THE INFLUENCE OF IMMUNOGLOBULIN-CONTAINING DIETARY PROTEIN ON ASPECTS OF GROWTH PERFORMANCE AND GASTROINTESTINAL IMMUNITY IN WEANER PIGS.

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CANDIDATE'S DECLARATION

This is to certify that the research carried out for my Doctoral thesis entitled "The Influence of Immunoglobulin-Containing Dietary Protein on Aspects of Growth Performance and Gastrointestinal Immunity in Weaner Pigs" in the Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand is my own work and that the thesis material has not been used in part or in whole for any other qualification.

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CERTIFICATE OF REGULATORY COMPLIANCE

This is to certify that the research carried out in the Doctoral Thesis entitled: "The Influence of Immunoglobulin-Containing Dietary Protein on Aspects of Growth Performance and Gastrointestinal Immunity in Weaner Pigs" in the Institute of Food, Nutrition and Human Health at Massey University, Palmerston North, New Zealand:

(a) is the original work of the candidate, except as indicated by appropriate attribution in the text and/or in the acknowledgements;

(b) that the text, excluding appendices/annexes, does not exceed 100,000 words;

(c) all the ethical requirements applicable to this study have been complied with as required by Massey University, other organisations and/or committees which had a particular association with this study, and relevant legislation.

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SUPERVISOR'S DECLARATION

This is to certify that the research carried out for the Doctoral thesis entitled "The Influence of Immunoglobulin-Containing Dietary Protein on Aspects of Growth Performance and Gastrointestinal Immunity in Weaner Pigs" in the Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand. The thesis material has not been used in part or in whole for any other qualification, and I confirm that the candidate has pursued the course of study in accordance with the requirements of the Massey University regulations.

Supervisor's Name: Dr. Patrick C.H. Morel

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"Another damned, thick, square, book! Always scribble, scribble, scribble!
Eh, Mr. Gibbon?"
(William Henry, Duke of Gloucester, upon receiving the second volume of Decline and Fall of the Roman Empire from the author, 1781)

ABSTRACT

The main objective of this study is to investigate and compare the effects of spray-dried plasma and bovine colostrum on the intestinal health and growth performance of weaner pigs, providing more information on their possible mechanisms of action and determining if these mechanisms are common to both products. A secondary objective was to evaluate the potential benefits of dietary spray-dried plasma and colostrum for broiler chickens. In general, the study tested the hypothesis that dietary spray-dried plasma and colostrum act via a common mechanism to improve intestinal morphology and immunity in pigs.

In Chapter 1, the effect of weaning on the intestinal immune system of the pig was reviewed. This included an introduction to immunology in the pig; discussion of the detrimental effects of weaning on pig performance, the morphology of the small intestine and the intestinal immune system; and an exploration of the two main hypotheses that attempt to account for the deleterious effects of weaning: (1) lack of luminal nutrition, and (2) hypersensitivity to dietary soy proteins. It is suggested that the primary factor influencing the status of the gastrointestinal immune system is the degree of luminal nutrition, and that hypersensitivity to soy proteins is a secondary factor.

In Chapter 2, the current literature on spray-dried plasma and colostrum was reviewed. This included: an overview of the compositions of both products; their effects on feed intake, growth rate and feed conversion ratio in pigs after weaning; a discussion of their possible active components; and a discussion of hypotheses accounting for their mechanisms of action, including: (1) the stimulation of feed intake, (2) the provision of passive immunological protection in the intestine, and (3) beneficial effects due to hormones and growth factors contained in both products. It is suggested that a passive immunoprotective effect of bovine plasma and colostrum can account for the majority of the observed effects of dietary spray-dried plasma and colostrum.

Chapter 3 investigated the effect of two diets (containing either 0 (control) or 5% spray-dried bovine colostrum) on indices of growth performance, intestinal morphology and intestinal immunity in early-weaned pigs from 14-28 days of age. No dietary effect on pig growth or feed intake was observed. Consumption of the bovine colostrum diet increased villus height, reduced crypt depth, and increased the ratio of villus height to crypt depth in all areas of the

small intestine, compared to pigs offered the control diet. Consumption of the bovine colostrum diet also decreased small intestine weight and increased the density of mid jejunal lamina propria $CD4^+$ and $CD8^+$ T lymphocytes. These results demonstrate positive effects of dietary bovine colostrum on small intestine morphology, and suggest that consumption of colostrum may have provided passive immune protection in the small intestine. These data also show that the consumption of bovine colostrum caused expansion of T lymphocyte subsets, which may be due to the induction of oral tolerance to novel proteins within colostrum, accompanied by a secretory immune response.

Chapter 4 examined the effects of 7.5% dietary inclusion of bovine colostrum, bovine plasma and porcine plasma on performance and intestinal health of weaner pigs from 21-28 days of age. These were compared to observations taken from pigs at the point of weaning (21 days of age) and pigs consuming a standard weaning diet from 21-28 days of age (control). Pigs killed at weaning had larger stomach weights, longer villi, shallower crypts, greater ratio of villus height to crypt depth, reduced epithelial cell height, and an altered distribution of intestinal goblet cells, compared to all pigs killed a week after weaning. Pigs killed at weaning also displayed reduced density of lamina propria $CD4^+$ and $CD8^+$ T lymphocytes compared to pigs killed one week after weaning, with the exception of those consuming the bovine plasma diet, which were similar. No dietary effect on feed intake or growth rate was demonstrated. No consistent effect of the test proteins on intestinal morphology or lymphocyte subsets was observed, although some positive effects were demonstrated in more distal areas of the small intestine. Crypt goblet cell density was consistently increased in the proximal and mid-jejunum of the intestine by consumption of diets containing the test proteins. Generally positive relationships were observed between villus height and both average daily feed intake and average daily gain, regardless of dietary treatment. These results demonstrate the deleterious effects of weaning on intestinal morphology and expansion of subsets of the intestinal immune system in the first 7 days after weaning. They also support a link between the level of voluntary feed intake and some indices of intestinal inflammation. The results suggest that dietary colostrum and plasma may have beneficial effects in various areas of the intestine possibly due to the provision of passive immune protection and the induction of crypt goblet cell expression, which may be linked to the induction of oral tolerance to novel dietary proteins.

Chapter 5 evaluated the effects of 7.5% dietary bovine colostrum and bovine plasma on indices of intestinal and humoral immunity in enteropathogenic *E. coli* challenged weaner pigs (21 days of age), compared to unchallenged and challenged pigs consuming a standard weaning diet (control). Pigs were acclimatised to the diets for 12 days prior to oral administration of 10^9 colony-forming units of *E. coli* O149:K88. Diets continued to be offered for 7 days after administration of the challenge, whereupon all pigs were killed and measurements taken. No dietary nor challenge effects on faecal *E. coli* count, rectal temperature, feed intake, growth rate and humoral immune status were observed. Consumption of bovine plasma increased large intestine weight compared to any other group. Post-weaning diarrhoea was present in all challenged groups, although the diarrhoea score of pigs consuming the control diet was significantly higher than unchallenged pigs, whereas the diarrhoea score of pigs consuming the bovine plasma and colostrum diets was not different from unchallenged pigs. The challenge induced some mild signs of intestinal inflammation in pigs consuming the control diet, whereas greater signs of intestinal inflammation were present in pigs consuming the bovine plasma and colostrum diets. These results suggest that pigs consuming bovine plasma and colostrum exhibit immunological hyper-responsiveness, which is indicative of a lack of immunological "priming" by exposure to antigens. This supports the hypothesis that plasma and colostrum products provide passive immune protection in the intestine, increasing the immunological "naïveté" of animals. However the results also show that this may increase the injurious effects of subsequent major immune challenges.

Chapter 6 tested the effects of dietary bovine plasma and bovine colostrum on the performance of pigs in a commercial production situation. Weaner pigs (28 days of age) were offered either a standard weaning diet (control), or a diet containing 6% bovine plasma or colostrum, for 7 days after weaning. After this time, all pigs were offered the same sequence of diets until they reached market weight (85kg live-weight), during which time growth rate was monitored. Offering the bovine colostrum and plasma diets increased voluntary feed intake in the week after weaning, compared to pigs offered the control diet. Numerical improvements in growth rate were also observed in pigs offered the bovine colostrum and plasma diets. Positive effects of dietary bovine plasma and colostrum on growth rate were greatest for pigs that were lighter at weaning. From 42-70 days of age, growth rate of pigs offered the bovine colostrum and plasma diets in the week after weaning was lower than that of control pigs, but diet offered in the week after weaning had no effect

on any other performance parameters, nor the length of time taken to reach market weight. These results demonstrate beneficial effects of both dietary bovine colostrum and plasma on the performance of pigs immediately after weaning, but suggest that these products are of greatest benefit for lighter pigs at weaning. Given the similar response of pigs to dietary inclusion of plasma and colostrum, and their similar immunoglobulin composition, it is suggested that they may share a common mode of action.

Chapter 7 evaluated the effect of dietary inclusion of the test proteins bovine colostrum, bovine plasma or porcine plasma, on the growth performance and intestinal morphology of broiler chickens. Four diets, consisting of a standard control broiler diet, and diets containing 5% of the test protein were offered from 1-14 days of age, thereafter and a common broiler diet was offered until 35 days of age. Intestinal morphology was measured at 14 days of age in a cohort of birds offered each of the diets. No consistent effect of the test proteins on intestinal villus height or goblet cell density was observed, although birds consuming the porcine plasma diet displayed taller villi compared to those consuming the control diet. A common effect of the test proteins was to increase intestinal crypt depth relative to birds consuming the control diet, suggesting an increase in epithelial cell mitosis due to consumption of these products. No dietary effects on feed intake and growth rate were observed, but consumption of diets containing the test proteins improved feed conversion ratio from day 1-14 compared to birds consuming the control diet. These results suggest that plasma and colostrum may be useful dietary ingredients for the broiler industry, which may have beneficial effects during the period of their consumption.

It is concluded that bovine colostrum may be a suitable alternative to plasma products, and moreover, that these products are potentially of benefit to the poultry industry. It is concluded that the results presented in this thesis generally support the hypothesis that the beneficial effects of dietary plasma are due to the provision of passive immune protection in the intestinal lumen. They also support the use of this hypothesis to account for the beneficial effects of dietary bovine colostrum. However, these results suggest that other mechanisms may also be involved, such as antigenic stimulation of the intestinal immune system by novel proteins present in colostrum and plasma products, which may stimulate goblet cell differentiation and potentially a secretory immune response, both of which may improve immune protection in the small intestine.

PUBLICATIONS

Studies completed during candidature, some of which are reported in this thesis have been presented in the following publications:

King, M.R., D. Kelly, P.C.H. Morel and J.R. Pluske, 2003. Aspects of intestinal immunity in the pig around weaning. In: Pluske, J.R., M.W.A. Verstegen and J. Le Dividich (editors), Weaning the Pig: Concepts and Consequences. Wageningen Academic Publishers, The Netherlands, pp. 219-257. (Chapter 1)

King, M.R., 2002. Ethics in the education of animal-based scientists – a postgraduate student perspective. Proceedings of The Physiological Society of New Zealand, Palmerston North, N.Z.

King, M.R., J.R. Pluske, E.A.C. James, M.J. Birtles and P.C.H. Morel, 2002. Dietary colostrum alters pig intestinal morphology. Proceedings of the Nutrition Society of New Zealand, pp. 36. (Chapter 2)

King, M.R., J.R. Pluske, W.H. Hendriks and P.C.H. Morel, 2002. Immunoglobulin-fortified protein for piglets. Massey University Technical Update Seminar "Advancing Pork Production", Palmerston North, N.Z., pp. 48-52. (Chapters 3, 4, 5 and 6)

King, M.R., W.H. Hendriks and P.C.H. Morel, 2002. Increasing weaner health and productivity in New Zealand pig herds using spray-dried bovine plasma. Research Report for the New Zealand Pork Industry Board. (Chapters 2, 5 and 6)

King, M.R., P.C.H. Morel, D.V. Thomas, B.J. Camden and V. Ravindran, 2002. Evaluation of immunoglobulin-fortified protein sources in broiler diets. Proceedings of the Australian Poultry Science Symposium No 14, pp. 182. (Chapter 7)

King, M.R., P.C.H. Morel, E.A.C. James, W.H. Hendriks, J.R. Pluske, R. Skilton, and G. Skilton, 2001. Inclusion of colostrum powder and bovine plasma in starter diets increases voluntary feed intake. In: Cranwell, P.D. (editor), Manipulating Pig Production IIX. Australasian Pig Science Association, Werribee, Australia, pp. 213. (Chapter 6)

King, M.R., P.C.H. Morel, B.J. Camden, D.V. Thomas and V. Ravindran, 2001. Evaluation of immunoglobulin-fortified protein sources in starter diets for broiler chickens. Massey University Technical Update Seminar "Advancing Poultry Production", Palmerston North, N.Z. (Chapter 7)

Johnson, M.J. and M.R. King, 2001. Effects of dry sow stall use for a limited period after mating: A literature review for the New Zealand Pork Industry. Research Report for the New Zealand Pork Industry Board.

Hendriks, W.H. and M.R. King, 2001. A review of the literature on boar taint for New Zealand PORK. Research Report for the New Zealand Pork Industry Board.

King, M.R., P.C.H. Morel, D.K. Revell, E.A.C. James, M.J. Birtles and J.R. Pluske, 1999. Bovine colostrum supplementation increases villous height in sucking piglets. In: Cranwell, P.D. (editor), Manipulating Pig Production VII. Australasian Pig Science Association, Werribee, Australia, pp. 255.

Pluske, J.R., H.R. Gaskins, P.C.H. Morel, D.K. Revell, M.R. King and E.A.C. James, 1999. The number of villus and crypt $CD4^+$ T cells in the jejunum of pigs increases after weaning. In: Cranwell, P.D. (editor), Manipulating Pig Production VII. Australasian Pig Science Association, Werribee, Australia, pp. 244.

Pluske, J.R., G. Pearson, P.C.H. Morel, M.R. King, G. Skilton and R. Skilton, 1999. A bovine colostrum product in a weaner diet increases growth and reduces day to slaughter. In: Cranwell, P.D. (editor), Manipulating Pig Production VII. Australasian Pig Science Association, Werribee, Australia, pp. 256.

King, M.R., P.C.H. Morel, D.K. Revell, E.A.C. James, M.J. Birtles and J.R Pluske, 1999. Improved gut morphology does not reduce the effect of weaning on villous height. Proceedings of the 2nd Southwest Pacific Nutrition & Dietetic Conference, Auckland, N.Z., pp. 153.

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TABLE OF CONTENTS

GENERAL INTRODUCTION

In pig production, weaning occurs at a unnaturally young age and is therefore accompanied by numerous problems, many of which are related to the developmental immaturity of the animal at weaning. Examples of this include a reduction in voluntary feed intake and the occurrence of intestinal inflammation and post-weaning diarrhoea. The net effect of these problems is poor health and growth performance of the pig in the immediate post-weaning period.

One approach for improving the response of pigs to weaning is to include, in weaning diets, products that may enhance feed intake and health over the weaning period. A product that has demonstrated potential to perform these functions is spray-dried plasma. The plasma is derived by centrifugation from the blood of animals at slaughter, and is spray-dried to produce a powder that may be included as a protein source in the grain-based diets commonly fed to pigs. Evidence suggests that the beneficial effects of spray-dried plasma are due to the fact that it contains immunoglobulins.

Another product that has demonstrated potential to improve the performance of pigs over the weaning period is spray-dried bovine colostrum. This is harvested from cows immediately after parturition, and spray-dried to produce a powder that may be included as a protein source in weaning diets in the same way as spray-dried plasma. It also contains a similar immunoglobulin composition to spray-dried plasma, which suggests that they may benefit the weaner pig through a similar mechanism of action.

The main objective of this study is to investigate and compare the effects of spray-dried plasma and bovine colostrum on the intestinal health and growth performance of weaner pigs. This may help to elucidate their mechanisms of action, and determine any potential commonalities between the two products in this regard. The potential for these products to be of benefit to broiler chicken immediately after hatching will also be evaluated.

SECTION I

General Literature Review

Chapter 1

ASPECTS OF INTESTINAL IMMUNITY IN THE PIG AROUND WEANING

M.R. King[1], D. Kelly[2], P.C.H. Morel[1] and J.R. Pluske[3], 2003. Aspects of intestinal immunity in the pig around weaning. In: Pluske, J.R., M.W.A. Verstegen and J. Le Dividich (editors), Weaning the Pig: Concepts and Consequences. Wageningen Academic Publishers, The Netherlands, pp. 219-257.

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1.1 INTRODUCTION

Two equally important functions performed by the small intestine are the digestion and absorption of dietary nutrients, and the defence of the body from infection via the gastrointestinal mucosa. Some antagonism exists between these tasks, since any increase in the digestive and absorptive area of the intestine also enlarges the area that must be protected by the intestinal immune system. From a teleological perspective, the intestine has sought to perform its functions by providing a physical barrier to most luminal antigens while areas which specialise in sampling of antigen enable controlled induction of immune responses, and by providing a vast area for nutrient absorption which necessitates an equally vast immune system to effectively protect it from infection. The intestinal immune system is constantly exposed to a barrage of antigenic material, ranging from dangerous antigens associated with pathogenic bacteria and viruses to harmless antigens present in a normal diet. This has led to the development of a sophisticated system enabling the induction of active immune responses against harmful antigens and tolerance towards those that are innocuous.

Because the pig is born with an immature gastrointestinal immune system, the early postnatal period is of particular developmental significance. The modern practice of abrupt weaning at an early age is highly unnatural for the piglet, which would, under normal circumstances, be gradually weaned at a much greater age and degree of developmental maturity. Modern weaning practices abruptly remove the passive protection of maternal milk-derived immunoglobulins and other protective immune factors exposing the piglet to a plethora of novel dietary and environmental antigens. Along with these changes, the piglet is required to rapidly adapt to differences in diet presentation and composition, and social environment, while maintaining a high level of growth and productive efficiency. Given these psychological and physiological hurdles, the weaning period is, unsurprisingly, often accompanied by poor performance. Weaning is also accompanied by significant alterations in intestinal immunity (Vega-López et al., 1995; McCracken et al., 1999; Pluske et al., 1999; Solano-Aguilar et al., 2001) and intestinal immune responses, in particular inflammatory responses directed against dietary and bacterial antigens, which have been implicated in the pathogenesis of the post-weaning 'growth check' (Li et al., 1990; Pluske et al., 1997).

1.2 OVERVIEW OF IMMUNE SYSTEMS

1.2.1 Active immunity

1.2.1.1 Innate immunity

During their evolutionary development, vertebrates and invertebrates were subjected to selection pressure conveyed by infectious pathogens, which resulted in the early development of the non-specific, or innate immune system (Mushegian and Medzhitov, 2001). Functioning independent to prior exposure to bacterial pathogens, innate immunity can respond to bacterial invasion extremely quickly, and may be considered the 'first line of defence' against bacterial infection. The predominant leukocytes that mediate the actions of the innate immune system are natural killer cells, mast cells, macrophages and neutrophils, which are derived from the myeloid descendants of the hematopoietic stem cells that reside in bone marrow. Constituting approximately 50% of the leukocytes found in blood, neutrophils are considered the most active of the cells involved in innate responses, and circulate constantly in the blood.

In the gut, epithelial cells provide the first point of contact for both bacterial and dietary antigens. These cells play a pivotal role in initiating inflammatory immune responses by secreting chemokines and cytokines that promote the activation and recruitment of myelolymphoid effector cells to sites of infection or damage. An important feature of the epithelial cell is its ability to discriminate between harmful and innocuous antigens; with respect to antigens associated with gut bacteria, various receptor recognition systems expressed on apical and basolateral surfaces fulfil this function. An important receptor class in bacterial recognition is the toll-like receptor (TLR) (Cario et al. 2000); these receptors recognise pathogen-associated molecule patterns (PAMPS) such as gram negative lipopolysaccharide and gram positive peptidoglycan and trigger downstream signalling cascades that activate epithelial transcription factors which drive inflammatory gene expression. Gene products including IL-8 and MIP-2α are chemotactic for neutrophils and macrophages (McCormick et al. 1993; Hang et al. 1999). Epithelial cells also produce anti-microbial peptides referred to as beta-defensins, an important constituent of the innate immune system, that kill bacteria thus limiting their translocation across the epithelial barrier during infection and invasion (O'Neil et al. 1999).

