%)						
DE (kcal/kg)	3491	3490	3490	3490	3490	3490
CP (%)	27.4	27.4	27.4	27.4	27.4	27.4
Lysine	1.6	1.6	1.6	1.6	1.6	1.6
Threonine	1.4	1.3	1.3	1.3	1.3	1.3
Methionine	0.7	0.7	0.7	0.7	0.7	0.7
Tryptophan	0.4	0.4	0.4	0.4	0.4	0.4
Anti-K88 titer[e]	1800	<100	1800	<100	<100	<100
Anti-F18 titer[e]	1500	<100	900	<100	<100	<100
Others[f]	<100	<100	<100	<100	<100	<100

[a]Spray-dried porcine plasma (SDPP), egg-yolk antibody (EYA), zinc oxide (ZnO), fumaric acid, antibiotics (AB). SDPP-EYA (E1); PPI-EYA (E2); PPI+EYA (E3); PPI+ZnO (E4); PPI+FA (E5); PPI+AB (E6).

[b]Premix provided per kg of diet: 9 000 IU vitamin A, 1 500 IU vitamin D3, 18 mg vitamin E, 1.5 mg vitamin K, 250 mg choline, 30 mg niacin, 27.5 mg calcium pentothenate, 9.4 mg B2, 2 mg B6, 25 µg B12, 80 µg biotin, 0.5 mg folic acid, 5.75 g Ca, 2.6 g phosphate, 3.5 g sodium chloride, 27.5 mg Mn, 105 mg iron, 125 mg Cu, 0.6 mg.

[c]Egg-yolk powder did not contain the specific anti-K88 and anti-F18 antibodies

[d]Antibiotics, used in commercial grower diets. Provided 55 mg carbadox/kg diet to specifically control *E. coli* infection.

[e]Titer is defined as the dilution of antibody preparation that gives 50% of maximal absorption in the ELISA assay.

[f]Others, assays were for *E. coli* strains K99, 987P, and F41, (titers were <100).

Table 13. Analyzed DM, CP and AA composition (%) of experimental diets

	Dietary Treatments [a]					
Components	SDPP-EYA	PPI-EYA	PPI+EYA	PPI+ZnO	PPI+FA	PPI+AB
DM	89.9	90.1	89.6	89.8	90.0	89.7
CP	27.0	26.7	26.5	26.8	26.5	26.4
Aspartic acid	2.6	2.5	2.4	2.3	2.3	2.4
Threonine	1.6	1.4	1.4	1.4	1.5	1.4
Serine	1.5	1.4	1.3	1.3	1.3	1.3
Glutamic acid	4.6	4.4	4.3	4.3	4.4	4.3
Proline	1.6	1.5	1.4	1.3	1.4	1.4
Glycine	1.4	1.3	1.2	1.2	1.3	1.3
Alanine	1.4	1.3	1.2	1.3	1.2	1.3
Cysteine	0.4	0.3	0.3	0.3	0.3	0.3
Valine	1.0	0.9	0.8	0.8	0.8	0.8
Methionine	0.4	0.5	0.6	0.5	0.6	0.7
Isoleucine	0.7	0.7	0.6	0.5	0.6	0.6
Leucine	2.2	1.8	1.6	1.6	1.6	1.6
Tyrosine	0.8	0.7	0.7	0.6	0.6	0.6
Phenylalanine	1.1	0.9	0.8	0.8	0.8	0.8
Histidine	0.7	0.5	0.5	0.5	0.5	0.6
Lysine	2.0	1.9	1.9	1.9	1.9	1.9
Arginine	1.5	1.4	1.3	1.3	1.3	1.3

[a]Spray-dried porcine plasma (SDPP), Egg-yolk antibody (EYA), zinc oxide (ZnO), Fumaric acid, antibiotics (AB). SDPP-EYA, E1; PPI-EYA, E2; PPI+EYA, E3; PPI+ZnO, E4; PPI+FA, E5; PPI+AB, E6.

All experimental diets were formulated to meet NRC (1998) nutrient requirements for piglets weighing 3.0 to 6.0 kg BW and contained similar CP (27.4%), lysine (1.6%), methionine (0.7%), and threonine (1.4), (Tables 12 and 13). Pigs had unlimited access to feed and water at all times. ADG and ADFI were determined weekly from which FCE (F/G) were obtained. On d 0, 7 and 14 blood samples (10 mL) were collected from all pigs via jugular vein puncture into vacutainer tubes (Becton Dickinson, Rutherford, NJ), and immediately centrifuged at 2000 x *g* for 10 min at 5°C to recover plasma, which were immediately stored at –20 °C until analysed for PUN.

Bacteria Culture, Oral Challenge and Health Status

A local strain of ETEC expressing the K88 (F4) fimbriae, was obtained from the Animal Health Center, Veterinary Services Branch, Manitoba Department of Agriculture, Winnipeg, MB, Canada. The K88 strain of *E. coli* was used because it is the leading cause of diarrheal disease in EWP (Nagy and Fekete, 1999). Primary cultures of the ETEC strain were grown overnight in tryptic soya broth (TSB, CASO-Bouillon, Mikrobiologie Darmstadt, Germany) at 37°C using 1% inoculum from stocks stored at –20°C in 30% glycerol. The K88 *E. coli* strain was prepared as described by Marquardt et al. (1999). Briefly, ETEC K88 was grown overnight in blood agar plate (Atlas Laboratories Co. Ltd. Winnipeg, MB. Canada) at 37°C using 1% inoculum from stock. Cells were washed twice with 2-mL sterilized saline solution (0.9%, pH 7.2), and then the suspension (10^{12} colony forming unit [CFU] mL^{-1}) used for oral challenge. On day

7 of the experiment (17 d old pigs), each pig received 6 mL of bacterial suspension contained in a syringe attached to polyethylene tube held in the oral cavity. Severity of diarrhea was characterized by using the fecal consistency (FC) score system described by Marquardt et al. (1999). FC scoring (0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea) performed by 2 trained personnel with no prior knowledge of dietary treatment allocation were used to assess the health status of pigs.