Intestinal inflammation leads to expression of adhesion molecules on endothelial cells lining the tissue capillaries, to which blood-borne neutrophils bind by virtue of complimentary cell-

surface receptors that they express (Osborne, 1990; Butcher, 1991). Bound neutrophils infiltrate the tissue via the capillary wall by a process known as diapedesis, which allows the cell to fit through a pore much smaller than its size. After entering the infected tissue, neutrophils also recognise PAMPs via specific cell-surface pattern recognition receptors including TLRs (Kelly and King, 2001). Neutrophils migrate towards the source of these antigens by a process known as 'chemotaxis' and non-specifically engulf the invading bacteria. Recognition of bacterial antigens activates the neutrophil, resulting in an 'effector' phase characterised by activation of the complement system, and secretion of inflammatory agents such as chemokines and cytokines including interleukin (IL) -1, IL-6, interferon (IFN) -γ, tumour necrosis factor (TNF) -α and reactive oxygen metabolites, all of which have direct or indirect anti-bacterial actions (Sandborg and Smolen, 1998; Zhang et al., 2000; Morein and Hu, 2001). However, although present in low numbers at birth, blood-borne neutrophils do not reach adult levels until 21 days after weaning (McCauley and Hartmann, 1984), also the chemotactic mechanism of neutrophils (and macrophages) is reported to be impaired in young pigs, and the complement system may not reach adult concentrations until 4 weeks of age (Stokes et al., 1992).

Another component of the innate immune system is the mast cell. Mast cells are present in the lamina propria of the intestine and respond to antigen and non-antigen-dependent stimulation, releasing a broad range of bioactive mediators which serve to recruit further leukocytes such as neutrophils, and promote the development of the intestinal inflammatory response (Befus et al., 1988; Malaviya and Abraham, 2001; Yu and Perdue, 2001). Mast cells are of particular importance in the pathogenesis of allergic reactions, in which they play a central role (Befus et al., 1988; Malaviya and Abraham, 2001; Yu and Perdue, 2001).

In addition to the innate immune system, which provides a generic response to repeated bacterial invasion, there exists the adaptive immune system, providing what is known as 'acquired' immunity. This comprises two arms of the immune system, which are primed by initial exposure to antigens, allowing an antigen-specific immune response that provides long-term immunity. These immune systems are functionally distinct, and their activation is dependent on the nature of the antigen involved. In the case of viral infection, in which a mammalian 'host cell' and its machinery is exploited to enable viral replication, the infected cell must be destroyed in order to eliminate the viral pathogen. These actions are performed by the cellular arm of the immune system. In the case of bacteria, which replicate

independently in most environments, such cytotoxic responses alone are largely ineffectual and the antibody-mediated response of the 'humoral' arm of the immune system is engendered. These systems will now be discussed.

1.2.1.2 Adaptive immunity

1.2.1.2.1 Humoral immunity

Activation of the adaptive immune response begins with the processing and presentation of intracellular antigens to either the humoral or cellular arm of the immune system. In the case of humoral immunity, bacterial or other soluble antigens are taken up by specialised antigen-presenting cells (APCs) such as tissue macrophages and dendritic cells (Kagnoff, 1987), which use proteolytic enzymes to degrade (process) the antigen into immunogenic peptides. These peptides are presented on the surface of the APC, associated with specialised antigen-receptor molecules referred to as major histocompatibility complex (MHC) class II molecules. The MHC class II-antigen complex is subsequently recognised by antigen-specific helper T cells. T cells are commonly identified by specific 'cluster of differentiation' (CD) molecules which are expressed on their cell surfaces – in the case of helper T cells this is CD4, and on this basis helper T cells are often referred to as $CD4^+$ T cells. Antigen recognition by helper T cells causes them to secrete specific lymphokines. These lymphokines stimulate antigen-specific B cells to undergo clonal expansion (multiplication) and differentiation, producing large numbers of antibody-secreting plasma cells (Gaskins and Kelley, 1995). The immunoglobulins (antibodies) secreted by plasma cells recognise and bind specific antigens associated with the pathogenic agent that initiated the immune response, and effect removal of the agent through such processes as opsonisation and complement-mediated direct cytotoxicity (Gaskins and Kelley, 1995). The humoral immune system therefore provides a potent, antigen-specific response to extracellular infection.

1.2.1.2.2 Cellular immunity

Viral infection, which subverts the cellular machinery of host cells to enable viral replication, necessitates the destruction of the infected cell, using the cytotoxic actions of the so-called 'cellular' immune response. Most somatic cells are susceptible to viral infection, and most are therefore also able to process and present viral antigen to the cellular arm of the immune system. The process of antigen presentation begins with the intracellular processing of a subset of viral antigens into immunogenic peptides, which are then presented on the cell

surface as MHC class I-antigen complexes (Jackson and Peterson, 1993). In contrast to the humoral immune system, the cellular immune system employs MHC class I molecules in antigen presentation, which mediate recognition of antigen by antigen-specific cytotoxic T lymphocytes. Cytotoxic T lymphocytes express the CD8 surface molecule, and are therefore often referred to as CD8+ T cells. Recognition of the antigen-MHC class I complex 'activates' the cytotoxic T lymphocyte, causing it to multiply by clonal expansion, and to synthesise and secrete bioactive factors that destroy the infected cell (Gaskins and Kelley, 1995). As in the case of humoral B lymphocytes, once activated, the cytotoxic action of the T cell is antigen-specific, meaning it will only kill cells expressing the stimulating antigen in conjunction with the same MHC class I molecules involved in induction of the immune response (Kagnoff, 1987). The cell-mediated immune response therefore specifically targets and destroys only the infected cells that are the source of viral replication, effectively removing the intracellular pathogenic threat while leaving healthy cells unperturbed.

1.2.2 Passive immunity

The use of preformed antibodies derived from an individual to provide temporary protection against infection in another individual, is termed 'passive' immunity. The acquisition of passive immunity is crucial to the survival of the neonatal pig for several reasons. First, the epitheliochorial placentation of the pig foetus prevents the transfer of maternal antibodies during gestation (Sterzl and Silverstein, 1967) resulting in a pig that is agammaglobulinemic at birth (Salmon, 1984). Second, although the cellular components of the immune system are qualitatively represented at birth (Binns, 1973), they are quantitatively and functionally immature (Stokes and Bourne, 1989; Stokes et al., 1992). The acquisition of passive immunity therefore provides crucial protection from pathogens while the cellular components of the immune system mature.

Passive immunity is provided by maternal immunoglobulins, which are selectively concentrated in the mammary gland towards the end of gestation and absorbed intact across the 'open' small intestine of the neonatal pig upon the initiation of suckling (Holland, 1990). The open gut of the piglet can endocytose macromolecules such as immunoglobulins in massive quantities within the first forty-eight hours after birth, resulting in serum antibody titres similar to those of sow (Holland, 1990), and a spectrum of antibodies indistinguishable from that of the sow (Bourne, 1977). The absorbed antibodies circulate in the serum, providing short-term passive systemic protection from infectious agents. However, the

passively acquired antibody repertoire of the neonate is necessarily restricted to those antigens to which the sow has been exposed, and developed memory B cells (Porter, 1986). The predominant immunoglobulin isotype in colostrum reflects that of the serum from which it is derived – immunoglobulin-G (IgG) (Jensen and Pedersen, 1979; Butler and Brown, 1994). Immunoglobulins A (IgA) and M (IgM) are present in colostrum in much smaller concentrations than IgG (Jensen and Pedersen, 1979; Butler and Brown, 1994), and are derived from both serum and local synthesis within the mammary gland (Bourne and Curtis, 1973).

The macromolecular endocytosis of the open gut is non-selective, and its gradual cessation (referred to as 'gut closure') is complete by 48 hours after birth (Murata and Namioka, 1977; Weström et al., 1984). This prevents further large-scale absorption of immunoglobulins, but has the benefit of also preventing further absorption of macromolecules that might be antigenic or pathogenic in nature. After colostrum formation, established lactation proceeds and the character of immunoglobulins present in mammary secretions changes, reflecting a change in the site of their synthesis with most immunoglobulins in milk derived from local synthesis within the mammary gland (Stokes et al. 1992; Salmon, 1999). This is associated with a decrease in total immunoglobulin concentration in milk, and an alteration in the relative concentration of milk immunoglobulins, with IgA predominating (Jensen and Pedersen, 1979; Stokes et al., 1992; Butler and Brown, 1994; Salmon, 1999). These changes coincide with gut closure, and mark a change in the major function of maternally-derived immunoglobulin for the piglet.

Prior to gut closure, a secondary function of unabsorbed maternal immunoglobulin is the provision of local passive protection against the many pathogenic agents encountered at the intestinal mucosa; after gut closure this becomes the predominant function of maternal immunoglobulins. However, IgG antibodies are relatively ineffective at mucosal surfaces (Gaskins and Kelley, 1995; Gaskins, 1998), whereas IgA is largely resistant to the action of digestive and bacterial proteolytic enzymes and binds to mucous components (Kerr, 1990) where it functions largely to bind antigens, prevent bacterial and viral colonisation and invasion at mucosal surfaces, and neutralise bacterial enterotoxins (Porter, 1986; Kagnoff, 1993; Salmon, 1999). Although a minor component of the immunoglobulins present in colostrum and milk, maternal IgM antibodies nonetheless bolster local passive protection by

virtue of a lower adherence to the mucous lining of epithelial surfaces, making IgM particularly suitable for opsonizing pathogens in the gut lumen (Salmon, 1999).

As with passive systemic immunity, the protection afforded by passive local immunity only extends to antigens to which the sow has been exposed and developed active immunity. Other protective factors present in milk and colostrum, such as lactoferrin and lactoperoxidase, perform non-specific antimicrobial functions, however a discussion of these factors falls outside of the scope of this review, and the reader is directed to recent reviews of the topic (Chierici, 2001; van der Strate et al., 2001; van Hooijdonk et al., 2000; Wagstrom et al., 2000). Both forms of passive immune protection extend for the entirety of the lactation period, and their removal at weaning marks a significant breach in the immune protection of the piglet, which will be discussed later in this review.

1.3 THE INTESTINAL IMMUNE SYSTEM

The intestinal epithelium provides an extensive and complex interface between the piglet's immune system and its environment, which must function simultaneously to absorb digested nutrients and provide a barrier against a vast array of ingested antigens. The barrier is composed of the basement membrane underlying epithelial cells, the epithelial cells themselves, the tight junctions that join adjacent cells, and the cell glycocalyx (Kagnoff, 1987; Perdue 1999; Podolsky, 1999). In addition to its barrier function the epithelium also functions in surveillance, communicating information regarding the contents of the intestinal lumen to the underlying mucosal immune system through the production of cytokines (Gaskins 1998; Perdue 1999; Lu and Walker, 2001; Sanderson, 2001). Innate defence is provided by epithelial goblet cells, which secrete mucin and trefoil peptides that form a viscoelastic gel which covers the mucosal surface, providing a barrier which protects the mucosa from luminal bacteria and antigens (Kindon et al., 1995; Podolsky, 1999; Deplanke and Gaskins, 2001). Trefoil peptides have also been implicated in mucosal repair as well as prevention of injury (Babyatsky et al., 1996; Playford, 1997; Podolsky, 1999). A further innate defence mechanism is performed by epithelial Paneth cells, which secrete antimicrobial peptides into the gut lumen, contributing to a biochemical barrier against colonisation (Ouellette, 1999; Zhang et al., 2000). The continual and rapid migration of epithelial cells from the crypts of Lieberkühn, culminating in their extrusion from the villus tip into the gut lumen, removes damaged or infected cells and also provides a mechanism for

rapid epithelial restitution after mucosal injury (Podolsky, 1999). Further protection is provided by the intestinal immune system, which is the largest immune organ in vertebrate species (Gaskins, 1998; Kraehenbuhl and Neutra, 1992). Approximately 25% of the intestinal mucosa consists of lymphoid tissue (Kagnoff, 1987), which in turn constitutes approximately 50% of the total body lymphoid tissue (James, 1993).

The gut-associated lymphoid tissue, commonly abbreviated as GALT, contains three major lymphoid compartments consisting of (1) dispersed or non-organised cells residing in the lamina propria and epithelium (lamina propria leukocytes and intraepithelial T lymphocytes); (2) collections of highly organised lymphoid follicles, such as Peyer's patches and lymph nodes; and (3) scattered individual or small aggregates of lymphoid follicles (Kagnoff, 1987; Gaskins, 1998). Approximately 20-30 discrete Peyer's patches exist in the jejunum and upper ileum of the pig, which increase only slightly in number but significantly in size and cellularity, during the post-natal period (Pabst et al., 1988; Stokes et al., 1994). One continuous patch, which can extend for 2.5 metres, exists in the distal ileum, but this involutes at approximately 1 year of age (Pabst et al., 1988; Stokes et al., 1994).

Antigen transport function is performed by specialised antigen-transporting cells known as M-cells, which are expressed in the epithelium overlying organised lymphoid follicles such as Peyer's patches (Neutra et al., 1980; Neutra, 1999; Kraehenbuhl and Neutra, 2000). M-cells efficiently endocytose and transcytose luminal antigens, bacteria and viruses, which then interact with APCs in the underlying lymphoid follicle, which acts as an antigen 'sampling site' (Neutra et al. 1980; Neutra, 1999; Kraehenbuhl and Neutra, 2000). APCs process the antigen into immunogenic peptides, which are then presented in association with class II MHC molecules to helper T lymphocytes. Antigen presentation causes T lymphocytes to secrete lymphokines that induce B lymphocytes to undergo immunoglobulin class-specific switching within the lymphoid follicle, dedicating themselves to production of a single class of antibody. Class-switching of B-cells in Peyer's patches favours the IgA^+ phenotype, due to unknown factors within the follicular microenvironment (Kagnoff, 1987, 1993).

A proportion of the activated T and B lymphocytes then migrate from the Peyer's patch through the lymphatic system before entering the systemic circulation, thereupon 'homing' to the lamina propria and intraepithelial region of the small intestine (Kagnoff, 1987; Thiele, 1991; Gaskins and Kelley, 1995; Corthesy and Kraehenbuhl, 1999). Upon reaching the

lamina propria, activated B lymphocytes differentiate into plasma cells, capable of secreting large quantities of IgA antibody, a process controlled by cytokines (such as TGF-β, IL4, IL-5 and IL-6) which are produced by helper T lymphocytes in response to reintroduction of antigen (Corthesy and Kraehenbuhl, 1999).

The dimeric IgA produced by plasma cells in the lamina propria interacts with a specialised receptor on the basal surface of intestinal epithelial cells, and the bound IgA is then endocytosed and trancytosed across the cell to be released into the lumen, retaining a cleaved portion of the receptor known as the secretory component (Solari and Kraehenbuhl, 1985; Kerr, 1990; James, 1993). The presence of the secretory component stabilises the structure of the antibody, known as secretory IgA, and increases its resistance to proteolysis (Lindh, 1975; James, 1993), making it particularly suitable for activity in the gut lumen. The main action of secretory IgA is at the mucosal surface, where it binds antigens and prevents viral and bacterial invasion of epithelial surfaces (Williams and Gibbons, 1972; Kagnoff, 1987, 1993; Kraehenbuhl and Neutra, 1992). There is also evidence that secretory IgA can act on antigens within the lamina propria, causing them to be expelled into the gut lumen via the IgA secretory pathway described previously (Kaetzel et al., 1991; Mazanec et al., 1993). A further feature of dimeric IgA is that it is relatively nonphlogistic compared to other immunoglobulins, participating in neither complement activation nor antibody-directed cytotoxic responses (Kagnoff, 1987, 1993). Since a vast number of the antigens commonly present in the gut lumen are likely to be harmless and non-pathogenic, from a teleological perspective it is sensible that the predominant immunoglobulin at mucosal surfaces functions through antigen binding and exclusion rather than induction of mucosal inflammation (Kagnoff, 1987, 1993).

In addition to professional APCs, processing and presentation of luminal antigens to the immune system has been postulated to occur via small intestine epithelial cells expressing class II MHC (Bland and Warren, 1986a, b; Hoyne, et al. 1993; Kaiserlian, 1999). Although epithelial cells have been shown to display class I and II MHC antigens (Olivier et al., 1994), their role in antigen presentation remains contentious (Dvorak et al., 1987; Vega-López et al. 1993, 1995; Chianini et al., 2001).

1.3.1 Intestinal inflammation

Despite the protection afforded by the aforementioned mechanical barriers and secretory IgA, enterocytes can transport a small proportion of luminal antigenic material to the underlying tissues by transcytosis (Wheeler et al. 1993; Heyman, 2001). Similarly, enteric pathogens and antigenic material can invade the intestinal epithelium and the underlying lamina propria via paracellular routes, particularly during times of compromised mucosal integrity such as intestinal infection and inflammation (Heyman, 2001).

The epithelial monolayer is interspersed with a heterogenous population of intraepithelial T lymphocytes, which are predominantly cytotoxic, although so-called double-negative T cells (which express neither the CD4+ nor CD8+ surface antigen) are also present, particularly in the neonate (Vega-López et al., 1993, 2001). Intraepithelial T cells, which represent around 50% of all intestinal lymphocytes in the mature pig (Vega-López et al., 2001), are capable of mediating antibody dependent and direct cytotoxic activity (see Stokes et al., 1994; MacDonald, 1999) and, because of their proximity to the intestinal lumen, are ideally positioned to potentially effect and regulate immune responses (Vega-López et al., 2001). The function of intraepithelial T cells is not well established, although it is hypothesised that they may maintain epithelial integrity by destroying damaged, virally infected or parasitised epithelial cells (Kraehenbuhl and Neutra, 1992; MacDonald, 1999), or promote epithelial growth and renewal through the production of cytokines during active immune responses (Mowat and Viney, 1997).

The lamina propria is populated by a wide range of diffuse immune cells, such as T lymphocytes, antibody-forming B lymphocytes and plasma cells, macrophages, dendritic cells, mast cells, eosinophils, neutrophils, and biologically active fibroblasts (Kagnoff, 1987; Gaskins and Kelley, 1995; Gaskins 1997). The distribution of T cells in lamina propria of the pig appears to be distinctly compartmentalised by 6 months of age (Vega-López et al., 1993; Olivier et al., 1994), with cytotoxic (CD8+) T cells generally positioned in and around the epithelium, and helper (CD4+) T cells generally situated deeper in the lamina propria. The ontogenesis and functional significance of this distribution is yet to be established.

Antigenic material that is present in the lamina propria as a result of disruption of the epithelial barrier is processed by lamina propria APCs such as dendritic cells and macrophages (Stokes et al., 1992, 1996; Iwasaki and Kelsall, 1999; Haverson et al., 2000).

Antigen is then presented as immunogenic peptides in the context of class II MHC and co-stimulatory molecules to helper T lymphocytes either in the lamina propria or, in the case of mature dendritic cells, after the APC has migrated to the mesenteric lymph nodes, (Haverson et al., 2000; Guermonprez et al., 2002). Antigen recognition by helper T lymphocytes causes activation and secretion of a range of cytokines (Murtaugh, 1994; Wood and Seow, 1996). Activated T lymphocytes in the mesenteric lymph nodes proliferate in response to inflammatory signals and migrate to the mucosa to interact with antigen-specific B-cells (Jenkins et al., 2001). The cytokines released by activated T lymphocytes recruit and activate further lymphocytes and the cellular components of the innate immune system, such as eosinophils, neutrophils and lamina propria mast cells, which in turn produce pro-inflammatory cytokines (such as IL-1, IL-4, IL-8, IFN-γ, TNF-α, and granulocyte-monocyte colony stimulating factor), neurotransmitters, and other inflammatory mediators such as complement, nitric oxide and granulocyte proteins which perform or aid antimicrobial functions (Murtaugh, 1994; Elwood and Garden, 1999; Miller and Sandoval, 1999; Zhang et al., 2000).

Proinflammatory cytokines and other mediators produce an array of enteropathic effects in the mucosa: induction of matrix metalloproteinase expression in macrophages, which destroys supporting elements in the mucosa; induction of MHC class II expression in APCs; increased ion secretion into the gut lumen; increased epithelial permeability; and induction of goblet cell differentiation and crypt cell mitosis (MacDonald and Spencer, 1988; Goetzl et al., 1996; Elwood and Garden, 1999; Ferreira et al., 1990, Pender et al., 1997; MacDondald et al., 1999; Monteleone et al., 1999). A detailed discussion of the nature of the inflammatory process in intestinal mucosa is outside the scope of this review, and the previous explanation represents an extreme simplification. Physiological and immunological processes in the intestine represent a complex interaction between immune and non-immune cells and the extracellular matrix, and pathological inflammation may result from dysfunction of one or more of these components, resulting in a homeorhetic response involving all constituents (see Fiocchi, 1997). Evidence of intestinal inflammation has been observed in pigs after weaning (McCracken et al., 1999), and this will be explored later in this review.

1.3.2 Oral tolerance

The gastrointestinal immune system is constantly sampling antigenic material from the intestinal lumen, a large proportion of which is dietary protein. It is essential for the

gastrointestinal immune system to be able to discriminate between antigens of dietary origin, to which immunological tolerance must be induced, and those derived from pathogenic bacteria, against which an active immune response must be mounted. Failure of this discriminatory system results in inappropriate immune responses to innocuous antigens, which can take the form of allergic reactions to dietary components. There is also evidence that a similar state of tolerance exists with regard to harmless commensal gut bacteria (Duchmann et al., 1995, 1996), and perturbations of this state may be crucial for the development and maintenance of chronic intestinal inflammation (Duchmann et al., 1997).

The most common example of the induction of oral tolerance is the feeding of a novel protein antigen to an animal, which results in systemic immunological hyporesponsiveness when the animals are subsequently challenged with the same antigen (Challacombe and Tomasi, 1980). The mechanisms by which oral tolerance is induced are the subject of active research, and are not yet fully understood; in particular, the specific cellular and molecular interactions which generate mucosal tolerance, and their localisation, have yet to be fully identified, and the mechanisms which allow the mucosal immune system to accurately discriminate between innocuous and hazardous antigen are unclear (Bailey et al., 2001a). For detailed discussions of current concepts of oral tolerance, the reader is directed to recent reviews of the topic (Strobel and Mowat, 1998; Weiner, 2000; Garside and Mowat, 2001).

Mucosal exposure to antigen from living and multiplying pathogens generally leads to priming of the local or systemic immune system, whereas exposure to soluble antigen most commonly results in the development of oral tolerance (Kagnoff, 1993; Strobel and Mowat, 1998; Strobel, 2001). The current understanding of the development of oral tolerance implicates several possible mechanisms of induction: clonal anergy, clonal suppression or regulation, and clonal deletion (Strobel and Mowat, 1998; Czerkinsky et al., 1999; Strobel, 2001; Bailey et al., 2001a). Apoptosis of T lymphocytes has also been suggested as a mechanism by which mucosal unresponsiveness may be maintained (Bu et al., 2001). It is thought that multiple mechanisms are likely to be involved in induction and maintenance of oral tolerance, many of which may not necessarily be mutually exclusive (Strobel and Mowat, 1998; Weiner, 2000; Garside and Mowat, 2001; Strobel, 2001).

Antigen processing and presentation may be a central determinant of whether active immunity or tolerance to an antigen is induced. Soluble antigens may be processed and

presented to helper T lymphocytes by APCs which express class II MHC, but not the full range of co-stimulatory molecules (such as B7-1 (CD80) or B7-2 (CD86)) which are normally required for induction of an active immune response, resulting in clonal anergy and oral tolerance (Strobel, 2001; Strobel and Mowat, 1998). Alternatively, the MHC class II^+ APC may express a specialised inhibitory receptor (such as cytotoxic T lymphocyte-associated antigen-4), which may be required to induce tolerance (Bluestone, 1997; Frauwirth and Thomson, 2002), possibly through interaction with regulatory T cells (Toms and Powrie, 2001). At low doses of antigen, tolerance may also be induced through non-professional APCs that express class I MHC or possibly non-classical class I restriction elements, which present immunogenic peptides to suppressor T lymphocytes, producing a dose-dependent induction of T lymphocyte-mediated clonal suppression (Strobel and Mowat, 1998; Strobel, 2001).

T lymphocyte-mediated regulation or suppression of immune responses is likely to be effected by production of transforming growth factor (TGF) -β, and other immunosuppressive cytokines such as IL-4 and IL-10 (Khoury et al. 1992; Chen et al., 1994; Friedman and Weiner, 1994). Interestingly, these cytokines are also implicated in the class switching of B lymphocytes to the IgA^+ phenotype (Murray et al., 1987; Defrance et al., 1992; van Vlasselaer et al., 1992), which is compatible with the observation that systemic tolerance and humoral secretory immune responses can develop concurrently (Challacombe and Tomasi, 1980).

There is significant evidence that the intestinal immune system of the pig is highly regulated, and perhaps biased in favour of the induction of mucosal tolerance rather than active immune responses to antigen. Activation of porcine T cells *in vitro* has been shown to induce secretion of the immunosuppressive lymphokines IL-4 and IL-10, and only low levels of IL-2, which implies preferential induction of tolerance and secretory immune responses rather than cellular immunity (Bailey et al., 1994, 1998; Whary et al., 1995). There is also some evidence that isolated pig lamina propria lymphocytes undergo increased apoptosis in response to activation compared to similarly isolated splenic lymphocytes (Stokes et al., 2001). Increased susceptibility to apoptosis is consistent with the observation that lamina propria T cells are generally in an advanced state of differentiation indicating memory or recent activation status (Haverson et al., 1999), which may predispose T cells to apoptosis after activation (Salmon et al., 1994). Furthermore, many of the MHC class II^+ cells in the

pig lamina propria are non-professional APCs, such as endothelial cells and eosinophils, which may induce anergy by presenting antigen in the absence of appropriate co-stimulatory molecules (Haverson et al., 1994; Stokes et al., 1996; Wilson et al., 1996; Haverson et al., 2000).

The induction of oral tolerance is likely to result from a complex interaction of many immunological factors, many of which are only partially understood at present. Failure of the mucosal immune system to develop oral tolerance towards innocuous antigen leads to induction of an active immune response in the lamina propria, resulting in pathological inflammation of the intestine, as described previously. An example of this condition is gluten intolerance resulting in coeliac disease in humans (Ferguson et al., 1984). In the pig, it has been suggested that the induction of oral tolerance is perturbed by the process of early-weaning, resulting in hypersensitivity reactions to dietary proteins including soy protein (Miller and Stokes, 1994; Bailey et al., 2001a, b), which will be discussed later. Supporting this hypothesis, weaning has been reported to be associated with reduced ease of oral tolerance induction in mice (see Strobel, 1996).

1.3.3 Development of intestinal immunity

The neonatal pig may be considered essentially immunoincompetent at birth, due to low numbers of intestinal MHC class II^+ cells that are required for the presentation of antigen, and $CD4^+$ and $CD8^+$ T cells, which are necessary for the induction of active immune responses (Bianchi et al., 1992; Vega-López et al., 1995, 2001; Pabst and Rothkötter, 1999; Rothkötter et al., 1999). However T cells (which express the characteristic CD2 surface antigen and are therefore classified as $CD2^+$ cells) are present in the intestine, but these cells express neither the CD4 or CD8 surface antigen, and are therefore predominantly of the double-negative phenotype $CD2^+CD4^-CD8^-$ (Rothkötter et al. 1991, Vega-López et al., 1995, 2001; Whary et al., 1995). The presence of this double-negative population of T cells in pigs has been reported elsewhere (Pescovitz et al., 1985; Saalmuller et al., 1989; Binns et al., 1992, Vega-López et al., 1993), as has a double-positive ($CD2^+CD4^+CD8^+$) population of T cells (Whary et al. 1995; Zuckermann and Gaskins, 1996; Zuckermann, 1999; Solano-Aguilar et al., 2001), however currently their function is unclear. Intraepithelial T cells are present at birth, accounting for around 40% of all intestinal T cells, and are also predominantly of the double-negative phenotype (Chu et al. 1979; Vega-López et al., 2001). A small number of IgM^+ and IgA^+ cells are present in the intestine at birth (Bianchi et al. 1992). Components of the innate

immune system are present at birth, with low numbers of macrophage and granulocyte cells present in an even distribution throughout the villus and crypt regions (Vega-López et al., 1995), however they may not yet be functionally mature (Stokes et al., 1992). As previously described, jejunal and upper ileal Peyer's patches are present at birth in approximately the same number and positions as adult animals, although the single continuous Peyer's patch present in the distal ileum involutes at around 1 year of age.

During the postnatal period the intestinal immune system undergoes extensive change, as the pig is exposed to a plethora of environmental antigens, both injurious and innocuous in nature. The presence of macrophages and polymorphonuclear cells increases after birth, becoming more concentrated in the crypt rather than villus region of the lamina propria and reaches adult levels at five weeks of age (Vega-López et al., 1995). The number of dendritic cells likewise increases after birth although, in contrast to macrophages, dendritic cells become prevalent in the villus lamina propria rather than the crypt (Stokes et al., 1992). This is confirmed by the development of MHC class II+ cells, which are about twice as abundant in the villus compared to the crypt area of lamina propria by one week of age (although significant numbers are still present in crypt area), and reach adult levels at 5 weeks after birth (Vega-López et al., 1995). At present it is unclear what may be the functional significance of this apparent compartmentalisation of APCs in the lamina propria.

The lymphocyte profile of the intestinal mucosa alters dramatically after birth, with the number of lamina propria T lymphocytes doubling in the first four weeks after birth (Rothkötter et al., 1991; Stokes et al., 1992). This process is driven largely by exposure to microbial antigens, with gnotobiotic pigs displaying only minor increases in intestinal lymphocytes despite being exposed to nutritional antigens (Rothkötter et al., 1991, 1994, 1999; Pabst and Rothkötter, 1999). Immature lamina propria T lymphocytes begin to differentiate into $CD4^+$ and $CD8^+$ subsets by the fifth day of age, with their collective number equalling that of $CD2^+$ T cells by 12 days of age (Rothkötter et al., 1994). However the $CD4^+$ and $CD8^+$ T cell subsets display different developmental patterns, with the presence of $CD4^+$ T cells rapidly increasing immediately after birth, while $CD8^+$ T cell numbers increase in a comparatively sedate fashion in the first 5-7 weeks after birth (Rothkötter et al., 1991; Bianchi et al., 1992; Stokes et al., 1992; Vega-López et al., 1995, 2001). By 6 months of age, lamina propria $CD4^+$ and $CD8^+$ T cells display distinct patterns of localisation within the lamina propria, as previously described, and are four times more

concentrated in the villus area of the lamina propria than the crypt area (Vega-López et al., 1993).

The number of intraepithelial T lymphocytes increases significantly with age and exposure to microbial antigen, and intraepithelial lymphocytes represent around 50% of all intestinal lymphocytes by 5 weeks of age (Rothkötter et al., 1999; Whary et al., 1995; Vega-López et al., 2001). Lymphocytes accumulating in the epithelium in early life are predominantly $CD2^+$, with significant numbers of $CD8^+$ T cells only detectable later in life (Vega-López et al., 1995, 2001). The number of intraepithelial T cells remains comparatively low during the first 5 weeks of life which, combined with the paucity of $CD8^+$ T cells, may predispose the young pig to enteric infection (Vega-López et al., 2001). Adult numbers of intraepithelial T cells are not reached until around 24 weeks of age, at which time approximately half are $CD8^+$ T cells and half double-negative T cells, with some granular lymphocytes also present (Vega-López et al., 2001).

B lymphocytes in the lamina propria respond to antigenic stimulation during the post-natal period, with IgA^+ plasma cells increasing rapidly in number from 6 to 28 days of age, around which time adult levels are reached (Brown and Bourne, 1976). The presence of IgM^+ plasma cells is also significant, outnumbering IgA^+ cells until around 4 weeks of age, after which time IgA^+ cells predominate (Brown and Bourne, 1976; Allen and Porter, 1977; Butler et al., 1981; Rothkötter et al., 1991; Bianchi and van der Heijden, 1994; Pabst and Rothkötter, 1999). In contrast to T cells, plasma cells are more concentrated in the crypt rather than villus area of the lamina propria (Brown and Bourne, 1976; Rothkötter et al., 1991; Pabst and Rothkötter, 1999).

Peyer's patches undergo enlargement during the postnatal period, with their length increasing around three times in the first 38 days after birth (Pabst et al., 1988). The enlargement and lymphocyte composition of Peyer's patches is at least partially determined by environmental conditions such as disease load. Germ-free pigs display a smaller increase in size of the ileal Peyer's patch in the first 38 days after birth, whereas jejunal patches showed no change (Pabst et al., 1988). Similarly at 6 weeks of age the vast majority of lymphocytes present in Peyer's patches of conventional pigs are B cells, whereas in germ-free pigs, T cells predominate (Rothkötter and Pabst, 1989; Pabst and Rothkötter, 1999).

The swift and extensive accumulation of MHC class II^+ APCs and both the $CD4^+$ and $CD8^+$ subsets of T lymphocytes in the postnatal period indicates that the piglet rapidly develops the potential for direct antigen recognition and subsequent induction of active immune responses in the lamina propria of the small intestine. However, the components of the intestinal immune system do not resemble that of the adult pig by three weeks of age, the time when weaning usually occurs. In particular, the ratio of $CD4^+$ to $CD8^+$ T cells at this time is the reverse of that which is observed in the adult pig. In adult pigs this ratio is less than 1, whereas in younger pigs the disparity in the relative proliferation of these T cell subsets produces a ratio greater than 1, which could potentially influence the capacity of the piglet to regulate normal intestinal immune responses (Miller and Stokes, 1994). Furthermore, absolute levels of lymphocytes in the lamina propria and intraepithelial compartments are far below adult levels at weaning, which may compromise immunological defence against enteric infections in the intestine (Vega-López et al., 1995, 2001). Withdrawal of the maternal supply of immunoglobulins at this time eliminates their passive immunological function in the intestinal lumen, leaving the mucosa vulnerable to opportunistic infections such as haemolytic *E. coli* and rotavirus (see van Beers-Schreurs et al., 1992; Pluske et al., 1997). Weaning is associated with numerous other sources of stress for the young pig, throughout which the animal must attempt to maintain homeostasis. It is therefore unsurprising that weaning has a significant effect on intestinal immunity, which will now be explored.

1.4 THE EFFECT OF WEANING ON THE INTESTINAL IMMUNE SYSTEM

1.4.1 Overview of the weaning process

In modern pig production systems weaning generally occurs at 2 to 4 weeks of age. Common characteristics of the weaning process are: separation of the sow and piglets, ensuring immediate and complete cessation of piglet access to sow's milk; relocation of the piglets to a nursery facility, which is recommended to be thoroughly cleaned and disinfected prior to piglet entry and maintained at thermoneutral temperature; mixing of different litters of piglets in nursery pens, which may be random or based on common live weight or sex within a pen; provision of a high nutrient density compound diet, which may be offered in dry (pelleted or meal) or liquid form from easily accessed feeders; water is provided *ad libitum* from specialised drinkers or water-nipples, and an electrolyte solution may also be provided. This weaning system provides a stark contrast to that which occurs under 'natural' conditions, where piglets, after a gradual decline in intake, generally cease consumption of

sow's milk between 15 and 22 weeks of age (Jensen and Stangel, 1992), during which time they have learned foraging behaviour to provide nutrition to supplement and eventually replace the declining maternal supply of nutrition.

Weaning under the majority of commercial conditions currently practiced worldwide generally results in a 'growth check', which is characterised by low voluntary feed intake, poor weight gain or weight loss and occasionally diarrhoea, morbidity and death (see Pluske et al. 1995). The impaired growth performance may persist for up to 14 days after weaning, with growth during this time reduced by 25-40% compared to that observed in unweaned piglets of the same age (Musgrave et al. 1991; Pajor et al., 1991). This phenomenon is variously attributed to nutritional stress, due to removal of sow's milk and provision of a novel replacement diet; psychological stress, caused by removal of the sow, relocation and mixing, and unfamiliarity with the nature of the weaning diet; and environmental stress, caused by fluctuations in ambient temperature, air-borne dust, and the presence of environmental antigens.

A further cause of the post-weaning growth check may be postulated to be that of immunological 'stress'. Since the immunological state of the animal is affected by alterations in nutritional, psychological, and environmental factors (Kelley, 1980), immunological stress may be considered to interpenetrate the effects of all of these variables, forming a potential secondary cause of growth stasis at weaning. The notion that immunological stress can inhibit animal growth is well established, with pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α implicated as primary mediators of this effect, through their modulation of intermediary metabolism of fat, protein and carbohydrate, inhibition of voluntary feed intake, stimulation of hepatic acute-phase protein synthesis, and other physiological and behavioural effects (Kelley et al., 1994; Johnson, 1997).

1.4.2 Alteration of intestinal morphology

The post-weaning period is usually characterised by villus atrophy and crypt hyperplasia in the small intestine (see Kelly et al., 1992; Pluske et al., 1997). Villus height has been shown to decrease rapidly to around 75% of pre-weaning values within 24 hours of weaning in pigs weaned at 21 days, and this villus atrophy was observed to continue, albeit at a slower rate, until 5 days after weaning (Figure 1; Hampson, 1986). Crypt elongation was observed to occur at a slower rate over the first 11 days of the weaning period, indicating an increase in

epithelial cell mitosis (Hampson, 1986). Similarly, reductions in the length of microvilli have been reported after weaning (Cera et al., 1988). After the small intestine has recovered from the weaning process, the long thin villi that are typical of the neonate have been remodelled into the shorter tongue or leaf-shaped villi characteristic of the adult intestine. In more natural conditions, this transition is likely to have occurred slowly over the course of a gradual weaning process, however the abrupt weaning system employed in modern pig farming induces more precipitous morphological restructuring.

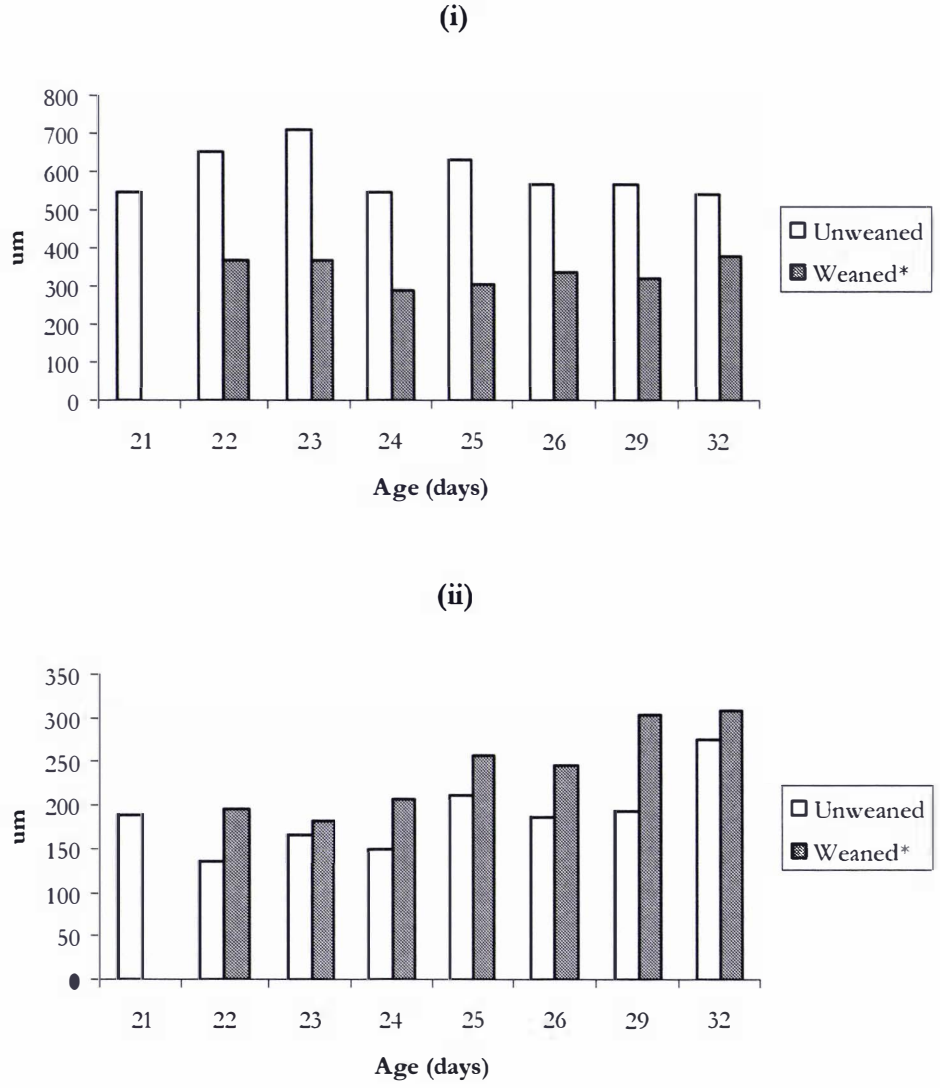

Figure 1. Comparison of villus height (i) and crypt depth (ii) at 2% along the small intestine, between unweaned pigs and pigs weaned at 21 days of age. * Significant difference between values for weaned vs. unweaned pigs ($P < 0.001$) (from Hampson, 1986).

The deleterious effects of weaning on gut architecture have been associated with a reduction in the specific activities of brush-border enzymes such as lactase, isomaltase and sucrase, within 4 to 5 days after weaning (Hampson and Kidder, 1986; Miller et al., 1986). The combined effect of reductions in brush-border enzyme activity and small intestinal absorptive area are likely to impair the absorptive function of the intestine after weaning. This has been confirmed in several studies, measuring absorption of a standard dose of D-xylose (Miller et al., 1984a, b; Hampson and Smith 1986), alanine (Smith, 1984; Miller et al., 1986), and a solution containing glucose and electrolytes (Nabuurs et al., 1994). However, contrary to these results, Kelly et al. (1990, 1991a) and Pluske et al. (1996c) observed no reduction in the ability of villi to absorb xylose after weaning. Nevertheless, decreases in the absorptive capacity of the intestine may be a central determinant of the severity of post-weaning growth stasis. Supporting this notion, several authors have reported a high correlation between post-weaning growth rate and small intestine villus height (Li et al., 1991b; Pluske et al., 1995, 1996b).