Histological and Other Measurements

On day 14, 3 pigs per treatment were sacrificed to determine the effect of dietary treatments and oral ETEC challenge on weights of visceral organs and morphology of the gastrointestinal tract. Pigs were held under general anesthesia and killed by an intra-cardiac injection of sodium pentobarbital (50 mg/kg BW). Stomach, spleen, small intestine and liver were removed from the animals and 20 mL of digesta each from the stomach and the small intestine was obtained for pH measurement. The organs or sections were flushed with ice-cold phenylmethyl sulfonyl fluoride (PMSF) saline (2 L of 0.9% saline, pH, 7.4 + 2-mL of 100 mM PMSF). The pH of the digesta was determined by inserting a combination electrode directly into aqueous suspension. The pH meter (Fisher Scientific Company) was calibrated using a pH 7.0-reference standard. The weights and length (small intestine) of these organs were determined. A 10-cm segment of the small intestine was removed 150 cm from the pylorus junction to represent the jejunal region. The sections were stored in 10% formalin to fix the

villous and the crypt for subsequent histological measurement. Six cross-sections were obtained from each formalin-fixed sample and processed for histological examination using the standard Hematoxylin and Eosin (H&E) method. The measurement of villous height (VH), and crypt depth (CD) was made on 10 well-oriented villi per specimen using a Zeiss photomicroscope equipped with a Sony 3 chip CCD colour camera. The images were captured using Empix's Northern Eclipse Image Processing Software (Empix Imaging, Inc., Mississauga, ON., Canada). The height of the villous was measured from the tip to the crypt-villous junction and the depth of the crypt from the crypt-villous junction to the base.

Detection of Antibodies Titer

Enzyme-linked immunosorbent assay (ELISA) with purified fimbrial antigen was used to determine anti-K88 (F4), -K99 (F5), -987P (F6), -F41 and -F18 antibody titers in SDPP, PPI, EYA and all experimental diets using the procedure of Kim et al. (1999) and as outlined in Chapter 4.

Chemical Analyses

All analyses were done in duplicate. All experimental diets were ground through a 1-mm screen (Cyclotec 1093, sample mill, Tecator, Hoganas, Sweden), prior to analysis. Samples were dried in a convection oven at 105°C for 16 h for DM determination, while CP (N x 6.25) content was determined using Leco NS 2000 Nitrogen Analyzer (LECO Corporation, St. Joseph, MI., USA). A 100-mg sample was prepared for acid hydrolysis according to AOAC (1984) and analysed for AA

as modified by Mills et al. (1989). The method involved digestion in 4 mL of 6*N* HCl in vacuo for 24 h at 110°C followed by neutralization with 4 mL w/v NaOH and cooling to room temperature. The mixture was then made to 50 mL volume with sodium citrate buffer (pH 2.2). Methionine and cysteine were analyzed as methionine sulfone and cysteic acid, respectively, after oxidation with performic acid. AA were then analyzed using a LK 4151 Alpha analyzer (LKB Biochrom, Cambridge, UK). Plasma samples were analyzed for urea nitrogen concentrations according to Crocker (1967), using a standard kit (Procedure No. 535, Sigma Diagnostic, St. Louis, MO., USA).

Polymerase chain reaction (PCR).

Fecal swab samples for microbial analysis were collected in duplicate from all pigs using Culture Swab Transport System (Difco) prior, 8, 24 and 48 h, as well as 7 d post-ETEC challenge. Samples were plated onto TSB and the PCR method was used on the individual colonies for the detection of K88 adhesive *E. coli*. The PCR-technique was based on the procedure described by Sambrook et al. (1989). The sense and anti-sense primers that encoded the specific K88 fimbrial gene were obtained from GenBank (www.ncbi.nlm.nih.gov/entrez) for primer design. The PCR was performed following a standard procedure in a thermocycler (Techne Genius, model FGENO2TP, Duxford, Cambridge, U.K.) with the following program; 30 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, and an extension step at 72°C for 5 min at the end of the cycle. The product of the PCR reaction was then electrophoresed on a 0.8% agarose gel

and viewed following exposure to UV light. The resultant PCR product corresponded in size (2.6 kDa) to structural subunit of the K88 operon that was selected. The sample was deemed positive for the K88 fimbrial gene when it produced a distinctive band consistent with its expected migration on the agarose gel as determined by comparison with the DNA fragment standards.

Calculations and Statistical Analysis

Villi height (VH) and crypt depths (CD) were determined by averaging the individual measurements in similarly treated pigs. Mean VH and CD were obtained by averaging the measurements from 3 pigs. All data (ADG, ADFI, FCE, scour scores, VH and CD) were analyzed as a completely randomized design. For ADFI and FCE the pen was considered the experimental unit, while the pig was the experimental unit for all other parameters. Treatment means were compared using Duncan Multiple Range procedure (Cody and Smith, 1991). Statistical significant was accepted at $P < 0.05$. All statistical analyses were performed using the GLM procedures of SAS (1988).

RESULTS AND DISCUSSION

Crude protein and AA acid compositions were similar in PPI and SDPP as reported in Chapter 4. The analysed and calculated composition indicated that all of the experimental diets had similar CP and AA (Table 13). Also, analysis of the experimental diets showed that with the exception of SDPP-EYA and PPI+EYA, all other dietary treatments contained no anti-K88 or anti-F18 antibodies titers (Table

12). This was not unexpected and agrees with our earlier report that PPI-based diets are devoid of specific ETEC antibodies reported in Chapter 4.

Performance data for the pre-challenge (d 0 to 7, week 1), post-challenge (7 to 14, week 2) and the entire experimental period (overall, d 0 to 14) are shown in Table 14. The initial and final BW were similar among treatments, ranging from 3.8 to 3.9, and 5.4 to 6.1 kg, respectively. The ADG during the pre-challenge (week 1), the challenge (week 2) and the overall periods were higher *($P < 0.05$)* for piglets fed SDPP-EYA, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB, compared with those fed PPI-EYA. The specific increase in ADG over the 2-wk experimental period of pigs fed diets containing SDPP or PPI-based diets that were supplemented with EYA, ZnO, FA or AB compared to those fed PPI-EYA (pea protein isolate without K88 and F18 antibodies were 62, 57, 65, 61 and 58%, respectively. A similar pattern of increase was observed for wk 1 and 2 of the experiment. Also, with the exception of piglets fed PPI-EYA, all treatment groups (SDPP-EYA, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB) had similar *($P > 0.05$)* ADG.