1.4.3 Activation of the intestinal immune system

Activation of the gastrointestinal immune system during weaning has been described in various animal species, including the rat (Cummins et al., 1988a, b, 1991; Thompson et al., 1996; Masjedi et al., 1999) human (Machado et al. 1994; Cummins and Thompson, 1997), and pig (Vega-López et al., 1995; McCracken et al., 1999; Pluske et al., 1999; Solano-Aguilar et al., 2001).

Using values derived at weaning (day 0) as a comparison, McCracken et al. (1999) observed that weaning in the pig at 21 days of age is associated with an increase in jejunal lamina propria $CD4^+$ and $CD8^+$ T lymphocytes within 2 and 7 days after weaning, respectively. These authors also reported increased expression of the active form of the matrix metalloproteinase stromelysin in jejunal explants during the initial 7 days after weaning, and a decrease in jejunal expression of MHC class I and II mRNA (McCracken et al., 1999). Similarly, Pluske et al. (1999) observed an increase in jejunal lamina propria $CD4^+$ T cells within 24 hours of weaning at 21 days of age, but no change in $CD8^+$ T cells, compared to values obtained at weaning.

In contrast to these results, Vega-López et al. (1995) reported that by 4 days after weaning, piglets weaned at 21 days of age displayed an increase in lamina propria T cells ($CD2^+$)

compared to unweaned control piglets, but no increase in $CD4^+$ or $CD8^+$ T cells, indicating that the infiltrating cells were of the $CD2^+CD4^-CD8^-$ phenotype. Vega-López et al. (1995) also observed an increase in granulocyte/macrophage cells in the crypt and villus regions of proximal small intestine lamina propria, and an increase in MHC class II^+ APCs, after weaning. Somewhat paradoxically, despite the observed influx of immunocytes into the mucosa of piglets in this study, there was no evidence of increased activation of T cells or macrophages after weaning, as determined by IL-2 receptor expression.

Similar results to Vega-López et al. (1995) were reported by Solano-Aguilar et al. (2001), who observed gradual changes in $CD4^+$ and $CD8^+$ T cells, monocytes granulocytes and macrophages (cells expressing the SWC3 surface antigen), and expression of the SLA-DQ surface antigen in the month after weaning at 17 days of age. However the study of Solano-Aguilar et al. (2001) did not provide an unweaned control for comparison, and sampling occurred somewhat erratically due to animal and/or lymphocyte yield limitations, making discrimination between age and weaning related changes problematic. Similarly determination of the time-course of immunological activity in the immediate post-weaning period (i.e. 1-7 days after weaning) is difficult due to long intervals between sampling and, in many cases, the absence of data taken at the point of weaning. In this study the final pre-weaning sample was taken 6 days prior to weaning, and the first post-weaning sample was taken 1 day after weaning. Since significant changes in immunological variables, such as T lymphocytes and MHC mRNA expression, have been observed in the first 24 hours after weaning (McCracken et al., 1999; Pluske et al., 1999), rigorous temporal sampling is required to illustrate the dynamic alterations in intestinal immunity in the immediate post-weaning period. However the study of Solano-Aguilar et al. (2001) provides useful and extensive data characterising relatively long-term alterations in mucosal lymphocyte subsets in the first month after weaning.

Linking activation of the intestinal immune system at weaning with metabolic inflammatory responses on a systemic level, McCracken et al. (1995) observed that the decreasing ratio of villus height to crypt depth immediately after weaning is accompanied by an increase in plasma concentrations of the proinflammatory cytokine IL-1, the acute-phase protein fibrinogen, and glucagon, as well as increased liver weight, which is associated with acute-phase responses.

Determining the causes of immune system activation at weaning has become a major focus in the pursuit of methods to ameliorate the physiological responses of piglets to the weaning process. In this context, two main hypotheses have emerged: (1) that anorexia of the piglet during the weaning period compromises the integrity of the intestine, allowing luminal antigens to penetrate the epithelial barrier initiating an active immune response in the underlying lamina propria; and (2) that the intestinal immune system is in an immature state at weaning, which impairs its ability to discriminate between harmful and innocuous antigen, and to generate appropriate active immune responses These hypotheses will now be discussed.

1.4.1.1 Compromised epithelial barrier function

Transient anorexia during the immediate post-weaning period is common in modern pig production. Voluntary feed intake after weaning is also extremely variable; group-housed pigs weaned at 27 days of age have been reported to take an average of around 15 hours to start eating, although the interval between weaning and eating varied from close to zero to around four days after weaning (Bruininx et al., 2001). In a summary of several data sets, Le Dividich and Herpin (1994) concluded that the intake of piglets weaned at 21 days of age does not meet the daily metabolisable energy requirement for maintenance until the fifth day after weaning, and that the daily metabolisable energy intake achieved by the piglet during the pre-weaning period is not reached until two weeks after weaning.

Increasing evidence supports the notion that luminal nutrition plays a pivotal role in determining the structure and function of the small intestine. For example, exclusion of luminal nutrients by total parenteral nutrition in piglets results in small intestinal villus atrophy and reduced crypt depth (Park et al., 1998; Ganessunker et al., 1999; Burrin et al., 2000); increased jejunal and ileal lamina propria $CD4^+$ and $CD8^+$ T cells, ileal MHC class II mRNA expression, and jejunal goblet cell numbers (Ganessunker et al., 1999); reduced epithelial cell mitosis (Burrin et al., 2000); reduced specific activity of mucosal sucrase and lactase (Park et al., 1998); and a negative protein accretion rate in the intestine (Stoll et al., 2000), compared to piglets offered luminal nutrition. Similar studies using rats have demonstrated that total parenteral nutrition increases epithelial permeability and bacterial translocation across the epithelial barrier, an effect that can be prevented through provision of luminal nutrition (Omura et al., 2000; Mosenthal et al., 2002). Similar symptoms are observed in piglets after weaning, from which it was inferred that the level of luminal

nutrition received over the post-weaning period may play a key role in determining the structure and function of the piglet intestine during this time, as initially proposed by Kelly et al. (1984) and McCracken and Kelly (1984).

The effect of feed intake on the pig intestinal mucosa has been illustrated in numerous studies (Kelly et al., 1984, 1991a, b; McCracken and Kelly, 1984; Pluske and Williams, 1995; Núñez et al., 1996; Pluske et al., 1996a, b, c; McCracken et al., 1999; Spreeuwenberg et al., 2001; Verdonk et al., 2001a, b). Taken together, these studies show that a reduction in luminal nutrition produces atrophy of the intestinal mucosa, and that mucosal atrophy over weaning can be ameliorated by maintaining a continuous supply of luminal nutrition during this time. Furthermore, it is hypothesised that transient anorexia over the weaning period compromises intestinal barrier function, allowing luminal antigens to penetrate the lamina propria, inducing intestinal inflammation which exacerbates the adverse morphology (McCracken et al., 1999).

Supporting this hypothesis, Verdonk et al. (2001a) observed an increase in paracellular, but not transcellular, transport within 2 days of weaning at approximately 26 days of age, which continued until at least 4 days after weaning, when measurement was ceased. This was accompanied by the characteristic post-weaning reduction in villus height, and increase in crypt depth. Furthermore, Verdonk et al. (2001a) showed that piglets maintained on a low level of nutrient intake after weaning had significantly increased paracellular, but not transcellular, transport, and reduced villus height in the proximal small intestine, compared to piglets maintained on a high level of nutrient intake. Similar results were reported by Spreeuwenburg et al. (2001), who concluded that diminished enteral stimulation and stress at weaning compromise small intestinal barrier function in pigs within 2 days of weaning at 26 days of age. Commensurate with the increase in paracellular transport observed in this study was a numerical increase in crypt lamina propria $CD8^+$ T cells, which was positively correlated to both paracellular and transcellular transport (as measured by transport of mannitol and GlySar, respectively), and a numerical decrease in $CD4^+$ T cells, leading to a significantly reduced ratio of $CD4^+$:$CD8^+$ T cells (Table 1). Spreeuwenburg et al. (2001) observed that these changes were accompanied by atrophy of intestinal villi, with villus height and ratio of villus height to crypt depth negatively correlated with lamina propria $CD8^+$ T cell numbers and tending to be negatively correlated with lamina propria $CD4^+$ T cell numbers. Collectively, these results suggest that low nutrient intake after weaning impairs the integrity

of the tight junctions between epithelial cells lining the small intestine, increasing paracellular permeability, and potentially allowing luminal antigens into the underlying lamina propria where an active immune response may be initiated.

Table 1. Transepithelial transport and T lymphocyte subsets in the small intestine of piglets fed a liquid milk replacer after weaning (from Spreeuwenburg et al., 2001).

	Trans-epithelial transport			T lymphocyte subsets			
	n	GlySar	Mannitol	n	$CD4^+$	$CD8^+$	$CD4^+$:$CD8^+$
		10^{-6} cm/s			$n/10^6$ μm² crypt lamina propria		
Days post-weaning							
0	12	16.6	6.6[b]	12	216	117	2.2[a]
1	12	15.6	8.1[b]	18	125	116	1.1[c]
2	12	16.8	12.2[a]	18	195	168	1.4[bc]
4	12	19.8	11.9[a]	18	226	167	2.0[ab]
SEM		1.52	0.88		30.7	28.9	0.26
P-value of model		NS	0.01		NS	NS	0.05

[a,b,c] Values with different superscripts are significantly different ($P < 0.05$).

Similar results were reported by McCracken et al. (1999), who observed expansion of lamina propria $CD4^+$ and $CD8^+$ T cells within 2 days of weaning at 21 days of age (Figure 2), which coincided with a reduction in villus height of around 65% compared to that observed at the point of weaning. This was accompanied by increased expression of the matrix metalloproteinase stromelysin, which peaked at 4 days after weaning, suggesting a link between expression of inflammatory matrix metalloproteinases and intestinal atrophy in the weaner piglet. McCracken et al. (1999) also observed reduced expression of MHC class I mRNA in the day after weaning, which was attributed to an effect of increased plasma cortisol concentration caused by weaning stress, as observed by Wu et al. (2000). In the study by McCracken et al. (1999), increased expression of MHC class I mRNA, which generally occurs during inflammatory responses, did not occur in the first 7 days after weaning. However McCracken et al. (1999) claim this may reflect cortisol-induced inhibition of the AP-1 family of transcription factors, which activate MHC class I gene expression, since the post-weaning cortisol surge may not decline until between 2 and 8 days after weaning (Wu et al., 2000).

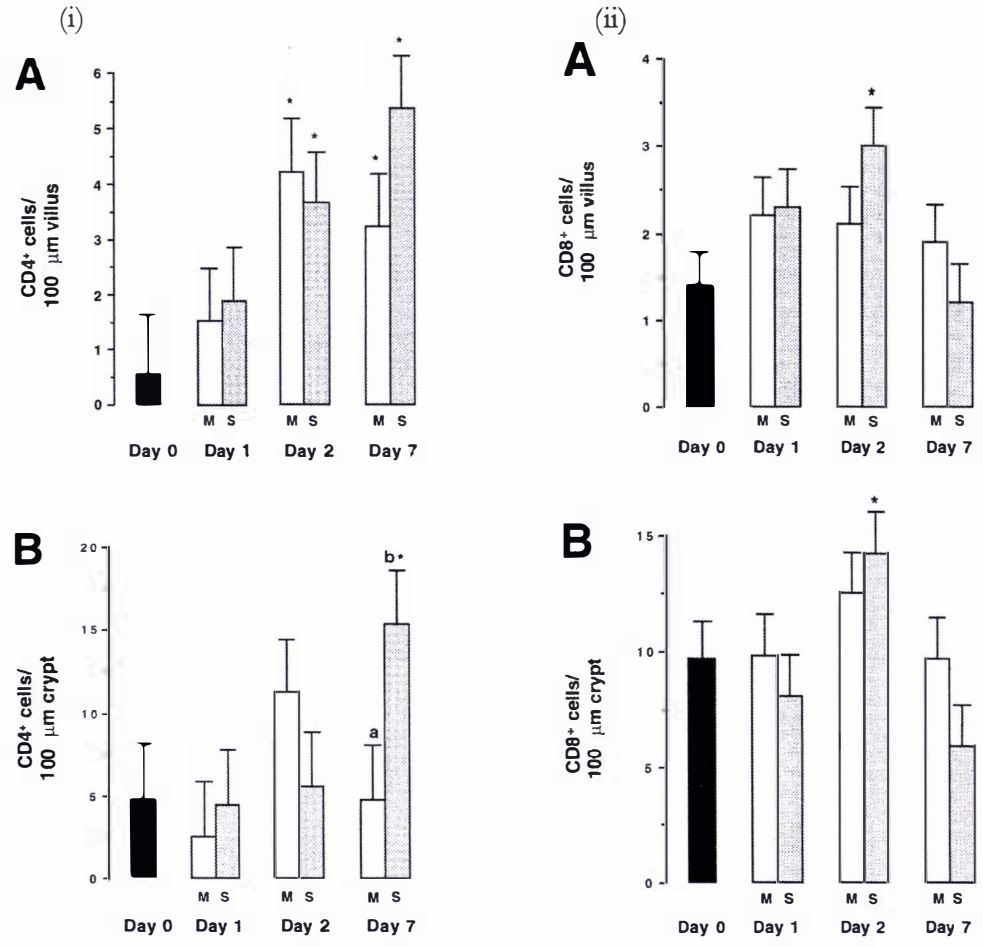

Figure 2. Enumeration of $CD4^+$ (i) and $CD8^+$ (ii) T cells in jejunal villus (A) and crypt (B) lamina propria of pigs offered either a milk (M)- or soy (S)-based diet after weaning. * Values differ significantly ($P < 0.05$) from those at Day 0; Different letters (a,b) indicate a significant difference ($P < 0.05$) between groups M and S (from McCracken et al., 1999).

Expansion of T cell subsets in the lamina propria after weaning reported by Spreeuwenburg et al. (2001) and McCracken et al. (1999) was also reported by Pluske et al. (1999), who observed an increase in lamina propria $CD4^+$ T cells within 24 hours after weaning at 28 days of age, although $CD8^+$ T cell numbers were unchanged compared to that observed at the point of weaning. Expansion and activation of $CD8^+$ T cells can indicate induction of a cellular immune response, which promotes secretion of various proinflammatory cytokines, such as INF-γ and TNF-α, which bolster the inflammatory response and cause injury to the gut tissue (MacDonald, 1999). T cell activation in this manner has been shown to induce crypt hyperplasia, villus atrophy and matrix metalloproteinases that degrade extracellular matrix proteins (MacDonald and Spencer, 1988; Ferreira et al., 1990, Pender et al., 1997; MacDondald et al., 1999; Monteleone, 1999). The data of Spreeuwenburg et al. (2001), in

which $CD8^+$ and to a lesser degree $CD4^+$ T cell numbers were negatively correlated to intestinal villus height, implicate anorexia-induced expansion of T cell subsets in the pathology of mucosal atrophy at weaning. Furthermore, the observation by McCracken et al. (1999) that weaning anorexia is associated with increased expression of matrix metalloproteinases elucidates a mechanism through which the observed tissue remodelling could be mediated in pigs. The decreased expression of MHC class I mRNA observed by McCracken et al. (1999) may also suggest a reduction in presentation of viral antigens and the associated cytotoxic T lymphocyte recognition and destruction of host cells, resulting in an increased susceptibility to viral disease during the post-weaning period.

1.4.1.2 Hypersensitivity to dietary antigen

Villus atrophy and crypt hyperplasia similar to that observed at weaning has been documented in cases of human dietary allergies, such as coeliac disease (Ferguson et al., 1984), where inappropriate active mucosal immune responses are mounted against dietary antigens. Immune responses to dietary proteins have often been observed after weaning in the pig, where weaning onto a soy protein-based diet has been shown to result in appearance of serum IgG antibodies specific for glycinin and β-conglycinin, which are major storage proteins of the soybean (Wilson et al., 1989; Li et al., 1990, 1991a, b; Dréau et al., 1994). Consumption of soy protein containing glycinin and β-conglycinin after weaning has also been associated with increased density of intestinal $CD2^+$, $CD4^+$ and $CD8^+$ T cells (Figure 3) and plasma cells (Dréau et al., 1995), as well as villus atrophy and crypt hyperplasia (Dréau et al., 1994; Li et al., 1990, 1991a, b). This immune response has been linked to depressed growth rate after weaning, with a significant amount of the variation in weight gain after weaning explained by systemic immune responses to soybean meal, as indicated by delayed-type skin hypersensitivity reactions and serum antibodies (Li et al., 1990). In this study, weight gain after weaning was negatively correlated with delayed-type hypersensitivity reactions to intradermal soybean protein, which is consistent with the hypothesis that cellular immune responses after weaning impair performance.

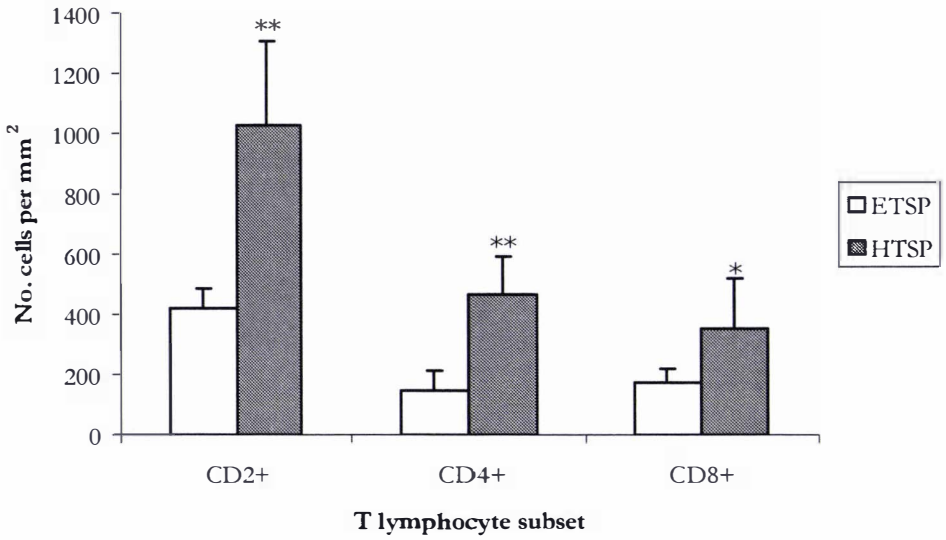

Figure 3. Lamina propria lymphocyte subsets of pig offered diets containing low and high concentration of conglycinin and β-conglycinin (ethanol-treated soy protein (ETSP) and heat-treated soy protein (HTSP), respectively), for 7-9 days after weaning at 21 days of age. *, ** Significantly different from ETSP group $P < 0.05$ and $P < 0.01$, respectively (from Dréau et al., 1995).

Immune response to dietary soybean protein appears to be most evident in younger pigs, with antibody responses decreasing with age (Wilson et al., 1989). This suggests that the immune system of the young pig is particularly prone to mounting inappropriate immune responses to dietary antigen. Soy protein-induced primary immune responses appear to be followed by immunological tolerance rather than priming, with pigs injected with soy protein after primary exposure displaying minimal immune responses compared to those that are naïve to soy protein (Bailey et al., 1993). This suggests that the primary immune mechanism controlling soybean hypersensitivity is classical 'oral tolerance' (Bailey et al., 2001a), as previously described. For more comprehensive reviews of this area of research the reader is directed to the work of Stokes et al. (1987), Miller and Stokes (1994) and Bailey et al. (2001a, b).

Exactly what immunological factors may predispose the weaner pig to development of dietary hypersensitivity is currently unknown. However, it has been suggested that weaning, which is associated with depression of humoral immune responses (Blecha et al., 1983; Wattrang et al., 1998); mixing and housing changes, which have been reported to alter

parameters of immunity and immune response (Hicks et al., 1998; Kelly et al., 2000); weaning stressors such as low temperatures or draughts, which have been shown to influence T cell responses to non-specific mitogens (Blecha and Kelley, 1981; Scheepens et al., 1994); and stress-induced cortisol release, which has been observed to have a suppressive effect on immune function (Westly and Kelley, 1984; Brown-Borg et al., 1993), may disturb the development of the intestinal immune system, impairing its ability to discriminate between harmful and innocuous antigen (Bailey et al., 2001a,b).

Although the hypothesis that soybean hypersensitivity is a common cause of growth stasis after weaning has generally gained acceptance in pig science, evidence of mucosal mast cell involvement in its pathogenesis has yet to be shown (Gaskins, 1997). Mucosal mast cells are an integral component of classical hypersensitivity reactions, producing a wide array of inflammatory mediators in response to antigenic stimulation, resulting in increased ion secretion into the gut lumen and increased epithelial and vascular permeability (Befus et al., 1988; Malaviya and Abraham, 2001; Yu and Perdue, 2001). Also, dietary and non-dietary factors have been implicated in post-weaning growth stasis of pigs offered a soy-containing diet, implying that, if present, hypersensitivity to soy protein may be one of several factors involved in poor performance after weaning (McCracken et al., 1995). Indeed, the observations of McCracken et al. (1999) led them to suggest that soy-hypersensitivity reactions at weaning may occur subsequent to inflammation induced by post-weaning anorexia, since expansion of T cell subsets occurred immediately after weaning regardless of whether soy or milk-based diets were consumed at this time (Figure 2). Given the apparently pivotal role of feed intake on intestinal morphology and immunity, the absence of individual feed intake measurements in many studies reporting immunological and morphological effects of soy hypersensitivity (Dréau et al., 1994; Li et al., 1990, 1991a, b) suggests that these two variables are often confounded.

To minimise or alleviate post-weaning inappetence through strategies that promote food intake is obviously an important target in pig production, however understanding the inputs required to stimulate functional maturity of the developing pig immune system, in relation to handling both dietary and bacterial antigens, is equally important. In this regard, factors that promote the regulatory systems governing tolerance, inflammation and active immunity require further investigation.

1.5 CONCLUSION

The modern practice of abrupt early weaning represents a formidable challenge for the intestinal immune system, a fact that is exemplified by the alterations in intestinal immunity that weaning causes. However, in a multi-factorial situation such as weaning, determination of the relative importance of different factors is inevitably difficult, and results are likely to vary due to subtle, and perhaps unpredictable, factors. This renders problematic the goal of establishing, with a high degree of certitude, the predominant cause of poor and variable post-weaning performance. Significant evidence exists supporting both the luminal nutrition and soybean hypersensitivity hypotheses, in some cases in the same experiment (McCracken et al., 1995; McCracken et al., 1999). This serves to emphasise that neither hypothesis precludes the other, and both problems are likely to significantly affect post-weaning growth performance. In the absence of irrefutable evidence contradicting either hypothesis, it is therefore prudent to make every effort to maintain a constant level of luminal nutrition over the weaning period, and to defer the inclusion of soybean protein in weaner diets until a greater of stage maturity has been reached. Weaning at a greater age, and hence degree of developmental maturity, can diminish weaning-induced immune responses (Wilson et al., 1989; Bianchi et al., 1992). Given the extensive physiological and behavioural effects of pro-inflammatory cytokines, which hinder growth during active immune responses (Kelley et al., 1994; Johnson, 1997; Stahly, 2001), control of the post-weaning immune response is a valuable objective in pig production.

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Chapter 2

THE USE OF SPRAY-DRIED COLOSTRUM AND PLASMA IN WEANING DIETS FOR PIGS

2.1 INTRODUCTION

Piglet performance over the weaning period is often well below potential. This is a multi-factorial problem, however two major causes are poor feed intake and post-weaning diarrhoea, which reduce piglet growth, particularly in the first week after weaning. Early weaning management systems, in which piglets are weaned at or below 21 days of age, reduce the exposure of piglets to environmental and maternal pathogens, thus reducing the incidence of post-weaning intestinal infection, but may exacerbate the problems of poor post-weaning piglet performance by reducing immunological and digestive maturity of pigs at weaning (Coffey and Cromwell, 2001). Early-weaned pigs do not express the full complement of digestive enzymes, particularly those required to digest complex proteins and carbohydrates (Pekas, 1991). Similarly, the early-weaned pig is in a state of immunological immaturity, which may have wide-ranging effects on performance at weaning (King et al., 2003).

In order to overcome these problems different feed additives have been employed to improve the palatability and digestibility of starter diets (the first diet fed at weaning), or to provide medication that may help reduce the incidence of post-weaning diarrhoea. In New Zealand, which has an inexpensive pasture-based dairy production system, dried skim milk is commonly used in starter and other weaning diets as a highly palatable and digestible source of protein. In countries like the United States where dried skim milk is relatively expensive, spray-dried plasma is included in weaning diets as an alternative source of highly digestible and palatable protein that also contains immunoglobulins and other compounds which may be beneficial to pig health. A similar product, spray-dried bovine colostrum, has been developed in New Zealand. Spray-dried colostrum is a high-quality source of dietary protein, and contains a similar composition of immunoglobulins and other compounds as spray-dried plasma. This review will summarise the production of spray-dried plasma and colostrum, their use as sources of dietary protein in weaning diets, and their possible mechanisms of action.

2.2 PROCESSING AND MANUFACTURE

2.2.1 Spray-dried bovine plasma

At slaughter, cattle and pigs yield approximately 12 and 2.5 litres of blood per animal, respectively (Wismer-Pedersen, 1979). Fresh whole blood, which has passed health inspection, is collected under aseptic conditions in slaughter houses, and mixed with an anti-coagulant such as sodium citrate. The blood is then fractionated into haem and plasma components by centrifugation, typically yielding approximately 65% plasma (Morrissey et al., 1989). The resultant plasma is refrigerated at approximately 4 °C and concentrated by ultrafiltration or reverse osmosis, usually achieving an approximate concentration factor of 3 (Grigorov et al., 1987). Generally, ultrafiltration serves to concentrate protein within the plasma, at the expense of non-protein nitrogen and low molecular weight mineral compounds (Delaney, 1975). The concentrated plasma is then spray-dried at temperatures typically ranging from 154-240 °C inlet temperature, and 68-99 °C outlet temperature (Delaney, 1975). Spray-drying temperature affects the functional properties of the resultant spray-dried plasma, and will therefore vary to suit desired specifications for solubility (lower spray-drying temperatures increase solubility) and other physical characteristics.

The haem component produced at fractionation (≈35% of whole blood) is mainly composed of haemoglobin (Morrissey et al., 1989), and may be spray-dried in a similar manner as plasma to produce spray-dried red blood cells.

2.2.2 Spray-dried colostrum

Bovine colostrum is collected from health inspected herds usually within 24 (Dunshea et al., 2002) to 36 (Chelack et al., 1993) hours after parturition, or from the first two post-partum milkings (Donnelly et al., 1988), with primiparous and multiparous cows yielding approximately 4 and 7 litres per milking, respectively (Chelack et al., 1993), resulting in a yield of approximately 15-20 L of colostrum per cow (Mitra et al., 1994). Whole colostrum is chilled to 4-7 °C, and may be skimmed to remove fat, thereby improving stability, and pasteurised (e.g. at 72 °C for 15 seconds) prior to concentration. The colostrum is then concentrated by ultrafiltration, reverse osmosis and/or low temperature evaporation before

spray-drying to produce a flowing colostrum powder suitable for storage, reconstitution with water, or use as dry powder.

2.3 COMPOSITION

2.3.1 Spray-dried plasma

Bovine, porcine and animal (mixed species) plasma contains approximately twice the concentration of crude protein found in dried skim milk (Table 1). The concentrations of amino acids also compare favourably with dried skim milk - reflecting the higher crude protein concentration - with the exception of methionine, which is present to a lesser degree than is found in dried skim milk. The mineral composition of spray-dried plasma varies somewhat between species, with porcine plasma containing a considerably higher sodium concentration than bovine plasma, and sodium concentration in animal plasma falling approximately mid-way between that of bovine and porcine sources, reflecting its mixed origin. Regardless of species of origin, spray-dried plasma contains a higher concentration of sodium, and a lower concentration of calcium than is present in dried skim milk.

Nutrient composition aside, spray-dried plasma contains a complex array of high molecular weight proteins, comprising α-globulins (≈15%), β-globulins (≈10%), γ-globulins (≈20%), fibrinogen (≈5%) and serum albumin (≈50%) (Morrissey et al., 1989). The γ-globulins comprise immunoglobulins G, A and M, which may provide antigen-specific immune protection. The specificity of the immunoglobulins present in spray-dried plasma was recently reported by Owusu-Asiedu et al. (2002), who evaluated the specific antibody titres for enterotoxigenic *E. coli* strains K88, K99, F18, 987P and F41 which are present to varying degrees in spray-dried animal and porcine plasma (Table 2). Glycoproteins are also present in plasma, and the oligosaccharide chains of these may perform antimicrobial functions (Sanchez et al., 1993). Porcine plasma has also been reported to contain considerable amounts of immunoreactive insulin-like growth factor-I (IGF-I) (0.8 ng/mg; de Rodas et al., 1995), an important component of the somatotrophic axis, which is involved in regulation of growth (Claus and Weiler, 1994; Butler and Le Roith, 2001), and can also induce local hypertrophic effects in the intestinal mucosa (Burrin et al., 1996; Houle et al., 1997).

Table 1. Protein, amino acid and mineral composition (% of product as-fed) of bovine, porcine and animal plasma, bovine colostrum and dried skim milk.

	Dried skim milk	Spray-dried animal plasma[1]	Spray-dried bovine plasma[2]	Spray-dried porcine plasma[2]	Spray-dried bovine colostrum[3]
Crude protein	35	78	68	67	81
Amino Acids					
Alanine	n.p.	n.p.	n.p.	n.p.	2.3
Arginine	1.24	4.55	4.24	4.36	2.5
Aspartic acid	n.p.	n.p.	n.p.	n.p.	5.2
Cystine	0.30	2.63	2.46	2.29	0.8
Glutamic acid	n.p.	n.p.	n.p.	n.p.	12.6
Glycine	n.p.	n.p.	n.p.	n.p.	1.8
Histidine	1.05	2.55	2.26	2.35	2.0
Isoleucine	1.87	2.71	2.20	2.60	5.5
Leucine	3.67	7.61	6.95	6.70	4.4
Lysine	2.86	6.84	6.50	6.10	4.5
Methionine	0.92	0.75	0.71	0.48	1.7
Phenylalanine	1.78	4.42	3.89	4.00	3.8
Proline	n.p.	n.p.	n.p.	n.p.	7.5
Serine	n.p.	n.p.	n.p.	n.p.	4.7
Threonine	1.62	4.72	4.65	4.04	3.5
Tryptophan	0.51	1.36	1.39	1.38	1.2
Tyrosine	1.87	3.53	3.67	3.64	3.9
Valine	2.33	4.94	4.95	4.66	4.5
Minerals					
Sodium	0.48	3.02	0.99	6.25	0.22
Calcium	1.31	0.15	0.11	0.14	1.06
Phosphorus	1.00	1.71	0.14	0.13	0.85

[1] National Research Council (1998).
[2] Hansen et al. (1993).
[3] Fonterra Corp., Specialty Ingredients Division, Hautapu, New Zealand.
n.p., not provided.

Table 2. Comparative antibody titre of spray-dried porcine plasma and spray-dried animal plasma[1].

	Antibody titre[2]				
	Anti-K88	Anti-F18	Anti-K99	Anti-987P	Anti-F41
Spray-dried porcine plasma	1.8×10^4	1.5×10^4	$<10^2$	$<10^2$	$<10^2$
Spray-dried animal plasma	0.5×10^3	$<10^2$	4.0×10^4	2.5×10^3	2.0×10^3

[1] Adapted from Owusu-Asiedu et al. (2002).
[2] Titre is defined as the dilution of antibody preparation that gives 50% of maximal absorption in the ELISA.

2.3.2 Spray-dried colostrum

Spray-dried colostrum contains over twice the concentration of crude protein found in dried skim milk, and a similarly high comparative concentration of many amino acids, with the notable exception of lysine, which is present at less than twice that found in dried skim milk (Table 1). The mineral composition of spray-dried colostrum is similar to that of dried skim milk with sodium, calcium and phosphorous present at only slightly lower concentrations in colostrum.

Colostrum also contains numerous compounds such as immunoglobulins G, A and M, lactoferrin, lactoperoxidase, oligosaccharides, glycoconjugates, and growth factors such as IGF-I, IGF-II transforming growth factor-β and epidermal growth factor which, in addition to their nutritive value, may be biologically active after ingestion. Immunoglobulins present in colostrum (such as IgG, IgA and IgM), can provide antigen-specific immune protection, whereas components such as lactoferrin, lactoperoxidase, oligosaccharides and glycoconjugates are capable of performing non-specific antimicrobial and/or antiviral defence functions (Schanbacher et al., 1997; Gopal and Gill, 2000; Shah, 2000; Van Hooijdonk et al., 2000). The effects of the many hormones and growth factors in colostrum have been the subject of numerous reviews detailing their involvement in gastrointestinal function and physiological development of the neonate (Baumrucker and Blum, 1993; Donovan et al., 1994; Xu, 1996; Schanbacher et al., 1997; Playford et al., 2000).

2.4 EFFECTS OF SPRAY-DRIED PLASMA

A large body of research published over the last ten years has shown that inclusion of low levels (3-9%) of spray-dried plasma in diets for pigs can significantly increase feed intake and growth rate during the immediate post-weaning period (Gatnau and Zimmerman, 1990; Sohn et al., 1991; Hansen et al., 1993; Kats et al., 1994; de Rodas et al., 1995; Chae et al., 1999). The effect of spray-dried plasma on feed conversion ratio during the immediate post-weaning period is less consistent, significantly improving (Jiang et al., 2000a,b), worsening (Owen et al., 1995a) and having no effect (Sohn et al., 1991; Hansen et al., 1993; Kats et al., 1994; de Rodas et al., 1995; Chae et al., 1999).

2.4.1 Feed intake & growth rate

The post-weaning period is characterised by low initial levels of feed intake, which are usually insufficient to cover the maintenance energy requirement of the piglet until at least the fifth day post-weaning (Pluske et al., 1995). This contributes to the characteristic post-weaning 'growth check' which can represent a 25-40% reduction in weight gain compared to piglets remaining on the sow (Pluske et al., 1995). Inclusion of spray-dried plasma in phase I weaner diets (typically fed from d0-14 post-weaning) has been shown to significantly increase feed intake and growth rate during the feeding period, when substituted for other protein sources such as dried skim milk, soybean meal, isolated soy protein, meat extract, and casein (Table 3). Reviewing published experiments investigating the growth performance of weaner pigs offered diets containing spray-dried plasma after weaning, Coffey and Cromwell (2001) and van Dijk et al. (2001a) calculated that inclusion of spray-dried plasma in weaning diets improved average daily gain by 25-26.8%, average daily feed intake by 21-24.5% and feed conversion ratio by 3.2-4% during the period of feeding.

Table 3. The performance of pigs fed diets containing different supplemental protein sources after weaning[1].

	Experiment 1[2]				Experiment 2[3]		
	CAS	MX	ISP	SDPP	SBM	DSM	SDPP
Week 0-2							
ADG[4]	247[a]	139[b]	153[b]	261[a]	191[a]	230[a]	345[b]
ADFI	292[b]	213[c]	204[c]	350[a]	267[a]	300[a]	462[b]
FCR	1.18	1.46	1.47	1.34	1.44	1.74	1.35
Week 0-4							
ADG	405[a]	249[c]	354[b]	371[ab]	371[a]	367[a]	438[b]
ADFI	571[a]	415[c]	486[b]	595[a]	507[a]	532[a]	652[b]
FCR	1.40[a]	1.65[b]	1.37[a]	1.59[b]	1.37[a]	1.45[b]	1.49[b]

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1] Adapted from Gatnau and Zimmerman (1990). All diets were formulated to contain 1.2% lysine.
[2] CAS = casein; MX = meat extract; ISP = isolated soy protein; SDPP = Spray-dried porcine plasma. All diets contained the tested protein source at 10%, with additional protein from 20% dried whey, corn and soybean meal.
[3] SBM = soybean meal; DSM = dried skim milk; SDPP = spray-dried porcine plasma. All diets contained a corn-soybean meal base with: diet 1, 20% soybean meal; diet 2, 30% dried skim milk; diet 3, 20% dried whey.
[4] ADG, average daily gain (g); ADFI, average daily feed intake (g); FCR, feed conversion ratio.

Significant preference of weaner pigs for diets containing spray-dried porcine plasma over those containing dried skim milk has been shown to exist from day two post-weaning, and increase throughout the 21-day duration of the experiment, indicating that the diet preference was based on palatability rather than novelty (Ermer et al., 1994). However it was also

shown that, when not offered a choice between these diets, pigs only consumed greater amounts of the spray-dried porcine plasma diet for the initial seven days post-weaning, leading to an increase in growth during day 0-7 which was not reflected in growth rate to day 21 post-weaning (Ermer et al., 1994). In this experiment the increase in feed intake of the pigs fed the plasma diet was shown to result from an increase in both rate of feed consumption and meal size.

2.4.1.1 Factors influencing feed intake and growth rate

The effect of inclusion rate of spray-dried plasma on feed intake has been investigated in numerous experiments, a summary of which is provided (Figure 1). This relationship has been described as non-significant (Coffey and Cromwell, 1995) significantly linear (Gatnau et al., 1991; Dritz et al., 1994), quadratic (Gatnau and Zimmerman, 1992), and with both linear and quadratic effects showing significance (Kats et al., 1994).

Increasing inclusion rate of spray-dried plasma was shown to produce linear (Dritz et al., 1994; Kats et al., 1994), as well as linear and quadratic (Gatnau et al., 1991; Gatnau and Zimmerman, 1992) increases in average daily gain in the first 14 days post-weaning. In one experiment, increasing plasma inclusion rate had no significant effect on pig growth from day 0-14 post-weaning (Coffey and Cromwell, 1995). A summary of the data from these experiments is presented (Figure 1).

Generally, a linear effect of increasing concentration of spray-dried plasma has been observed at inclusion levels ranging from 0% to 6-8%, however at inclusion levels above 6-8% a plateau or decrease in feed intake and growth rate may be observed. However, due possibly to the complex interactions between plasma and other dietary and environmental factors, as well as the variability in intake between piglets during the first two weeks post-weaning, consistent characterisation of the relationship between concentration of spray-dried plasma in the diet and feed intake is difficult. In a multiple regression analysis of 14 published studies, van Dijk et al. (2001a) concluded that at dietary inclusion levels of up to 6% a consistent improvement in average daily gain and average daily feed intake was evident, but that at levels higher than 6% the response was variable, leading overall to no significant relationship between inclusion rate of spray-dried plasma and average daily gain or feed intake.

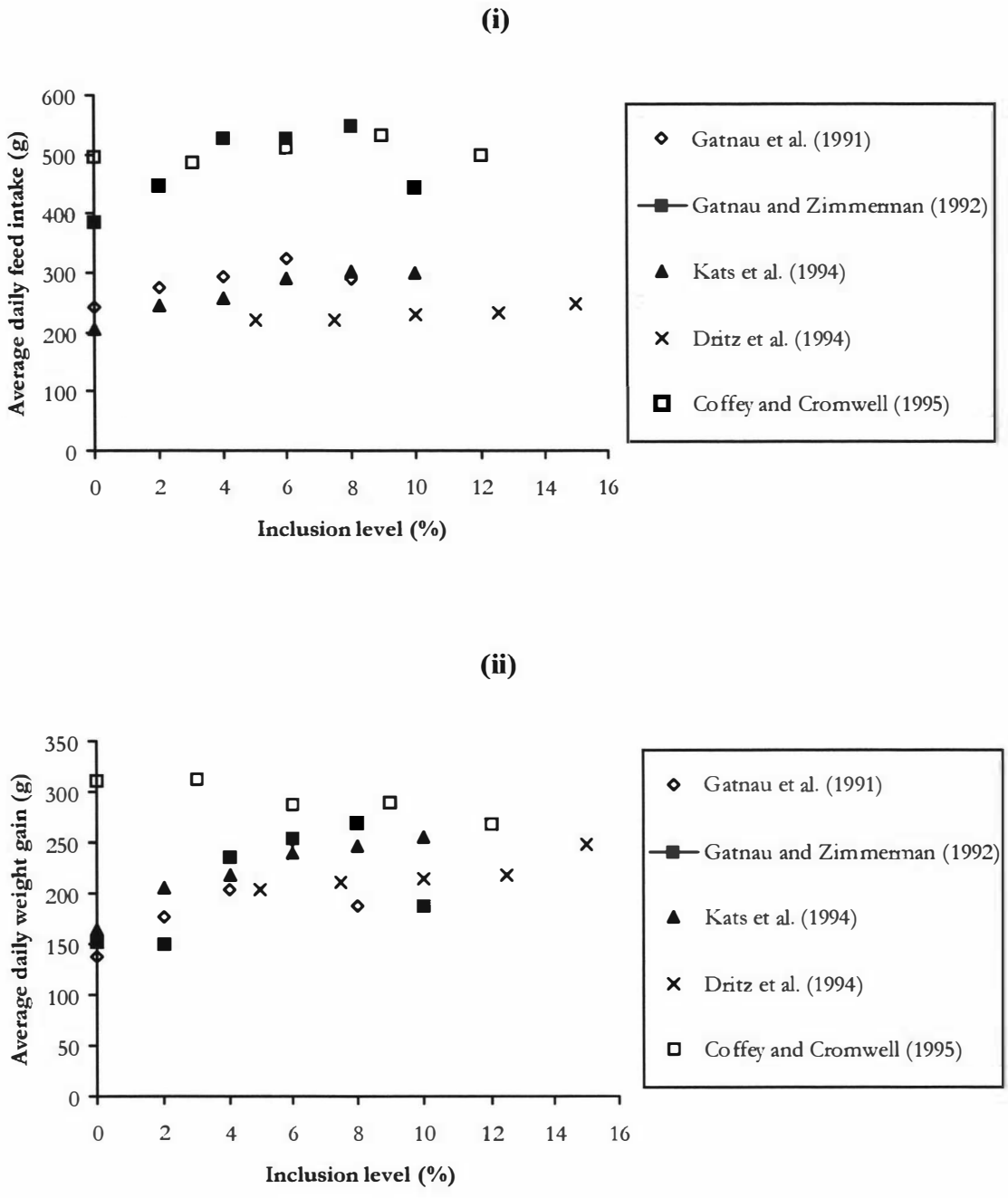


Figure 1. Effect of spray-dried plasma inclusion rate on feed intake (i) and weight gain (ii) from day 0-14 post-weaning.