The ADFI for wk 2 (post-challenge period) was from 80 to 102 % lower *($P < 0.05$)* in PPI-EYA-fed piglets compared with all other treatments (SDPP-EYA, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB). However, the ADFI were not different *($P > 0.05$)* among dietary treatments for pigs in the pre-challenge and the entire experimental period (Table 14). Although the ADFI for wk 1 and the overall period was not affected *($P > 0.05$)* by dietary treatments, the numerical improvements *($P >$*

Table 14. Effect of spray-dried porcine plasma, egg-yolk antibody, zinc oxide, fumaric acid and antibiotics supplementation on performance and plasma urea N level of 10-d old weaned pigs

	Dietary Treatments[a]						
Parameter	SDPP-EYA	PPI-EYA	PPI+EYA	PPI+ZnO	PPI+FA	PPI+AB	SEM
Body Weight (kg)							
Initial (d 10)[b]	3.90	3.80	3.80	3.80	3.80	3.80	0.11
Final (d 24)	6.10	5.40	5.92	6.03	5.98	5.84	0.14
ADG (g/d)							
Week 1 (10-14 d)	137.8^{x}	99.7^{y}	133.5^{x}	129.4^{x}	126.4^{x}	128.2^{x}	7.9
Week 2 (14-24 d)	188.9^{x}	94.3^{y}	166.2^{x}	188.6^{x}	185.5^{x}	170.3^{x}	15.6
Overall (10-24 d)	156.5^{x}	96.5^{y}	151.2^{x}	158.8^{x}	155.5^{x}	152.9^{x}	9.1
ADFI (g/d)							
Week 1	186.7	146.9	186.2	173.0	176.9	184.1	13.4
Week 2	276.0^{x}	136.5^{y}	245.7^{x}	264.9^{x}	259.2^{x}	255.1^{x}	19.4
Overall	213.2	141.0	208.1	214.7	211.6	222.4	15.3
FCE							
Week 1	1.35	1.47	1.39	1.34	1.39	1.44	0.04
Week 2	1.46	1.45	1.48	1.40	1.40	1.49	0.05
Overall	1.36	1.45	1.38	1.35	1.36	1.45	0.04
PUN (mg/dL)							
Initial	6.5	6.6	6.7	6.7	6.8	6.6	0.39
Week 1	13.8	15.0	14.5	14.6	14.1	14.2	0.82
Week 2	22.5^{x}	26.0^{y}	22.9^{x}	21.9^{x}	22.1^{x}	21.9^{x}	0.47

[a]Spray-dried porcine plasma (SDPP), Egg-yolk antibody (EYA), zinc oxide (ZnO), Fumaric acid, antibiotics (AB). E1). SDPP-EYA; E2). PPI-EYA; E3). PPI+EYA; E4). PPI+ZnO; E5). PPI+FA; E6). PPI+AB.
[b]Bracket values are age of the piglets
[xy]Means within a row, lacking a common superscript differ *($P < 0.05$)*.
SEM, standard error of the mean.

0.05) in all supplemented treatment groups (SDPP-EYA, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB) compared with the piglets fed PPI-EYA-based diet were from 18 to 27% during wk 1 and 49 to 59% for the two wk period. FCE was not affected by any of the dietary treatments *(P > 0.05)*, with maximum differences among all dietary treatments being 9%, which is considerably lower than those seen with ADG and ADFI.

The improved growth rate and feed intake is supported by the results of other researchers using EYA (Kim et al., 1999; Marquardt et al., 1999), ZnO (Poulsen, 1995; Mavromichalis et al., 2000), organic acids (Paulick et al., 1996; van Kol, 2000), and/or antibiotics (Roof and Mahan, 1982; Hill et al., 2001). Recently, Hill et al. (2001) evaluated the efficacy of different levels of dietary ZnO (0, 500, 1000, 2000 or 3000 mg Zn/kg) for a 28-d post-weaning period. They reported that ADG, ADFI and FCE in nursery pigs increased with increasing dietary ZnO concentrations and that the response reached a plateau at 2000-mg Zn/kg.

The PUN level during d 0 and 7 (pre-challenge period) were similar *(P > 0.05)* for all treatment groups (Table 14). However, the PUN on d 14 (post-challenge period) was higher *(P < 0.05)* for PPI-EYA-fed piglets compared to piglets fed the other dietary treatments (SDPP-EYA, PPI+EYA, PPI+ZnO, PPI+EYA and PPI+AB). Feed intake is greatly reduced due to over production of cytokines during infection or inflammation, resulting in redistribution of nutrients away from growth processes to support the immune system. Amino acids are liberated from muscle breakdown and are used to synthesis acute phase proteins in the liver, and as an energy source in such instances (Wannemacher, 1977). The higher (*P < 0.05*) PUN level (15.5 to

17.6%) obtained in the current study in the E2 (PPI-EYA) fed pigs compared to the other treatment groups after ETEC challenge on day 14 (Table 14), suggest that infection in the former group activated the immune system resulting in poor utilization of dietary AA and/or breakdown of muscle protein. In contrast, feeding SDPP or adding either EYA, ZnO, FA or AB to the diets of EWP reduced the severity of the ETEC K88 challenge which in turn might have prevented or reduced the severity of activation of the immune system in these groups resulting in more efficient utilization of dietary protein and AA for growth or body protein deposition.

All piglets appeared healthy and no scours were recorded during week 1. However, 8 h after oral ETEC challenge, PPI-EYA-fed piglets had severe diarrhea with a scour score of 2.4 that lasted for more than 7 d, resulting in 40% mortality. In contrast, all the other treatment groups (SDPP-EYA PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB) had only mild diarrhea and mild scour score and most of the piglets recovered 4 d after oral ETEC challenge (Table 15). Mortality in these treatment groups was only between 6 to 13% (Table 15). Fecal swap samples taken for PCR analysis prior to the oral ETEC challenge indicate that all piglets were free of ETEC-K88. However, as shown in Table 16, between 53 and 87% of all piglets groups gave positive identification of ETEC, 8 h after ETEC challenge. Also, 24 h after the ETEC challenge as much as 100% of PPI-EYA-fed compared with 53% PPI+EYA group continued to shed *E. coli* K88 in the feces. Although most piglets on either SDPP-EYA or PPI-supplemented with EYA, ZnO, FA or AB showed no signs of

Table 15. Effect of spray-dried porcine plasma, egg-yolk antibody, zinc oxide, fumaric acid and antibiotics supplementation on scours score, *E. coli* shedding and mortality of 10-d old weaned pigs