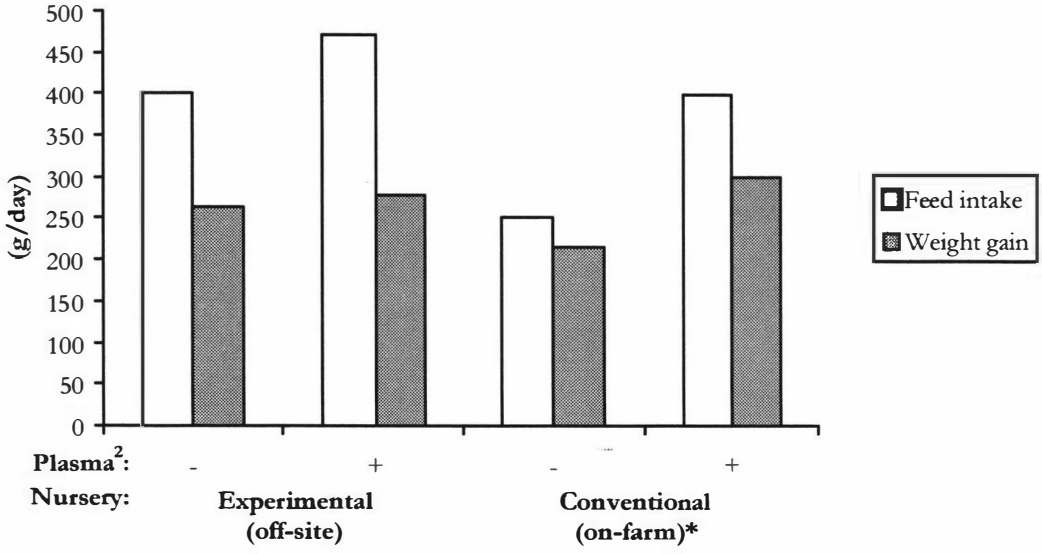
There is evidence that the results of Gatnau et al. (1991) and Gatnau and Zimmerman (1992), which indicated that the feed intake and growth response of weaner pigs to dietary spray-dried plasma was maximised at a level of 6-8% in the diet, may have been confounded by insufficient dietary methionine (≤ 0.28%) limiting production at inclusion rates greater than

6% (Kats et al., 1994). Investigation of the effect of concentration of dietary methionine in a plasma-containing diet has shown that feed intake and growth rate improve quadratically as total dietary methionine concentration is increased from 0.28 to 0.48%, and are maximised at concentrations of 0.41 to 0.42%, in a diet containing 10% spray-dried plasma, fed from day 0-14 post-weaning (Owen et al., 1995b). Kats et al. (1994), feeding diets containing 0.41% methionine and levels of spray-dried plasma ranging from 0 to 10%, observed a linear increase in average daily gain and used inflection point analysis to calculate that feed intake was maximised at a plasma inclusion rate of 8.5% in the first 14 days post-weaning.

The possibility that other dietary components may interact with spray-dried plasma to influence piglet feed intake and growth rate has been investigated in several recent experiments focusing on the interaction between plasma and lactose concentration in the diet (Owen et al., 1993; Touchette et al., 1995) and the interactions between spray-dried plasma, soy protein sources, and lactose (Touchette et al., 1996; Liu et al., 1997; Nessmith et al., 1997). Increasing the concentration of lactose in a starter diet containing soybean meal and no spray-dried plasma has been shown to produce a linear increase in piglet feed intake in the first seven (Nessmith et al., 1997) and fourteen days post-weaning (Owen et al., 1993; Touchette et al., 1995). Increasing the concentration of lactose in a diet containing soybean meal was not found to increase piglet weight gain from day 0-7 post-weaning (Nessmith et al., 1997), although a linear increase in weight gain with increasing lactose was found over day 0-14 post-weaning (Owen et al., 1993; Liu et al., 1997; Nessmith et al., 1997). In contrast to these results, Touchette et al. (1995) found a linear improvement in weight gain during day 0-7 post-weaning with increasing dietary lactose concentration, and a quadratic relationship between these variables over day 7-14 and 0-14 post-weaning. An interaction between lactose concentration and spray-dried plasma has been observed, such that piglet feed intake and growth rate peaked between 0 and 15% added lactose in a diet containing 6.75% spray-dried plasma, whereas feed intake peaked between 30 and 45% added lactose in a diet containing extruded soy protein concentrate in lieu of spray-dried plasma (Touchette et al., 1995).

However, determining interactions of dietary ingredients can be problematic. Evaluating the interaction between lactose and protein sources in weaner diets, Nessmith et al. (1997) found inconsistent results which were attributed to the use of different protein sources to replace the tested protein sources (spray-dried plasma and soybean meal). After two experiments (the latter of which attempted to address the possible effects of replacement protein sources)

Nessmith et al. (1997) found no lactose by plasma interaction for feed intake at lactose concentrations of up to 40%, in contrast to the results of Touchette et al. (1995). The reasons for the conflicting results are unclear, since both groups used similar diets (based on corn, extruded soy protein concentrate and soybean meal). In order to reduce the potentially confounding effect of different dietary lactose concentrations, experimental diets testing spray-dried plasma are usually formulated to contain equal amounts of lactose.



[1] Adapted from Coffey and Cromwell (1995).
[2] +, spray-dried plasma added at 8.33% replacing dried skim milk on an isolysine basis; -, No plasma added.
* Protein source by environment interaction for feed intake ($P < 0.005$) and weight gain ($P < 0.005$); Plasma inclusion improved feed intake and weight gain in the conventional nursery ($P < 0.001$), and feed intake in the experimental nursery ($P < 0.04$).

Figure 2. The effect of nursery environment on the average daily feed intake and weight gain response of piglets when spray-dried plasma is added to starter diets[1].

A further interaction, which has proved more replicable, is that of dietary spray-dried plasma and the experimental weaning environment. Extensive data on this interaction have been reported in the literature (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995; Stahly et al., 1995; Touchette et al., 1996; Bergstrom et al., 1997; Bregendahl et al., 1998; Chirra et al., 1999), and generally shows that dietary spray-dried plasma is most effective at stimulating feed intake and growth when pigs are housed in "practical conditions" (i.e. on-farm or continuous-flow nurseries), compared to experimental conditions (Figure 2). This effect was first observed by Gatnau and Zimmerman (1991) who reported that newly-weaned pigs housed in a continuous-flow nursery (8 pigs per pen) responded to the inclusion of 10%

spray-dried plasma in their diet with a significant increase in feed intake and weight gain of 76% and 102%, respectively, compared to pigs offered a control diet based on corn-soybean meal-dried whey. When these authors performed a similar experiment in an all-in-all-out nursery (pigs individually penned), they found no difference in feed intake and weight gain between pigs offered a diet containing 10% spray-dried plasma and pigs offered a control diet containing a mixture of meat extract and spray-dried plasma at 10%. Although the inclusion of spray-dried plasma in the control diet used in this experiment may have confounded the results of Gatnau and Zimmerman (1991), they were confirmed in a series of experiments by Coffey and Cromwell (1995) which compared pigs housed in a clean, experimental environment with pigs housed in less-clean, on-farm environment, using a control diet containing dried skim milk instead of spray-dried plasma.

Similar results have also been reported for pigs reared under medicated early-weaning and conventional weaning management regimes to produce pigs with comparatively low and high degrees of antigen exposure, respectively (Stahly et al., 1995). These authors found a spray-dried plasma by antigen exposure interaction ($P < 0.06$) such that pigs with high antigen exposure responded to inclusion of 6% plasma (replacing soybean meal on an isolysine basis) with a 19% increase in feed intake and a 33% increase in weight gain compared to pigs offered a control diet, whereas low antigen exposure pigs showed no change in feed intake or growth rate when offered the same diets.

The review, conducted by van Dijk et al. (2001a), of the effects of plasma on growth performance in 14 published studies demonstrated that the average daily gain of pigs offered a control diet can explain 59% of the variation in the percentage change in average daily gain of pigs offered dietary spray-dried plasma relative to those offered the control diet. Van Dijk et al. (2001a) observed that dietary spray-dried plasma improves average daily gain only when control piglets display suboptimal growth, leading to a negative relationship between growth of the control group and relative growth of the plasma group within studies. Many factors are capable of reducing the growth performance of the weaner pig, including the health status of the animal and its environment (Kelley et al., 1994; Johnson, 1997; Stahly, 2001), and it is possible that the observation of van Dijk et al. (2001a) is further evidence of the capability of spray-dried plasma to improve pig performance in immunologically challenging circumstances.

Another factor which may influence the effect of spray-dried plasma on piglet feed intake and growth rate is the species of plasma origin. A number of experiments have been conducted comparing the effects of spray-dried plasma of bovine and porcine origin (Hansen et al., 1993; Gatnau and Zimmerman, 1994; Rantanen et al., 1994; Russell, 1994; Smith et al., 1995; Pierce et al., 1996). Both porcine and bovine plasma have been shown to significantly increase piglet feed intake and growth rate during the first 7 (Rantanen et al., 1994) and 14 days post-weaning (Gatnau and Zimmerman, 1994; Rantanen et al., 1994; Pierce et al., 1996), when compared to control diets containing no plasma. However, over the 14-day post-weaning period porcine plasma produced a higher feed intake and growth rate compared to bovine plasma in several experiments (Gatnau and Zimmerman, 1994; Rantanen et al., 1994; Pierce et al., 1996). In contrast to these results, Russell (1994) found that bovine plasma was more effective at improving piglet feed intake and growth rate than porcine plasma in the first 7 and 21 days post-weaning, although in another experiment no difference between plasma protein source was found. Thus it appears from the reported data that both porcine and bovine spray-dried plasma are capable of stimulating piglet feed intake during the post-weaning period, and porcine plasma may be more effective in this regard than bovine plasma, however further data are required to establish this conclusively.

Due to the many interactions involved in the piglet response to dietary spray-dried plasma, reported data detailing this response vary considerably. All results should therefore be interpreted within the boundaries of the experimental design, and extrapolation, interpolation and integration of results must be performed with caution. Results from a selection of the multitude of trials conducted to evaluate spray-dried plasma are presented to show the variation in piglet response to inclusion of plasma in a starter diet (Table 4).

Table 4. Variation in experimental results evaluating the effects of dietary spray-dried plasma.

Reference	Plasma species	Inclusion level (%)	Control protein[1]	n	Time of feeding[2]	ADG (%)[3]	ADFI (%)	FCR (%)
A	Porcine	18.9	DSM	60	22-29	+59**	+44**	-6
B[4]	Porcine	10	ESP	64	14-22	+14	-3	-25
C	Porcine	10.3	DSM	60	21-28	+12	+21	+9
D	n.p.	4	DSM	96	24-31	+33**	+29**	-4
E[5]	Bovine	5	DSM	208	15-22	+35**	+25	-8
F	Porcine	8	SPC	36	14-21	-13	5	+21
G	Porcine	8	SPC	64	21-28	+62***	+49***	-13
H	Porcine	10	DSM	236	24-31	+22*	+24**	+2

A, Chae et al. (1999); B, Jiang et al. (2000b); C, Hansen et al. (1993); D, Sohn et al. (1991); E, Smith et al. (1995); F, Pierce et al. (1995a); G, Pierce et al. (1995b); H, Hansen et al. (1993).

*, **, *** Significant effect of dietary spray-dried plasma ($P < 0.10$), ($P < 0.05$) and ($P < 0.01$), respectively.

n.p., not provided.

n, number of pigs used in the experiment.

[1] DSM, dried skim milk; ESP, extruded soy protein; SPC, soy protein concentrate.

[2] Piglet age, in days.

[3] ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio (feed intake ÷ weight gain). Values indicate % change in variable relative to control.

[4] FCR calculated using protein intake in original text.

[5] Feed intake calculated from reported ADG and FCR values.

2.4.2 Feed conversion efficiency

The effect of dietary spray-dried plasma on feed conversion efficiency has been shown to vary in a seemingly unpredictable manner, with reported improvements of 25% to declines of 21% compared to control values, but these differences are often non-significant (Table 3). However, significant improvements in feed conversion efficiency due to inclusion of spray-dried plasma in weaning diets have been reported in pigs (Kats et al., 1994; Coffey and Cromwell, 1995; Smith et al., 1995; Campbell et al., 1998a,b; Jiang et al., 2000a,b), and also mice (Thomson et al., 1994a; Thomson et al., 1995). Coffey and Cromwell (1995) reported that pigs offered diets containing spray-dried plasma showed improved feed conversion efficiency only when pigs were housed in a 'conventional' nursery compared to pigs housed in an 'experimental' nursery, resulting in a protein source by environment interaction. However, these authors were unable to replicate this result, and a similar experiment showed no difference in feed conversion efficiency between pigs offered diets containing spray-dried plasma or dried skim milk (Coffey and Cromwell, 1995).

Two recent studies by Jiang et al. (2000a,b) demonstrated significant improvements in feed conversion efficiency over day 0-16 and day 0-24 post-weaning, respectively, in 14 day old pigs offered a diet containing 10% spray-dried plasma compared to those offered the control

diet which contained extruded soy protein. In one experiment this effect was shown to be independent of intake, with pigs pair-fed the plasma diet to the control diet intake level also showing significantly improved feed-conversion efficiency (Jiang et al., 2000b). The increase in feed conversion efficiency of plasma-fed pigs was reflected in a 16% increase in lean body mass compared to control animals (Jiang et al., 2000a), and a 40% decrease in plasma urea concentration, which is suggestive of reduced amino acid catabolism in plasma-fed pigs (Jiang et al., 2000a,b). Similar results have been observed in mice, where feed conversion efficiency was also improved by inclusion of spray-dried plasma at the expense of dried skim milk in *ad libitum*-fed weaning diets (Thomson et al., 1994a; Thomson et al., 1995). This was associated with increases in both nitrogen intake and retention as well as efficiency of nitrogen retention (Thompson et al., 1995).

2.5 EFFECTS OF SPRAY-DRIED BOVINE COLOSTRUM

At present, spray-dried colostrum is not commonly included in weaner diets and research evaluating its potential use as a dietary ingredient in the pig industry is limited. However, some research investigating its use as a liquid supplementary feed for piglets during the suckling period has been conducted by King et al. (1999a,b) and Pluske et al. (1999b). These authors demonstrated that the provision of liquid bovine colostrum during the suckling period can increase small intestine villus height (King et al., 1999a) and alter the profile of inflammatory T cell populations in the lamina propria (Pluske et al., 1999b) in the pre-weaning period, although King et al. (1999b) observed that the improvement in intestinal morphology disappeared within 24 hours of weaning onto a conventional weaning diet. The use of bovine colostrum as a dry ingredient in weaning diets has been investigated by Pluske et al. (1999a) and Dunshea et al. (2002), and will now be discussed.

2.5.1 Feed intake, growth rate and feed conversion efficiency

Inclusion of spray-dried bovine colostrum in weaner diets has been shown to produce similar results to spray-dried plasma. Pluske et al. (1999a) demonstrated that inclusion of 5 and 10% spray-dried colostrum in a weaner diet can improve feed intake, average daily gain and feed conversion ratio, and reduce the number of days taken for pigs to reach slaughter weight, compared to pigs offered a diet containing no colostrum (Table 5). Given that growth rate was increased by 12 and 25% respectively for pigs offered diets containing 5 and 10%

colostrum, the authors suggested that spray-dried colostrum is a potential alternative to spray-dried plasma products for use in weaner diets (Pluske et al., 1999a).

A more recent study by Dunshea et al. (2002) measured growth rate, feed intake and feed conversion ratio of piglets weaned at 14 days of age and offered diets containing either soy protein, dried skim milk, freeze-dried porcine plasma or freeze-dried bovine colostrum for 35 days after weaning. Overall, the performance of pigs offered the soy protein diet was poorer than those offered the other diets (Dunshea et al., 2002), which supports the current hypothesis that soy proteins impair growth after weaning by causing adverse changes in intestinal morphology and function due to induction of an immunological hypersensitivity reaction (see King et al., 2003). Performance of pigs offered the plasma or colostrum diets did not differ from that of pigs offered the dried skim milk diet in the first week after weaning, however in the second week after weaning pigs offered the colostrum or plasma diets tended to grow faster than those offered the other diets, and were significantly heavier by 35 days of age (Dunshea et al., 2002). An interesting aspect of this study is the comparable performance of pigs consuming diets containing bovine colostrum and those consuming porcine plasma, which suggests that colostrum may be a potential alternative to plasma products in pig diets.

Table 5. Growth rate, feed intake, feed conversion ratio and number of days taken to reach slaughter weight (83kg) of weaner pigs offered diets containing 0, 5 and 10% spray-dried bovine colostrum for the first week after weaning at 28 days of age[1].

	Dietary spray-dried bovine colostrum		
	0%	5%	10%
ADG	114[a]	161[b]	204[c]
ADFI	184	206	230
FCR	1.66	1.40	1.14
Days to slaughter	121.6[a]	118.7[b]	117.4[b]

ADG, average daily gain (g); ADFI, average daily feed intake (g), FCR, feed conversion ratio.
[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).
[1] Adapted from Pluske et al. (1999a).

2.6 ACTIVE COMPONENTS

2.6.1 Spray-dried plasma

Plasma proteins comprise a dynamic system and possess varied biological functions, making it difficult to determine which component of plasma may be causing the observed effects when it is fed to animals. The experimental approach to this problem has generally entailed fractionating the plasma into three fractions, based on molecular weight. The resultant fractions can be generally labelled as fibrinogen and globulin protein (high molecular weight, ≥ 100 kDa), albumin (medium molecular weight, 10 – 100 kDa) and low molecular weight peptides (≤ 10 kDa).

The effect of the three molecular weight class fractions on animal performance has been evaluated in several experiments with pigs (Gatnau et al., 1995; Owen et al., 1995a; Pierce et al., 1995b; Weaver et al., 1995) and mice (Thomson et al., 1994b; Godfredson-Kisic, 1998). A summary of the data gained from pig trials (Table 6) shows that pigs fed the low molecular weight fraction perform consistently worse than the positive control, whereas pigs fed the medium molecular weight fraction show a variable response, often not significantly different from the positive control. However the response of pigs fed the high molecular weight fraction consistently matches the response shown by pigs fed whole spray-dried plasma. Further research into the effects of the medium and high molecular weight fractions using mice showed a similar performance response to dietary inclusion of either plasma component alone, and that, when included together, a partially additive effect is observed which resulted in significantly greater performance compared to mice offered diets containing the medium and high molecular weight fractions alone (Thomson et al., 1994b). In the absence of further research addressing the effect of the medium molecular weight fraction it is difficult to draw any conclusions as to its role in the function of spray-dried plasma.

Due to the consistent effect of the high molecular weight fraction, further work was conducted to assess the relationship between its inclusion rate and pig performance. Generally, this relationship is shown to be quadratic, with the greatest response in piglet feed intake and growth observed when the high molecular weight fraction is included at concentrations approximating those found in a diet containing 8% spray-dried porcine

plasma (Pierce et al., 1995a,b). However, one experiment showed that a dietary concentration of the high molecular weight fraction approximating that found in a diet containing 4% spray-dried plasma was most effective (Pierce et al., 1996). It is perhaps noteworthy that the former experiments (Pierce et al., 1995a,b) were conducted using porcine plasma and the high molecular weight fraction derived from porcine plasma, whereas the latter experiment was conducted with bovine plasma and bovine plasma-derived high molecular weight fraction. It is unclear what effect this may have had on the results of these experiments.