	Dietary treatments [a]						
	SDPP-EYA	PPI-EYA	PPI+EYA	PPI+ZnO	PPI+FA	PPI+AB	SEM
Scours scores[b]							
8 h after *E. coli* challenge	1.9^{xy}	2.4^{x}	1.5^{y}	2.3^{y}	2.1^{y}	1.4^{y}	0.15
24 h after *E. coli* challenge	2.0^{x}	2.7^{y}	1.6^{x}	1.9^{x}	1.9^{x}	1.6^{x}	0.18
48 h after *E. coli* challenge	1.6^{x}	2.7^{y}	1.3^{x}	1.4^{x}	1.3^{x}	1.1^{x}	0.15
7 d after *E. coli* challenge	0.5^{x}	2.2^{y}	0.3^{x}	0.6^{x}	0.5^{x}	0.2^{x}	0.15
PCR (pig shedding *E. coli*, %)							
8 h after *E. coli* challenge	73	80	67	87	80	53	-
24 h after *E. coli* challenge	80	100	53	67	73	67	-
48 h after *E. coli* challenge	53	85	27	62	64	29	-
7 d after *E. coli* challenge	29	81	23	42	31	21	-
Scouring days	4	7	3	4	5	4	-
Mortality (%)	6	40	6	13	6	13	-

[a]E1, SDPP-EYA; E2, PPI-EYA; E3, PPI+EYA; E4, PPI+ZnO; E5, PPI+Fumaric acid (FA); E6, PPI+Antibiotic (AB).
[b]Scour scores; 0, normal; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea.
[xy]Means within a row, lacking a common superscript differ *(P < 0.05)*.
SEM, standard error of the means

scours on d 7 post-challenge, a few of piglets in these treatment groups continued to shed *E. coli* (k88) (Table 16). In contrast, as much as 80% of those fed PPI-EYA continued to shed the *E. coli* strain K88 in the feces. This indicates that *E. coli* (K88) was able to colonize and proliferate in the small intestine of this treatment group. Nagy and Fekete (1999) proposed that colonization of the small intestine by ETEC adhering to the epithelium is responsible for most of the digestive tract disorders seen in EWP.

Visceral organ weights, digesta pH, villous height, crypt depth and villous:crypt ratio for the different treatments are shown in Table 16. Liver weight, small intestinal length and crypt depth were similar *($P > 0.05$)* for all dietary treatments. Piglets fed PPI-EYA diet from d 0 to 14 had higher *($P < 0.05$)* digesta pH (both in the stomach and small intestine) compared to piglets fed SDPP-EYA or PPI-based diets supplemented with EYA, ZnO, FA or AB. The consequences of higher digesta pH and scours have been explained in a number of ways. A rise in gastric pH have been suggested to allow increased proliferation of *E. coli* (Smith and Jones 1963), which is associated with scours and increased mortality (White et al., 1969; Thromlinson and Lawrence, 1981). Fuller (1977) showed that low gastric pH has a bactericidal effect on potentially deleterious bacteria by producing a more favorable environment for *Lactobacilli. Lactobacilli* in the ileal content have been reported to reduce to almost zero within 2 d after weaning, while, coliform population increased significantly and was strongly correlated to increased pH of the ileal content (Mathew et al., 1991).) *Lactobacilli* may inhibit the colonization and proliferation of *E. coli* by blocking possible intestinal *E. coli* receptors (Danielson et

al., 1989), as well as, producing toxic metabolites such as hydrogen peroxide which is reported to have anti-microbial effects (Reiter et al., 1980). Also, higher gastric pH, resulting from inefficient digestion of protein, has been speculated to provide an optimal environment for the ETEC to colonize the surface of the villi, resulting in the initiation of scours in post-weaned piglets (Smith and Jones, 1963). Acidifiers have been shown to be partially effective in the diet of EWP as piglets are not able to maintain the appropriate gastric pH during the transition from sow's milk to dry plant-based proteins (Risley et al., 1992; Roth et al., 1992).

Piglets fed diets containing SDPP-EYA, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB generally had longer jejunal villi and a greater villous height to crypt depth ratio *($P < 0.05$)* compared to those fed PPI-EYA with the difference in villous height being 66, 58, 37, 61 and 60%, respectively. These observations and those previously discussed demonstrate that the integrity of the villous is not maintained and that diarrhea and associated effects develop when PPI was fed without the presence of anti-microbial (anti-*E. coli*) agents such as EYA, Zn, organic acid, antibiotics or SDPP. The addition of these feed additives overcomes the negative effects associated with the feeding PPI-based diet alone. Other researchers have also reported that the feeding of plant based protein is associated with the occurrence of diarrhea, damage of the microvilli and post-weaned lag in growth (Hornich et al., 1973; Cera et al., 1988; Li et al., 1990,1991). In addition, Nunes do

Table 16. Effect of spray-dried porcine plasma, egg-yolk antibody, zinc oxide, fumaric acid and antibiotic supplementation on organ weights and morphology of the gastrointestinal tract of 10-d old weaned pigs

	Dietary Treatments[a]						
	SDPP+EYP	PPI+EYP	PPI+EYA	PPI+ZnO	PPI+FA	PPI+AB	
Parameter							SEM
Small intestine pH	6.2	5.7	6.1	6.7	6.4	6.5	0.17
Stomach pH	2.7^{yz}	3.8^{x}	2.6^{yz}	2.6^{yz}	2.6^{z}	2.9^{y}	0.07
Spleen weight, g/kg BW	2.7^{x}	2.5^{xy}	1.9^{z}	2.7^{x}	2.5^{xy}	2.1^{yz}	0.13
Liver weight, g/kg BW	37	32	32	32	33	31	1.3
Stomach weight, g/kg BW	7.9^{yz}	10.1^{x}	7.3^{yz}	7.1^{z}	9.0^{xy}	7.3^{yz}	0.44
Small intestine weight, g/kg BW	52.6^{x}	49.0^{x}	47.2^{x}	40.1^{y}	52.6^{x}	53.1^{x}	1.6
Small intestine length, g/kg BW	160	147	145	149	148	162	5.9
Villous height (V)[b], μm	590^{x}	355^{z}	564^{x}	488^{y}	573^{x}	570^{x}	20.0
Crypt depth (C)[b], μm	168	204	183	190	207	204	10.1
Villous : Crypt[b]	3.5^{x}	1.7^{z}	3.1^{xy}	2.6^{y}	2.8^{xy}	2.8^{xy}	0.11

[a]E1, SDPP+EYP; E2, PPI+EYP; E3, PPI+EYA; E4, PPI+ZnO; E5, PPI+Fumaric acid (FA); E6, PPI+Antibiotic (AB).
[b]V, height of the villi; C, depth of crypt and V:C ratio of the villous to crypt on the wall of the jejunum.
[xy]Means within a row, lacking a common superscript differ *(P < 0.05)*.
SEM, standard error of the means

Prado et al. (1988) and Le Guen et al. (1991), respectively, reported that feeding raw pea flour to pre-ruminant calves and pigs resulted in formation of antibodies against pea proteins (legumin and vicilin), as a result an increase in gut permeability to these macromolecules. Walker et al., (1975) observed that under normal physiological conditions, the gut-wall prevented passage of dietary antigen from intestinal lumen into the blood circulation. The damage to the microvilli, seen in villous atrophy and crypt hyperplasia has been reported to result in immune response at the intestinal level, resulting in immediate or delayed type hypersensitivity and mucosal inflammation (Li et al., 1990). Although the reaction has been reported to be transient and variable, it can be severe and lead to reduced performance, immune system breakdown, with subsequent increase in the incidence of diarrhea (Lalles et al., 1993). In the current study, with the exception of E2 (PPI+EYA)-fed piglets, piglets fed all other treatments (SDPP-EYA, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB) had longer villi, mild diarrhea and grew faster, suggesting that these group of piglets experienced minimum post-weaning stress.

Anti-microbial agents in swine feed exhibit their effect through either a direct suppressive effect on microbes in the gastrointestinal tract, such as pathogenic *E. coli* or a nutrient-sparing effect (Dierick et al., 1986; Yen et al., 1987). Nutrient-sparing effects, most likely, are the result of an increase in the amount of absorbable nutrients, such as amino acids and carbohydrates, as a result of a reduction in nutrients used by bacteria for growth. The current observations suggest that ZnO, FA, EYA and SDPP all function as specific or general anti-microbial agents and the results are supported by numerous studies in the literature.

The anti-microbial properties of ZnO are well known in human medicinal practice (Sordeberg et al., 1990). ZnO-based ointment and ZnO has been respectively used for dermal application and wound healing (Prasad, 1983). In pigs, high doses of ZnO have been associated with reduced bacterial translocation from the small intestine to the ileal mesenteric lymph node (Huang et al., 1999), and with increased stability and homogeneity in coliform populations (Katouli et al., 1999). The efficacy of using pharmacological doses of ZnO in preventing and/or alleviating diarrhea in young pigs has also been documented in a number of studies (Poulsen, 1995; Kavanagh, 1992). McClain et al. (1988) demonstrated that one of the functional consequences of zinc deficiency in humans is diarrhea and suggested that possibly, zinc may be one factor in the etiology of PWD in pigs. Poulsen (1995) observed that significantly fewer piglets fed 2500 or 4000 ppm ZnO needed medical treatment for diarrhea. Carlson et al. (1999) also reported that both early- and later weaned pigs responded in a beneficial manner to supplementing ZnO from weaning to 14 d post-weaning and that the percentage of piglets that had PWD was significantly influenced by the dietary zinc content. In the current study, supplementing PPI-based diet with 2000 mg/kg ZnO not only reduced the incidence of diarrhea, but also piglets in this treatment group recovered within 4 d of ETEC administration. In contrast, piglets fed PPI-based diet without ZnO continued to shed *E. coli* K88 and had severe diarrhea.

Antibiotics are known to inhibit growth of bacteria and are widely used in weaner diets as means of reducing scours and improving post-weaning pig performance (NRC, 1979). The efficacy of supplementing carbadox on post-weaning

pig performance responses has been established (Yen et al., 1976; Roof and Mahan, 1982). However, the link between performance benefits of antibiotics and an anti-microbial effect have not been convincingly shown. Nevertheless, a number of speculations suggest an indirect involvement of the microflora on the performance of animals as affected by antibiotics (Visek, 1978; Roth-Maier and Kerchgessiner, 1993). Hill et al., (2001) indicated that the addition of anti-bacterial agent (carbadox) improve performance in 15-d old weaned pigs. The results from the current study support the hypothesis that antibiotics inhibit ETEC (K88).

Numerous studies have shown that the use of organic acids may reduce coliform burden along the gastrointestinal tract (White et al., 1969; Thomlinson and Lawrence, 1981; Bolduan et al., 1988a,b; Mathew et al., 1991) and reduce scouring and piglets mortality. In the current study, adding FA to PPI-based diet reduced incidence and severity of diarrhea compared to feeding PPI-EYA. Kershaw et al. (1966) reported that 1 % lactic acid addition to the drinking water improved growth rate and feed efficiency, and reduced the *E. coli* count in the duodenum and jejunum of pigs. Subsequently, Thromlinson and Lawrence (1981) demonstrated that the multiplication of *E. coli* 0141:K85 was reduced by acidification with a corresponding reduction in piglets mortality. Recently, Callessen et al. (1999) also observed a considerable decrease in frequency of diarrhea in piglets fed organic acid compared to that found in piglets fed control diet.

SDPP has been used widely in the diet of EWP as it has been shown to greatly improve weight gains (10 to 50%) as a result of increased feed intake (Coffey and Cromwell 1995; De Rodas et al., 1995; Jiang et al., 2000). It has also been

shown that the feeding of SDPP reduces incidence and severity of diarrhea in young pigs (Van der Peet-Schwering and Binnendijk, 1995; Owusu-Asiedu et al., 2000) and that its effects are more darmatic in low health as compared to high health herds (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995). It has been proposed that the immunoglobulins and complex protein fractions present in SDPP provide anti-microbial protection (Coffey and Cromwell, 1995; Godfredson-Kisc et al., 1997; Jiang et al., 2000) and might also influence intestinal immune status in the transition to weaning (Jiang et al., 2000). The latter might protect against the development of mucosal damage by enteric pathogens thereby restricting passage of inert large molecules through the intestinal wall (Van Dijk et al., 2001). Blood plasma immunoglobulins, by preventing bacterial damage of the intestinal gut wall help maintain optimal intestinal function and gastrointestinal growth which, in turn, benefits piglet health and performance (De Rodas et al., 1995; Gomez, et al., 1998).