Table 6. Performance effects of three molecular weight class fractions of spray-dried plasma, expressed as a percentage change relative to performance of pigs fed diets containing spray-dried plasma (positive control).

	HMW[bc]	MMW	LMW	Time of feeding[a]	Reference
ADG[d]	+18	-42	-63**		
ADFI	+4	-7	-27**	19-34	Gatnau et al. (1995)
FCR	-6	+33**	+34**		
ADG	-3	-10	-57**		
ADFI	-5	-8	-35**	15-22	Weaver et al. (1995)
FCR	-2	+3	+34**		
ADG	+5	-34***	-48***		
ADFI	-11	-39***	-35***	21-28	Pierce et al. (1995b)
FCR	-5	+4	+25		
ADG	+8	-6	-10*		
ADFI[e]	-2	-11	-21	21-35	Owen et al. (1995a)
FCR	-9*	-6*	-14*		

*, **, *** Significantly different from positive control value ($P < 0.10$), ($P < 0.05$) and ($P < 0.01$), respectively.
[a] Piglet age, in days.
[b] HMW = high molecular weight, MMW = medium molecular weight, LMW = low molecular weight.
[c] All plasma fractions were added to diets at concentrations which approximated their respective concentrations in the positive control diet.
[d] ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; Values indicate % change in variable relative to control.
[e] Feed intake calculated from reported ADG and FCR values.

The active compound or compounds present in spray-dried plasma appear to be predominantly present in the high molecular weight fraction. This fraction is composed primarily of IgG, measured at concentrations of 57% (Pierce et al., 1995a) to 61% (Pierce et al., 1995b). Due to the high proportion of immunoglobulins in this fraction it is often commonly referred to as the immunoglobulin or IgG-fraction, although it should be noted

that other compounds other than immunoglobulin G are present. Other proteins likely to be present in this fraction include IgA and IgM, the α-globulins ceruloplasmin and α_2-macroglobulin, the β-globulin complement component-C3, and fibrinogen (Morrissey et al., 1989).

2.6.2 Spray-dried colostrum

Like spray-dried plasma, spray-dried colostrum is composed of numerous compounds which perform complex and varied functions when ingested by neonatal animals (Donovan et al., 1994; Playford et al., 2000). However the extent to which these compounds are efficacious when colostrum is fed to the juvenile or adult animal is not well established. Colostrum has demonstrated potent growth-promoting effects in neonatal pigs, such as the stimulation of protein synthesis (Burrin et al., 1992, 1997), as well as growth and development of the intestine (Simmen et al. 1990; Wang and Xu, 1996; Xu, 1996) and other organs (Mubiru and Xu, 1997). The effects of colostrum have been shown to be due to nutrient-dependent and nutrient-independent factors (Burrin et al., 1995; Fiorotto et al., 2000). Many components of colostrum have been isolated and their effects after oral administration tested in animals (see Donovan et al., 1994; Playford et al., 2000), particularly growth factors such as IGF-I, EGF and TGF-α, which have variously been shown to induce epithelial proliferation in the gastrointestinal mucosa (Burrin et al., 1996; Houle et al., 1997), to protect the intestinal epithelium from injury (Playford et al., 1999; Berlanga et al., 2002), and to stimulate mucosal repair (Zijlstra et al., 1994; Rhoads et al., 1995; Blikslager et al., 1999).

The similar immunoglobulin composition and production method of spray-dried plasma and spray-dried colostrum suggests that immunoglobulins are likely to comprise an active component of colostrum, however colostrum has not yet been fractionated and fed to pigs after weaning to observe the relative activity of the different fractions. However, Playford et al. (1999) have fractionated colostrum to determine the effect of different molecular weight fractions on reducing non-steroid anti-inflammatory (NSAID) induced gastrointestinal injury in rats and mice, and stimulating rat intestinal epithelial cell proliferation and migration (as a model of wound repair) *in vitro*, and reported that the positive effect of whole colostrum on these variables was due primarily to the biological activity of the fraction with a molecular weight of greater than 30 kDa. This molecular weight would exclude many but not all of the growth factors present in colostrum, and would include colostral immunoglobulins (this will

be discussed in greater detail in the following section). These results are limited in their application to the feed intake and growth of pigs over the weaning period, however they are interesting in light of the intestinal inflammation and injury which accompanies weaning in pigs (King et al., 2003), and suggest a potential role for dietary colostrum in ameliorating the intestinal health of pigs over weaning.

2.7 POSSIBLE MECHANISMS OF ACTION

In summary, spray-dried plasma and colostrum generally increase feed intake and growth rate, and have the potential to improve the efficiency of feed conversion, during the immediate post-weaning period. The mechanisms through which they produce these effects in the pig are currently unknown, although some research has provided useful data on the metabolic and physiological effects of both products when included in weaner pig diets. There are at least three potential hypotheses which could account for the action of dietary spray-dried plasma, which will now be discussed.

2.7.1 Stimulation of feed intake

The characteristic increase in feed intake when spray-dried plasma is included in weaner diets may be attributed to an increase in palatability such as observed by Ermer et al. (1994), however there have been no palatability tests conducted on spray-dried colostrum. The growth stasis observed at weaning is, in part, due to a stress response that may be counteracted by ensuring adequate feed intake during the initial 2 days post-weaning, which will support continued growth (McCracken et al., 1995). High palatability may therefore account for the high feed intake and growth rate of pigs offered diets containing spray-dried plasma or colostrum.

Another possible explanation for the improvement in feed intake of pigs offered dietary spray-dried plasma or colostrum is that they may alter the expression of various neuroendocrine regulators of voluntary feed intake. Although this has not been investigated for spray-dried colostrum, it has been explored by Dyer et al. (1998) and Dyer et al. (1999), who demonstrated no effect of dietary spray-dried plasma on expression of mRNA for neuropeptide Y, adipose tissue leptin, orexin and orexin receptor type 2 in weaner pigs. These findings suggest that this is an unlikely mechanism of action for spray-dried plasma,

but the absence of similar research for spray-dried colostrum prevents any conclusions from being made with regard to this product.

2.7.2 Passive immunological protection

Other phenomena observed during the feeding of diets containing spray-dried plasma are not so easily explained by an increase in feed intake alone. For example, the efficacy of spray-dried plasma is increased in a 'dirty' or immunologically challenging environment (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995; Stahly et al., 1995; Touchette et al., 1996; Bergstrom et al., 1997; Bregendahl et al., 1998; Chirra et al., 1999), and feed conversion efficiency can improve when spray-dried plasma is included in diets (Kats et al., 1994; Smith et al., 1995; Campbell et al., 1998a,b; Jiang et al., 2000a,b), particularly if pigs are also housed in an environment which provides some immune stimulation (Coffey and Cromwell, 1995).

Since the high molecular weight fraction, which is composed predominantly of immunoglobulins, has been identified as the most active fraction of spray-dried plasma, a hypothesis based on immunological mechanisms may offer an explanation of the beneficial effects of spray-dried plasma. This was proposed by Coffey and Cromwell (1995) who suggested that that the immunoglobulins present in spray-dried plasma may provide passive immunological protection against enteropathogenic bacteria in the small intestine of the newly-weaned piglet. This could improve the immunocompetence of the gastrointestinal tract, and reduce mucosal damage caused by bacterial and viral colonisation, and help maintain mucosal integrity over the weaning period.

The immediate post-weaning period is accompanied by a decrease in the integrity of the intestinal epithelium, which can compromise the normal epithelial barrier function that prevents antigenic material from entering the underlying lamina propria (Spreeuwenberg et al., 2001; Verdonk, 2001). There is considerable evidence that this is initiated by transient anorexia after weaning (Kelly et al., 1984; McCracken and Kelly, 1984), and it is hypothesised that the compromised epithelial barrier allows antigenic material from the gastrointestinal lumen to invade the lamina propria and initiate an active immune response (McCracken et al., 1999). An active, inflammatory immune response in the intestine of young pigs produces pro-inflammatory cytokines and other inflammatory mediators which induce both local and systemic effects that can exacerbate the problem by further impairing epithelial integrity (MacDonald et al., 1999), depressing voluntary feed intake, and partitioning nutrients away

from growth processes towards immune function (Kelley et al., 1994; Johnson, 1997; Stahly, 2001).

According to this hypothesis, the immunoglobulins present in spray-dried plasma (and potentially colostrum) bind antigens in the gastrointestinal lumen, inactivating them and reducing the incidence and/or extent of intestinal infection, inflammation and injury during the immediate post-weaning period (Coffey and Cromwell, 1995). This diminishes the production of pro-inflammatory cytokines and other mediators that accompany active immune responses, reducing both their restrictive influence on voluntary feed intake and the partitioning of nutrients towards immunological activity (King et al., 2003)

Supporting this hypothesis, Jiang et al., (2000b) observed a reduction in lamina propria cell density and small intestine weight in pigs offered diets containing spray-dried plasma, an effect suggested by the authors to be indicative of reduced intestinal inflammation. Furthermore, Carroll et al. (2002) observed that the activation of the hypothalamic-pituitary-adrenal axis after weaning is reduced in pigs offered dietary spray-dried plasma, which accords with a reduction in pathogen exposure and immune system activation in these pigs after weaning. Similarly, Touchette et al. (1999b) observed that dietary spray-dried plasma alters the responsiveness of the hypothalamic-pituitary-adrenal axis of weaner pigs during an *E. coli* challenge. Touchette et al. (2002) and Carroll et al. (2002) also reported that dietary spray-dried plasma increases the proinflammatory cytokine and hypothalamic-pituitary-adrenal axis response of pigs to lipopolysaccharide and *E. coli* challenge, reactions which suggest greater immunological naïveté on the part of plasma-fed pigs, supporting the notion of a protective effect of dietary plasma. However, Dritz et al. (1996) reported that dietary spray-dried plasma had no effect on production of the acute-phase protein haptoglobin in lipopolysaccharide-challenged pigs, which suggests that cytokine expression may not have been influenced by dietary plasma in their study.

Despite the degree of research interest in this hypothesis there has been little attention given to characterisation of the populations of immune cells in the small intestine of pigs ingesting diets containing spray-dried plasma or colostrum during the post-weaning period. Of particular interest in this regard are helper ($CD4^+$) T cells and cytotoxic ($CD8^+$) T cells, whose profiles alter during inflammatory responses in the small intestine (see King et al., 2003). Pluske et al. (1999b) demonstrated a diet composed entirely of rehydrated spray-dried

colostrum offered to pigs before and after weaning reduced jejunal CD4+ T cell proliferation in the first 24 hours after weaning by 25% compared to those offered a conventional solid starter diet devoid of colostrum, while CD8+ T cell populations were unaffected by dietary treatment. This observation may indicate an anti-inflammatory effect of dietary colostrum during weaning, however it could also be attributed to greater dry matter intake observed in the colostrum-fed pigs (Pluske et al. 1999b), which may reduce the incidence of inflammation (McCracken et al., 1999).

Further support for the passive immune protection hypothesis is provided by Deprez et al. (1990, 1996) who reported a reduction in faecal excretion of haemolytic *E. coli* in pigs offered diets containing spray-dried plasma, regardless of whether the plasma contained antibodies specific to the challenge strain of *E. coli*. This suggests that non-specific factors such as glycoproteins may contribute significantly to the immune protection afforded by plasma. Further observations were reported by Nollet et al. (1999), who demonstrated that a high concentration of dietary spray-dried plasma (45-90 g plasma/pig/day) reduces faecal excretion of pathogenic *E. coli* in pigs after weaning. However a more recent experiment by van Dijk et al. (2002a), which used the more commercially viable inclusion rate of 8% plasma in the diet, showed no effect of dietary plasma on *E. coli* concentrations at different sites of the intestine, and Cain and Zimmerman (1997) also found no effect of dietary plasma on faecal shedding of haemolytic *E. coli*. However, despite the absence of a reduction in the presence of *E. coli* in the intestine, van Dijk et al. (2002a) demonstrated that challenged pigs offered a diet containing spray-dried plasma had improved weight gain, feed intake, faecal score and condition score compared to challenged pigs offered a diet containing no plasma, and Cain and Zimmerman (1997) reported a reduction in post-weaning diarrhoea due to dietary plasma. A lower incidence of post-weaning diarrhoea attributable to dietary spray-dried plasma was also noted by Gatnau and Zimmerman (1991) and Van der Peet-Schwering and Binnendijk (1995). In a recent series of experiments using *E. coli* K88 challenged piglets, Bosi et al. (2001) observed no reduction in faecal shedding of the challenge strain when pigs were offered dietary spray-dried plasma, however plasma-fed pigs did exhibit decreased K88-specific IgA titres in plasma and saliva and reduced mortality rate, suggesting that dietary plasma may have reduced the level of infection after *E. coli* challenge without affecting *E. coli* proliferation in the intestine.

Evidence that dietary spray-dried plasma can improve gut structure and function over the weaning period has been provided by studies of the morphology and enzyme activity of the small intestine. Dietary spray-dried plasma has been demonstrated to increase villus surface area (Gatnau et al., 1995), increase crypt depth (Touchette et al., 2000), increase villus height and ratio of villus height to crypt depth (Spencer et al., 1997; Touchette et al., 1997; Touchette et al., 1999b) and increase mucosal maltase and lactase activity (Cain et al., 1992; Gatnau et al., 1995). However, other studies have shown no effect of dietary spray-dried plasma on intestinal morphology (Touchette et al., 1999c; Jiang et al., 2000b; van Dijk et al., 2001b, 2002b) and intestinal disaccharidase activity (van Dijk et al., 2002b). These contradictory results are perhaps indicative of the complex and multifactorial interactions which may influence the effect of dietary spray-dried plasma. There is considerable evidence that intestinal morphology is greatly affected by the level of luminal nutrition (see King et al., 2003), with morphological parameters such as villus height commonly varying in direct proportion to the level of luminal nutrition received. Since most of the studies evaluating the effect of dietary plasma on intestinal morphology have not investigated the influence of the level of feed intake on this parameter, it is difficult to separate the effect of dietary plasma from that of dietary intake.

One study which did investigate the effect of dietary intake and spray-dried plasma on intestinal morphology was that of Touchette et al. (1997). In this experiment pigs were fed a diet containing no plasma either *ad libitum* or fed to the level of intake of pigs offered a diet containing 7% spray-dried plasma, and pigs fed the diet containing 7% spray-dried plasma were fed *ad libitum* or at the level of intake of pigs fed the no plasma diet. It was found that pigs fed the plasma diet *ad libitum* had greater feed intake and weight gain, longer villi and higher ratio of villus height to crypt depth compared to pigs fed the no plasma diet *ad libitum*, but when the intake of pigs fed the plasma diet was reduced to the level of pigs fed the no plasma diet *ad libitum*, the plasma diet had no effect on weight gain and intestinal morphology. However, pigs fed the no plasma diet to the level of intake of pigs offered the plasma diet *ad libitum* displayed equivalent weight gain but lower villus height, greater crypt depth, and lower ratio of villus height to crypt depth. These results demonstrate the significant effect of feed intake on intestinal morphology, but also show that dietary spray-dried plasma can improve intestinal morphology in a way that cannot be accounted for by an increase in the level of feed intake alone.

There is some evidence that dietary colostrum can increase small intestine villus height in pigs during the pre-weaning period when offered in liquid form as a supplementary feed (King et al. 1999a). However, this effect is lost during the first 24 hours after weaning onto a standard weaner diet devoid of colostrum (King et al., 1999b). These experiments also did not investigate the effect of feed intake on intestinal morphology, and King et al. (1999a) reported a higher intake in pigs offered the colostrum diet compared to those offered the liquid control diet of whey protein concentrate, which could account for some or all of the observed increase in villus height.

2.7.3 Hormonal effects

Plasma contains an array of hormones and growth factors (Antoniades, 1977), however research into the mechanism of action of spray-dried plasma has focused particularly on IGF-I (de Rodas et al., 1995; Dyer et al., 1998, 1999; Matteri et al., 2000), which is implicated in the improved growth performance of weaner pigs fed diets containing antimicrobial additives (Hathaway et al., 1996), and has been shown to improve intestinal morphology in pigs after oral administration (Burrin et al., 1996; Houle et al., 1997). However, there is at present little evidence to suggest that the positive effect of dietary spray-dried plasma on feed intake, growth rate and intestinal morphology is due to either the presence of IGF-I in the diet of plasma-fed pigs, or an influence of dietary plasma on circulating concentration of IGF-I. Experiments by de Rodas et al. (1995), Dyer et al., (1999) and Matteri et al. (2000) failed to show any significant alterations in circulating IGF-I or IGF binding protein (IGFBP) concentrations or tissue expression of IGF-I or IGFBP mRNA, regardless of whether plasma-fed pigs displayed improved performance or not. Dyer et al. (1998) observed no effect of dietary plasma on circulating IGF-I nor IGF-I mRNA expression in adipose tissue or liver, but hypothalamic IGF-I mRNA was reported to decrease in plasma-fed pigs. De Rodas et al. (1995) also demonstrated no effect of dietary plasma on circulating concentrations of insulin and glucose, although plasma-fed pigs did display a tendency for higher plasma growth hormone concentration compared to pigs offered diets containing no plasma.

Similar to spray-dried plasma, colostrum also contains IGF-I and IGF-II along with other growth factors such as TGF-α TGF-β, EGF (Playford et al., 2000) and bovine colostrum-derived growth factor, about which little is presently known other than its molecular weight (30-35 kDa), its structural relation to platelet-derived growth factor, and its ability to

stimulate proliferation of mouse fibroblast 3T3 cells (Shing and Klagsbrun, 1984; Shing and Klagsbrun, 1987). However, given that the use of spray-dried colostrum in weaning diets is a relatively new concept, there has been no research investigating whether these compounds may be responsible for the improvement in performance of pigs offered dietary spray-dried colostrum after weaning. Recent research has indicated that dietary bovine colostrum can reduce or prevent gastric injury and villus atrophy caused by non-steroidal anti-inflammatory (NSAID) drugs in a rat model of gastric injury and mouse model of small intestinal injury, respectively (Playford et al., 1999). Playford et al. (1999) demonstrated that this effect was largely attributable to compounds with a molecular weight of greater than 30 kDa, which eliminates many growth factors such as EGF and TGF-α, permitting the authors to suggest the high molecular weight form of TGF-β or bovine colostrum-derived growth factor as possible causative factors. The mechanisms responsible for NSAID-induced gastrointestinal injury are complex and their similarity to the inflammation and injury which occurs at weaning in pigs, which is yet to be fully characterised and understood (King et al., 2003), is uncertain. However the potential for bovine colostrum to prevent gastrointestinal damage in these models suggests that this might also be a mechanism by which it improves pig performance over the weaning period.

Given that orally administered IGF-I is only poorly absorbed by the newborn piglet prior to gut closure (Donovan et al., 1997) it is unlikely that the IGF-I present in dietary plasma and colostrum is capable of contributing to the pool of circulating endogenous IGF-I in weaner pigs. It has also been demonstrated that super-physiological doses of oral IGF-I are required to cause significant effects on gut morphology, although physiological doses may stimulate crypt cell proliferation (Burrin et al., 1996). IGF-I receptors are present throughout the small intestine of the pig, and have been shown to increase in number and activity at 21 days of age, which coincides with weaning in many production systems (Schober et al., 1990), so the possibility of dietary IGF-I affecting the physiology of the pig either locally in the intestine, or systemically, can not be discounted. However, given that the high molecular weight fraction ($\geq$ 100 kDa) of spray-dried plasma replicates the effect of feeding whole plasma, and the > 30 kDa fraction of colostrum appears to be the most biologically active in rat and mouse models of intestinal injury, IGF-I, which has a molecular weight of 7.649 kDa (Rinderknecht and Humbel, 1978), seems unlikely to be involved. The possibility that other bioactive compounds present in plasma and/or colostrum are responsible for some or all of their effects can not be excluded from consideration, in the absence of further research.

2.8 CONCLUSION

Characterising the exact nature of the action of spray-dried plasma poses an interesting challenge for scientists. It has proven to be a suitable protein source for use as a replacement for other, highly digestible sources of protein such as dried skim milk and casein. However, plasma contains a varying array of components with different biological functions, which commonly effect an increase in weaner pig feed intake and growth rate. These effects are manifested to the greatest degree when pigs are housed in conditions that provide a significant immune challenge to the pig, such as continuous-flow management systems, or unclean housing. The spray-dried plasma fraction predominantly composed of immunoglobulins has most consistently reproduced the effects observed when plasma is included in weaner diets. These observations support the development of a hypothesis based on immunological mechanisms to explain the action of spray-dried plasma.