Chicken EYA has successfully been used as a therapy to control rotavirus infection in mice (Bartz et al., 1980; Yolken et al., 1988), diarrhea caused by *E. coli* K88, K99, 987P in neonatal (Ikemori et al., 1992; Yokoyama et al., 1992; Jaradat, 1999) and weaned piglets (Zuniga et al., 1997; Marquardt et al., 1999). Ikemori et al. (1992) observed severe diarrhea resulting in 100% mortality in the control group, compared to no diarrhea and a survival rate of 100% in all calves challenged with K99 and treated with antibodies raised against *E. coli* K99 fimbriae. In another study, Zuniga et al. (1997) used EYA to control the intestinal colonization by *E. coli* F18 in weaned piglets. These authors observed full protection of piglets against this strain of *E. coli*, in a dose response manner. Recently, Jin et al. (1998) also demonstrated

that purified antibodies from the yolk of the chicken immunized against the fimbriae of *E. coli* (K88) were able to block the binding of ETEC K88 to the mucosal receptor and that the interaction of the antibodies with this strain of ETEC was fairly rapid as maximum protection was provided within 15 min. In the current study, piglets fed PPI+EYA-based diet recovered 3 d after oral ETEC challenge, indicating that the presence of the specific anti-ETEC antibody bind the *E. coli*, thus preventing colonization and proliferation, resulting in subsequent removal of the *E. coli* by the peristalsis action of the gastrointestinal tract.

Overall, the current observations support the earlier finding that feeding SDPP (Gatnau and Zimmerman, 1990; van der Peet-Schwering and Binnendijk, 1995), EYA (Yokoyama et al., 1992; Marquardt et al., 1999, 2001; Owusu-Asiedu et al., 2001), antiboitics (NRC, 1979), organic acid (van Kol, 2001) and ZnO (Carlson et al., 1999; Hill et al., 2001) from d 0 to 14 after weaning reduces the incidence of diarrhea and the number of piglets requiring treatment against gastrointestinal tract infections, thereby reducing post-weaning lag in growth. The current results further showed that fewer piglets in the groups receiving SDPP, EYA, ZnO, FA or AB excreted *E. coli* and that the excretion decreased with duration of the experiment. The result also demonstrated that antibodies prepared from the yolk of eggs from laying hens immunized with fimbrial antigens of *E. coli*, as well as, ZnO, FA, AB and SDPP are protective in piglets challenged with homologous ETEC strain K88. The severity of diarrhea and incidence of mortality was reduced in all cases by EYA, ZnO, FA, AB or feeding SDPP. It must also be mentioned that, these feed additives were more effective in a diseased condition (wk 2) compared to wk 1. Also adding

these additives probably improved the overall immunocompetence of weaned piglets by preventing colonization and proliferation of *E. coli*, as well as secretion of enterotoxins as suggested by Coffey and Cromwell (1995). This may explain the transient and mild scours observed in all treatment groups, except for PPI-EYA in the current study. By preventing colonization and proliferation of bacteria and subsequent damage of the intestinal wall, these supplements helped maintain optimal intestinal function and maturation of the GIT. This in turn, prevented villi atrophy with the corresponding improvement in piglets health and performance (De Rodas et al., 1995; Gomez et al., 1998).

IMPLICATIONS

Carbadox, EYA, ZnO, FA or SDPP can be used to control PWD and to prevent post-weaning lag in performance in early-weaned piglets. The positive effects of these additives appear to be more significant during infectious conditions. EYA may be a good alternative to the other treatments, as it will allow the use of less expensive feedstuffs such as PPI in diets for early-weaned pigs and may address the current regulatory, health and environmental concerns regarding the use of SDPP, antibiotics and minerals salts.

CHAPTER SIX

GENERAL DISCUSSION

Early-weaned pigs (EWP) are subjected to a combination of factors that increase their susceptibility to post-weaning diarrhea and depressed growth. Key among these factors include emotional and environmental stresses (Puppe et al., 1997), loss of maternal passive immunity (Deprez et al., 1986) and nutritional changes such as changing from highly digestible sow's milk to a poorly digestible dry diet containing complex carbohydrates and protein (Leece et al., 1983; Li et al., 1990; Miller et al., 1994). These factors can act in combination to increase the susceptibility of the EWP to enteric infection as caused by enterotoxigenic *E. coli* (ETEC) strains K88 and F18. This could affect the integrity of the gastrointestinal mucosa a reduction in feed intake and therefore rate of growth (Leece et al., 1983; Li et al., 1990; Miller et al., 1994). A major challenge currently facing the swine industry is to identify means for controlling diarrhea in young pigs that are not only cost-effective but also suitable for sustainable pork production. In the present research, attempts were made to verify whether the superior feeding value of SDPP compared plant based proteins was the due to the presence of specific anti-ETEC antibodies and also if SDPP can be replaced with a high quality protein (PPI) supplemented with EYA. Also, the ability of various anti-microbial growth promoting agents to control ETEC infection in 10-d old weaned piglets were invetsigated.

In manuscript 1, the objective of the study was to established whether the highly beneficial effects of using SDPP in the diet of EWP was attributable not only to its content of highly digestible and high quality protein but also to the presence of immunoglobulins that are able to neutralize the pathogenic effects of common intestinal microorganisms such as ETEC strains K88 and F18. It was hypothesized that SDPP would be superior to SDAP since the ETEC antibody profile of the two plasma preparations would be different, with SDPP being a reflection of ETEC that infect pigs (mainly ETEC strain K88 and F18) while those from SDAP (mainly from ruminants) would be high in anti-ETEC strain K99 antibodies, as ETEC-K99 commonly infects ruminant calves. SDPP should therefore be superior to SDAP for the control of the principal ETEC strains that infect young pigs. It was also hypothesized that the antibodies present in SDPP could be inactivated by heat treatment (autoclaving) and that specific antibodies from the egg of appropriately hyper-immunized hens could produce similar results to those obtained with non-heat treated SDPP, provided heat destruction of lysine and other AA in the plasma were replaced. The role of specific immunoglobulins as growth promoting agents in plasma protein and its replacement by supplementing a diet containing a high quality protein devoid of immunoglobulins with specific egg-yolk immunoglobulins was examined.

Spray-dried porcine plasma contained mainly anti-K88 and F18 antibodies while SDAP contained mainly K99 antibodies. Antibodies from specifically immunized hens had much higher titers (approximately 30-fold) of anti-K88 and anti-F18 antibodies. Heat treatment of SDPP resulted in a complete loss of

activity of all antibodies and a decrease in lysine availability. In these studies, it was observed that piglets fed diets devoid of specific anti-ETEC antibodies performed poorer in terms of feed intake and weight gain, had higher incidence of scours and had higher PUN concentartion compared to piglets fed anti-ETEC antibodies containing diets. The feeding studies suggested that the type and amount of specific antibodies present in animal plasma products (SDPP vs. SDAP) may affect piglets performance with SDPP being superior to SDAP and that the benefit of these antibodies can probably be met by adding other sources, such as those from egg-yolk. These observations are supported by a number of studies in the literature (Gatnau and Zimmerman, 1990; Hansen et al., 1993; Kats et al., 1994; Van der Peet-Schwering and Binnendijk, 1997). Immune protection (Gatnau et al., 1991) and prevention of pathogenic bacteria adhesion to the gastrointestinal mucosa (Nollet et al., 1999) are among the different modes of action proposed for SDPP. These two modes of action are used to explain the effect of EYA and also suggest that EYA and SDPP may be an alternative to anti-microbial growth promoting agents in EWP. This is further supported by the observations that higher responses are obtained in a high disease environment (Gatnau and Zimmerman, 1990; Coffey and Cromwell, 1995; Bergstrom et al., 1997). The lower PUN concentration in the anti-ETEC antibodies fed piglets could be explained by lower activation of the immune system in this group compared to piglets group fed diets containing no specific antibodies. This would result in less use of nutrients for aid immune response and also prevent muscle protein breakdown to synthesize acute phase proteins (Wannemacher, 1977).

In manuscript 2 and 3, the objectives were respectively, to evaluate the use of EYA-supplemented pea protein isolate as an alternative to SDPP in diets for EWP and to assess the effectiveness of EYA in controlling ETEC relative to conventional additives in EWP fed PPI-based diets. The results demonstrated that the ADG of EWP fed PPI-based diets supplemented with either EYA, SDPP, ZnO, FA or AB were greatly superior to those of piglets fed unsupplemented PPI-containing diet. The improvements in ADG as a results of feeding EYA, SDPP, ZnO, FA or AB observed in the current study with the different treatments are in close agreement with those reported by other researchers using SDPP (Gatnau and Zimmerman, 1990; Coffey and Cromwell, 1995), EYA (Kim et al., 1999; Marquardt et al., 1999), ZnO (Poulsen, 1995; Mavromichalis et al., 2000), organic acids (Paulick et al., 1996; van Kol, 2000), and antibiotics (Roof and Mahan, 1982; Hill et al., 2001). The results also suggest that the improvements obtained by the addition of either specific EYA, ZnO, FA or AB to the PPI diets was direct effect as these supplements were added in small amounts (less 0.5%) and, therefore, did not appreciably affect the content of other nutrients. Also ADFI and ADG but not efficiency of feed utilization increased when PPI-based diet was supplemented with the different anti-microbial agents.

All piglets appeared healthy and no scours were recorded before ETEC challenge, however, after oral ETEC challenge, piglets fed unsupplemented PPI-containing diet had severe diarrhea with a scour score of 2.4 that lasted for more than 7 d, resulting in 40% mortality. In contrast, all the other treatment groups (SDPP+EYP, PPI+SDPP, PPI+EYA, PPI+ZnO, PPI+FA and PPI+AB) had only mild diarrhea and mild scour score and most of the piglets recovered 4 d after oral ETEC

challenge. Mortality was also significantly lower ($P<0.05$) in these treatment groups. Fecal swap samples taken for PCR analysis prior to the oral ETEC challenge shoewd that all piglets were free of ETEC. However, all groups gave positive identification of ETEC, 6 to 8 h after ETEC challenge. Also, 24 h after the ETEC challenge, as much as 100% of the group fed PPI+EYP diet compared with 53.3% PPI+EYA group continued to shed *E. coli* K88 in the feces. Although a considerable number (42% less) of piglets fed either SDPP alone or PPI supplemented with EYA, ZnO, FA or AB based diets continued to shed *E. coli* (K88) they showed no signs of scours on d 7 post-challenge. In contrast, as much as 80% of piglets fed PPI alone continued to shed the *E. coli* strain K88 in their feces.

Piglets fed PPI+EYP based diet from d 0 to 14 had higher digesta pH (both in the stomach and small intestine) and PUN levels compared to piglets fed SDPP or PPI-based diets supplemented with EYA, ZnO, FA or AB. The effect of higher stomach and digesta pH and the subsequent incidence of scours have been explained in a number of different ways. A rise in gastric pH has been suggested to allow increased proliferation of *E. coli* (Smith and Jones 1963), which is associated with scours and increased mortality (White et al., 1969; Thromlinson and Lawrence, 1981). Fuller (1977) showed that low gastric pH has a bactericidal effect on potentially deleterious bacteria by producing a more favorable environment for *Lactobacilli.* Mathew et al. (1991) demonstrated that *Lactobacilli* in the ileal content were reduced to almost zero within 2 d after weaning. In contrast, coliform population increased significantly and was strongly correlated to increased pH of the ileal content. *Lactobacilli* may inhibit the colonization and proliferation of *E. coli* by

blocking possible intestinal *E. coli* receptors (Danielson et al., 1989), as well as producing toxic metabolites such as hydrogen peroxide which is reported to have anti-microbial effects (Reiter et al., 1980). Also, higher gastric pH, resulting from inefficient digestion of protein, has been speculated to provide an optimal environment for ETEC to colonize the surface of the villi, resulting in the initiation of scours in post-weaned piglets (Smith and Jones, 1963). This indicates that *E. coli* (K88) was able to colonize and proliferate the small intestine the group fed PPI alone-based diet and that the higher gastric pH might have aided this observation. Nagy and Fekete (1999) proposed that colonization of the small intestine by ETEC adhering to the epithelium is responsible for most of the digestive tract disorders seen in EWP.

During infection or inflammation feed intake is greatly reduced due to over production of cytokines, resulting in redistribution of nutrients away from growth processes in aid of the immune system. According to Wannemacher (1977), AA are liberated from muscle breakdown and are used to synthesis acute phase proteins in the liver, and as an energy source, in such instances. Furthermore, efficiency of protein utilization during inflammation response has been demonstrated to decrease in a 21-d old weaned pig injected with lipopolysaccharide (van Heugten et al., 1994). In the current study, PUN levels were higher in piglets fed diets devoid of antibodies or the other feed additives after oral ETEC challenge. It can therefore be speculated that the higher PUN in these treatment groups after ETEC challenge, is an indication that infection in this treatment group activated the immune system resulting in poor

utilization of dietary AA or breakdown of muscle protein. Clearly, this was not the case when diets containing SDPP, EYA, ZnO, FA or AB were fed.

In addition, compared to feeding SDPP, or PPI supplemented with EYA, ZnO, FA and AB, piglets fed unsupplemented PPI-containing diet had significantly shorter villi and villous height to crypt depth ratio. This observation is in close agreement with other studies and has been attributed to the presence of dietary antigens (Cera et al., 1988; Li et al., 1991; Dreau et al., 1994; Makinde et al., 1996). The damage to the microvilli, seen in villous atrophy and crypt hyperplasia has been reported to result in immune response at the intestinal level, resulting in immediate or delayed type hypersensitivity and mucosal inflammation (Li et al., 1990). Nunes do Prado et al. (1988) and Le Guen et al. (1991), respectively, reported that feeding raw pea flour to pre-ruminant calves and pigs resulted in formation of antibodies against pea proteins (legumin and vicilin), with an increase in gut permeability to macromolecules. In an earlier study, Walker et al., (1975) demonstrated that under normal physiological conditions, the gut-wall prevented passage of dietary antigen from intestinal lumen into the blood circulation. Thus in the current study, feeding SDPP or supplementing PPI with EYA, ZnO, FA or AB preserved the integrity of the gastrointestinal tract.

The only apparent differences between the various treatments were the content of anti-K88, anti-F18 antibodies, ZnO, FA, AB. Therefore, it should be possible to replace SDPP in the diet with a high quality plant-protein supplement such as PPI and a source of anti-K88 and possibly anti-F18 antibodies, as well as either ZnO, FA or AB. This effect may be attributed to the ability of the

antibodies and these feed additives to prevent intestinal infection by pathogens such as *E. coli*. This, in turn, will not only protect the integrity of the absorptive surface of the gastrointestinal tract, but also the overall health of the animal. Furthermore, the antigenic effect of PPI or that of any foreign protein may be a consequence of prior inflammation of the intestinal membrane, which in turn would permit the malabsorption of intact or partially hydrolyzed protein resulting in a subsequent immune and associated allergic response. Presumably this malabsorption of protein would not occur in piglets with an undamaged gastrointestinal tract mucosa.

The current studies suggest that feeding early-weaned pigs diets with SDPP, PPI plus SDPP, EYA, ZnO, FA or AB during d 0 to 14 post-weaning prevents enteric disorders associated with *E. coli* infection and allows weanling pigs to utilize plant proteins such as PPI. Considering the numerous problems and concerns associated with the use of these anti-microbial growth promoters (ZnO, FA, SDPP and AB), EYA appears to be an excellent therapeutic and prophylactic agent in control of PWD in piglets, and for the improvement of piglet performance after weaning. It also offers several advantages over other alternatives as large amounts of specific antibodies can be inexpensively produced, the product is environmentally friendly, is safe to use as it contains no toxic residues and more importantly is compatible with the current regulation of animal production.

CHAPTER SEVEN

CONCLUSIONS

Based on these studies it can be concluded that:

1. SDPP and SDAP have very different concentrations of different anti-*E. coli* (K88, K99, F18, 987P, F41) antibodies and therefore would target different types of *E. coli*.
2. Specific anti-K88 and -F18 antibody titers of SDPP and SDAP are considerably lower than those obtained from eggs of hens immunized against specific fimbrial antigens (K88 and F18).
3. The type and amount of antibodies present in animal plasma products (SDPP vs. SDAP) improved (7-10%) post-weaned piglets growth performance. The benefit of these antibodies can be obtained from other sources, such as those present in egg-yolk from hens immunized with the appropriate antigen(s).
4. The results also demonstrated that a diet containing both PPI as a source of high quality protein and egg-yolk from chickens that had been immunized against ETEC dramatically improved the performance of early-weaned piglets compared to that obtained with a diet that contains PPI plus egg-yolk powder that does not contain the specific anti-ETEC antibodies.
5. The results suggest that the use of SDPP, partially or completely replaced by EYA and other protein rich feedstuffs such as PPI. Such a product would be less expensive, have a known concentration of specific antibodies and

possibly would encounter less concern from regulatory agents regarding potential transmission of mammalian diseases.

6. Piglets fed diets containing PPI plus SDPP, EYA, ZnO, FA or AB yielded performance values greatly superior in terms growth rate, feed intake and reduced incidence of scours and mortality than those fed diets containing unspplemented PPI.
7. Supplementing PPI-based diets with SDPP, EYA, ZnO, FA or AB yielded similar results in terms of feed intake, growth rate and reduced incidence of scours.
8. Piglets fed diets containing PPI plus SDPP, EYA, ZnO, FA or AB had reduced PUN concentration (13-15%) and greater villi height (37-66%) than those fed unsupplemented PPI-containing diets.
9. A significantly fewer number (21-42%) of piglets fed diets containing PPI plus SDPP, EYA, ZnO, FA or AB shedded *E. coli* K88 and had a considerably lower incidence of scours compared with those fed unsupplemented PPI-containing diet (81%) at the end of the 14-d experimental period.
10. The results demonstrated that piglets can be passively protected against enterotoxigenic *E. coli* , an organism that can cause severe diarrhea and death, by using SDPP, ZnO, FA, AB or a homologous antibody from the EYA of a laying hen.
11. The studies further support the hypothesis that growth depression in weaned pig is related to the presence of certain intestinal pathogens especially the ETEC including those that express the K88 (F4) and F18 fimbrial antigens.

Recommendations:

1. Studies should be conducted out to test the activity and/or stability of EYA as it passes through the gastrointestinal tract of piglets of different ages. This will assist in establishing dose that should be used and the need to encapsulate it.
2. Studies should be carried to determine effect of heat treatment on stability of EYA and means to enhance it stability when heated. This is important when high temperature pelleting is used.
3. Determine if there is a synergistic interaction among the above-indicated treatments. These studies are important for comparative efficacy of different treatments and secondly interaction among treatments may produce synergistic or antagonistic effects. Synergistic interaction would increase efficacy of treatments and reduce costs.
4. Conduct dose response trial to establish relationship between level of *E. coli* challenge and specific antibody titer required to neutralize these challenges under different conditions. This is important, as cost of treatment is directly proportional to the antibody inclusion level.
5. Additional research should be carried out to verify the relationship between the integrity of the gastrointestinal tract and its effect on the digestibility of nutrients.
6. Future research should look at the effect of ETEC infection on absorption and transport of nutrients and intact proteins. The relationship between *E. coli* infection, destruction of villi, absorption of intact proteins, especially plant

proteins and the development of a systemic immune response to specific proteins should be established, as this would provide valuable information on digestive function in the gastrointestinal tract.

7. Conduct other areas of research and development including the use of EYA to control *E. coli 0157H7,* human pathogen, *Salmonella spp*, *Campylobacter*, etc.

CHAPTER EIGHT

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