Regardless of its mechanism of action, spray-dried plasma has proven itself a useful dietary ingredient in countries like the United States. It has provided a highly digestible source of protein, which can be produced in a consistent quantity and quality for pig producers, and a value-added alternative to dried blood meal for the rendering industry. It can also act as a useful management tool for producers whose production system provides an immunologically challenging weaning environment.

Unfortunately there is insufficient information detailing the effects of dietary spray-dried colostrum on pigs after weaning to draw any conclusions about its efficacy relative to spray-dried plasma, its effect in varying environments and at varying weaning ages, and its mechanisms of action. However, given the promising results from the few experiments conducted in this area, spray-dried colostrum shows potential for use as a specialty ingredient for inclusion in diets around the time of weaning.

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SECTION II

Experimental Research

Chapter 3

DIETARY BOVINE COLOSTRUM INCREASES VILLUS HEIGHT AND DECREASES SMALL INTESTINE WEIGHT IN EARLY-WEANED PIGS.

3.1 INTRODUCTION

In modern pig production, weaning generally occurs at two to four weeks of age and is commonly associated with a 'growth check' which varies in severity, but can last for up to two weeks after weaning (Pluske et al., 1995). Weaning is also associated with dramatic alterations in the morphology and histology of the small intestine, such as rapid villus atrophy and crypt hyperplasia, along with alterations in the specific activity of brush border enzymes, which may decrease the digestive and absorptive capacity of the intestine (Pluske et al., 1997).

The aetiology of these weaning problems is complex and multi-factorial, however low voluntary feed/nutrient intake has been identified as a major cause of the alterations in small intestine structure (Cera et al., 1988; Kelly et al., 1991a, b; McCracken et al., 1995, 1999; Pluske et al., 1996a, b, c), and maintenance of feed/nutrient intake over the weaning period is sufficient to maintain the structure and function of the small intestine (Pluske et al., 1996a, b, c). It has been proposed that the alterations in intestinal structure compromise the integrity of the mucosal barrier, allowing luminal antigens greater access to the intestinal lamina propria, inducing local inflammation (McCracken et al., 1999). The increased expression of matrix metalloproteinases that occurs during the intestinal inflammatory response may increase degradation of the extracellular matrix, resulting in further compromising changes in gut morphology (Goetzl et al., 1996). Increased expression of the matrix metalloproteinase stromelysin has recently been reported in association with villus atrophy and weaning anorexia in pigs (McCracken et al., 1999).

Increasing voluntary feed intake in the immediate post-weaning period may therefore reduce the intestinal damage associated with weaning, improving nutrient digestion and absorption as well as piglet health and growth during this time. Inclusion of spray-dried bovine colostrum in weaning diets has been shown to improve piglet feed intake and growth rate immediately after weaning (Pluske et al., 1999a). The improvement in feed intake is similar to that observed when spray-dried plasma is included in the diet, an effect that is generally attributed to the immunoglobulin fraction of plasma (Gatnau et al., 1995; Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995). Spray-dried bovine colostrum contains similar concentrations of immunoglobulins, so it is possible that the two products share a common mode of action. The presence of immunoglobulins in the weaning diet may also provide passive immunological protection in the small intestine, similar to that provided by immunoglobulins present in sows milk during the suckling period, which could bind and

inactivate luminal antigens, preventing their infiltration of the lamina propria and thereby reducing mucosal inflammation and adverse changes in small intestine morphology. Dietary immunoglobulin G has shown resistance to proteolysis in the small intestine of the pig (Morel et al., 1995) and human (Roos et al., 1995), and the use of spray-dried colostrum in diets for pre-wean piglets has been shown to increase villus height (King et al., 1999) and reduce intestinal T-cell proliferation (Pluske et al., 1999b). The hypothesis tested in this study was that inclusion of spray-dried bovine colostrum in a weaner diet would improve feed intake and improve intestinal health over the immediate post-weaning period, improving piglet growth during this time.

3.2 MATERIALS AND METHODS

3.2.1 Animals and conduct of the trial

Experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Recommendations and Minimum Standards for the Care and Use of Animals for Scientific Purposes (New Zealand Animal Welfare Advisory Committee, 1995).

The experiment used 12, 14-day-old mixed-sex piglets (Large White x Landrace; 3.6 ± 0.1 kg), which were obtained from a commercial piggery. Piglets were blocked by litter of birth and live weight and randomly assigned to receive either a control diet (CON) or a diet containing 5% spray-dried bovine colostrum (BC). Piglets were housed individually in stainless steel cages which permitted no contact between animals, and were separated from their waste products by perforated stainless-steel flooring. Airflow to the facility was controlled, and room temperature was maintained at 30°C with a 12-h light/dark cycle. The diets were offered *ad libitum* from day 1-14 of the experiment. Piglet live weight was measured at days 1, 7 and 14, and food intake was recorded at days 7 and 14.

3.2.2 Dietary Treatments

The experimental diets CON and BC were based on wheat and delactosed whey (Table 1), and were formulated to contain 14.8 MJ DE/kg, 1.26% available lysine, and to meet or exceed National Research Council (1998) recommendations for major nutrients, with lysine as the first limiting amino acid. Diet BC contained 5% bovine colostrum, providing a concentration of 7.5g/kg IgG. Diets were fed in meal form. Composition of the spray-dried

bovine colostrum product used in this experiment (Immulac15; Specialty Ingredients Division, Fonterra, Hautapu, New Zealand) has been provided elsewhere (Chapter 2, this thesis).

Table 1. Percentage composition and calculated analysis of the experimental diets.

	Experimental diets[1]	
Ingredient, %	CON	BC
Wheat	42.85	45.43
Delactosed whey powder	20.00	20.00
Fishmeal	10.00	7.50
Meat and bone meal	2.90	-
Ring-dried blood meal	2.50	2.50
Skim milk powder	5.00	5.00
Bovine colostrum[2]	-	5.00
Soybean meal	8.00	8.00
Sugar	5.00	5.00
Soybean oil	0.76	0.11
L-Lysine	0.19	0.20
D, L-Methionine	1.62	0.15
L-Threonine	0.37	0.31
Dicalcium phosphate	-	-
Salt	0.35	0.35
Tylan[3]	0.15	0.15
Vitamin and mineral premix[4]	0.30	0.30
Calculated Analysis		
DE[5], MJ/kg	14.8	14.8
Crude protein, %	22.5	22.6
Total lysine, %	1.72	1.75
Available lysine, %	1.26	1.26

[1] CON, control; BC, bovine colostrum.
[2] Immulac15 (Specialty Ingredients Division, Fonterra, Hautapu, New Zealand).
[3] Tylan (Elanco Animal Health, Auckland, New Zealand).
[4] Vitastart (Vitec Nutrition Ltd, Auckland, New Zealand). Supplied per kilogram diet: Mn, 45 mg; Zn, 120 mg; Cu, 125 mg; Co, 0.5 mg; I, 1 mg; Fe, 100 mg; Se, 300 µg; Vitamin A, 15 000 IU; Vitamin D_3, 2000 IU; Vitamin E, 70mg; Vitamin K, 2.5 mg; Vitamin B_1, 2 mg; Vitamin B_2, 3 mg; Vitamin B_6, 2 mg; Vitamin B_{12}, 30 µg; Calcium Pantothenate, 20 mg; Niacin, 20 mg; Biotin, 100 µg; Folic Acid, 500 µg; Choline 150 mg.
[5] DE, digestible energy.

3.2.3 Post-mortem procedure

On day 14 of the experiment (piglets 28 days of age) all piglets were euthanased and post-mortem measurements taken. Piglets were sedated with Stresnil (SmithKline Beecham Animal Health, Auckland, New Zealand), at a dosage of 1ml/5kg, 20 minutes prior to slaughter. Piglets were then euthanased by an intracardial injection of sodium pentobarbitone (125 mg/kg live-weight). The abdomen was opened immediately, from the sternum to the pubis, and the entire gastrointestinal tract removed. A pair of scissors was used to disconnect the small intestine at the gastric pylorus and the ileo-caecal valve, and the intestine was clamped. The small intestine was laid out on a stainless-steel tray and a section at proportionally 25, 50 and 75% along the intestine was clamped with haemostats, excised and placed immediately into a plastic container with Bouin's fluid (24% formalin, 5% glacial acetic acid, 71% picric acid). After fixation for 24 hours, the Bouin's fluid was replaced with 70% ethanol. The thymus, spleen, liver and pancreas were removed and weighed. The stomach, caecum, and large intestine were weighed upon removal with their contained digesta, then emptied, washed with water, blotted dry, and weighed again. The piglet was weighed again, to obtain the carcass weight. Total processing time, from killing to obtaining gut samples, was about 10 minutes. Sites at 0.25, 0.5 and 0.75 are referred to by their approximate position in the small intestine, i.e. proximal jejunum, mid-jejunum and distal ileum, respectively.

3.2.4 Histology and immunocytochemistry

After fixation, ring-shaped lengths of small intestine from all three sites were excised, dehydrated and embedded in paraffin wax. From each of these, 4 transverse sections (6 μm) were cut, stained with haematoxylin and eosin and alcian blue, and examined under a light microscope. Measurements of villus height and crypt depth were taken from sections where the plane of section ran vertically from the tip of each villus to the base of an adjacent crypt. For each section the image analysis software Sigma Scan (Jandel Scientific, San Rafael, CA), and a light microscope were used to measure 10 of the tallest, well oriented villi from villus tip to crypt mouth, and 10 associated crypts from crypt mouth to base. Measurement of epithelial cell height on the 10 villi was also performed, by taking 6 measurements of epithelial cell height at even distances along both sides of the villus length.

$CD4^+$ and $CD8^+$ T lymphocytes were identified by immunocytochemistry. Briefly, Bouin's fluid-fixed, paraffin wax-embedded samples from the mid-jejunum were transversely sectioned (6 μm) onto glass slides. Sections were de-paraffinised in two changes of xylene (7 minutes each), rehydrated through a graded series of alcohol washes and brought to water. Endogenous peroxidase was exhausted by immersing sections in 6% hydrogen peroxide for 30 minutes. Sections were then washed in 0.01M phosphate buffered saline (PBS) (pH 7.2), and antigen retrieval was performed by incubating sections in 0.1M phosphate-citrate buffer (pH 6.0) for 60 minutes at 60°C. Sections were then washed in three changes of PBS and non-specific binding sites were blocked by incubating in a humidity chamber with 1% bovine serum albumin for 5 minutes at room temperature. Sections were drained and a 1:100 dilution of murine polyclonal anti-porcine CD4 (clone 74-4-12) or anti-porcine CD8 (clone 76-2-11) antibody (VMRD Inc., Pullman, WA, USA) was applied. Sections were then incubated in a humidity chamber for 1 hour at room temperature. Sections were then washed in 3 changes of PBS and incubated in a humidity chamber with a 1:200 dilution of biotinylated anti-mouse IgG (Amersham Biosciences UK Ltd., Buckinghamshire, England) for 30 minutes at room temperature. Sections were then washed in 3 changes of PBS and incubated in a humidity chamber with a 1:200 dilution of biotin-streptavidin-peroxidase preformed complex (Amersham Biosciences UK Ltd., Buckinghamshire, England) for 15 minutes at room temperature. Sections were then washed in 3 changes of PBS, 3-3 diaminobenzidine solution was applied, and sections were allowed to react for 3 minutes. Sections were then washed in water, dehydrated through a graded series of alcohol washes, cleared in xylene and coverslipped. Black staining $CD4^+$ and $CD8^+$ T lymphocytes were counted at 10x magnification in the lamina propria of 5 well-oriented crypts (McCracken et al., 1999), using a light microscope and an eyepiece graticule. Lymphocyte density is expressed as the number of $CD4^+$or $CD8^+$ T lymphocytes per $0.1mm^2$ of crypt lamina propria.

3.2.6 Statistical analysis

The data were subjected to analysis of variance (ANOVA) by the General Linear Models procedures of SAS (SAS Institute, 2000) using piglet as the experimental unit. Organ weights were expressed as a percentage of the empty body weight.

The statistical model used in the analysis of growth rate, feed intake, organ weight and immunocytochemistry was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

Where:

y_{ijk} = observation from the k^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment.

μ = the population mean.

α_i = the fixed effect of the i^{th} dietary treatment.

β_j = the random effect of the j^{th} litter of birth.

ε_{ijk}= residual error of the k^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment.

The statistical model used in the analysis of small intestine histology was:

$$y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + \alpha_i\gamma_k + \varepsilon_{ijk}$$

Where:

$y_{(i)jk}$ = observation from the k^{th} site from the j^{th} pig nested within the i^{th} dietary treatment.

μ = the population mean.

α_i = the fixed effect of the j^{th} dietary treatment.

$\beta_{j(i)}$ = the random effect of the j^{th} pig nested within the i^{th} dietary treatment

γ_k = the fixed effect of the k^{th} site in the small intestine.

$\alpha_i\gamma_k$ = the interaction between the i^{th} dietary treatment and the k^{th} site of the small intestine.

$\varepsilon_{(i)jk}$= residual error of the k^{th} site from the j^{th} pig nested within the i^{th} dietary treatment.

To discern the effect of time on feed intake growth rate and feed conversion ratio, repeated measure analysis was performed using the following model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \pi_l(\alpha_i, \beta_j) + \chi_k + \chi_k\alpha_i + \chi_k\beta_j + \varepsilon_{ijkl}$$

Where:

y_{ijkl} = observation from the l^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment and the k^{th} time period.

μ = the population mean.

α_i = the fixed effect of the i^{th} dietary treatment.

β_j = the random effect of the j^{th} litter of birth.

$\pi_l(\alpha_i, \beta_j)$ = random effect of the l^{th} pig within the i^{th} dietary treatment and the j^{th} litter of birth.

χ_k = the fixed effect of the k^{th} time period (week 1 or week two after weaning).

$\chi_k\alpha_i$ = the interaction between the k^{th} time and the i^{th} dietary treatment.

$\chi_k\beta_j$ = the interaction between the k^{th} time period and the j^{th} litter of birth.

ε_{ijkl}= residual error of the l^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment and the the k^{th} time period.

Where a significant treatment effect was observed, Fisher's least significant difference test was performed to determine significant differences between least-square means of treatment groups. Level of significance was pre-set at $P < 0.05$, and trends were identified at $P < 0.10$. The effect of sex was tested in all models, found to be non-significant, and was therefore removed. Repeated measure analysis was used to discern the effect of time on feed intake, growth rate and feed conversion ratio during the experiment. Pearson correlation analysis was performed to evaluate a possible correlation between feed intake and organ weights, and morphological variables. Data are presented as least-square means with the associated pooled standard error of the mean (SEM).

3.3 RESULTS.

3.3.1 Feed intake, weight gain and feed conversion ratio

A summary of the feed intake, weight gain and feed conversion ratio data is presented (Table 2). Daily weight gain was not affected by litter of birth or dietary treatment during either the first or second week of the experiment, or both weeks combined ($P > 0.10$), although piglets offered the BC diet grew, on average, 38% faster during the first week, and 7% faster during the second week, and 15% faster in weeks 1 and 2 combined, compared to their counterparts offered the CON diet. Similarly, voluntary feed intake was not affected by litter of birth or diet during either week of the experiment ($P > 0.10$), although there was a trend for an effect of litter during both weeks combined ($P = 0.10$). However piglets offered the BC diet consumed, on average, 16% more feed during the first week, and 8% more feed during the second week of the experiment, compared to their counterparts offered the CON diet. Feed conversion ratio was not affected by litter of birth or diet during either week of the experiment, or both weeks combined ($P > 0.10$), although a 24% numerical improvement in average feed conversion ratio during the first week was observed in piglets offered the BC diet, compared to those offered the CON diet.

Table 2. Influence of dietary spray-dried bovine colostrum on the feed intake and growth performance of weaner pigs.

	Dietary treatment[a]		
	CON	BC	SEM
Daily gain[b], g/day			
Day 1-7	61[1]	84[1]	9
Day 7-14	176[2]	189[2]	7
Day 1-14	120	138	7
Voluntary feed intake, g/day			
Day 1-7	96[1]	111[1]	12
Day 7-14	197[2]	212[2]	8
Day 1-14	148	162	8
Feed conversion ratio			
Day 1-7	1.85	1.41	0.27
Day 7-14	1.13	1.12	0.06
Day 1-14	1.24	1.19	0.05

[a] CON, control diet; BC, bovine colostrum diet.
[b] Least-square mean values with pooled standard error of the mean (SEM).
[1,2] Values within a column with different superscripts are significantly different ($P < 0.05$).

Repeated measure analysis showed that average daily gain and average daily feed intake over the two measurement periods were affected by time period only ($P < 0.0001$), with feed intake increasing significantly in the second week of the experiment. No significant time by diet ($P > 0.10$) or time by litter of birth ($P > 0.10$) interactions were observed for average daily gain and average daily feed intake. Feed conversion ratio showed a trend for an effect of time period ($P = 0.07$), with average feed conversion ratio improving in the second week; no significant time by diet ($P > 0.10$) or time by litter of birth ($P > 0.10$) interactions were observed for this variable.

3.3.2 Empty body weight and organ weights

Empty body weights were unaffected by the addition of dietary colostrum ($P > 0.10$; Table 3), however a significant effect of litter of birth was observed ($P < 0.05$). The weight of all organs was unaffected by diet ($P > 0.10$), with the exception of small intestine weight, which was 12% lower in pigs offered the BC diet, compared to those offered the CON diet ($P < 0.05$). Litter of birth also affected small intestine weight ($P < 0.05$), and showed a trend for an effect on thymic weight ($P = 0.08$) and small intestine length ($P = 0.09$).

Table 3. Influence of dietary spray-dried bovine colostrum on empty body weight and organ weights (expressed as a percentage of the empty body weight) of weaner pigs.

	Dietary treatment[1]		
	CON	BC	SEM
Empty body weight, g	4884	5175	195
Organs[2]			
Pancreas, %	0.20	0.22	0.01
Liver, %	3.69	3.66	0.13
Thymus, %	0.10	0.10	0.01
Spleen, %	0.30	0.36	0.04
Stomach, %	0.98	0.92	0.04
Small intestine			
Weight, %	5.32[a]	4.666[b]	0.17
Length, %	0.19	0.166	0.01
Large intestine			
Caecum, %	0.23	0.250	0.02
Colon, %	1.51	1.41	0.09

[1] CON, control diet; BC, bovine colostrum diet.
[2] Least-square mean values with pooled standard error of the mean (SEM).
[a,b] Least-square means with different superscripts are significantly different ($P < 0.05$).

3.3.3 Histology

Villus height was significantly affected by small intestine site and pig nested within diet ($P < 0.0001$), and showed a site by diet interaction ($P < 0.05$), but not diet alone ($P > 0.10$; Figure 1). Consumption of the BC diet increased villus height in the proximal jejunum, mid jejunum and distal ileum of the small intestine compared to piglets offered the control diet ($P < 0.05$; Table 4). Increases varied from 8% in the proximal jejunum to 17% in the distal jejunum and 20% in the distal ileum ($P < 0.05$). Villus height of piglets offered the CON diet decreased ($P < 0.05$) from the proximal to the distal small intestine. However, consumption of the BC diet maintained mid-jejunal villus height to a level not significantly different from that of the proximal jejunum ($P > 0.10$), with distal ileal villus height lower than the more proximal areas ($P < 0.05$). Pig nested within treatment accounted for 35% of the total variation in villus height. Independent of treatment, a decreasing gradient of villus height was observed from the proximal to distal small intestine, with values in the proximal jejunum, mid-jejunum and distal ileum all significantly different ($P < 0.05$) (461, 431 and 354 μm, respectively, SEM 5.1 μm).

Crypt depth was significantly affected by diet and pig nested within treatment ($P < 0.0001$), and small intestine site ($P < 0.05$), but no site by diet interaction was observed ($P > 0.10$). Crypt depth of piglets offered the BC diet was lower at all sites in the small intestine, compared to their counterparts offered the CON diet ($P < 0.05$; Table 4). This was manifested as an 11% decrease in the proximal jejunum, a 13% decrease in the mid jejunum, and a 16% decrease in the distal ileum ($P < 0.05$), leading to a reduction in overall crypt depth of 13% in pigs consuming the BC diet ($P < 0.05$). The effect of pig nested within diet accounted for 20% of the total variation in crypt depth. Crypt depth of pigs consuming the CON diet was similar from the proximal to distal small intestine ($P > 0.10$), whereas crypt depth of pigs consuming the BC diet was lower in the distal ileum than in the proximal jejunum ($P < 0.05$). Independent of treatment, crypt depth was highest in the most proximal sampling site in the small intestine (proximal jejunum) ($P < 0.05$), with more distal sites (mid-jejunum and distal ileum) showing similar values (221, 212 and 213 μm, respectively, SEM 2.5μm)

The ratio of villus height to crypt depth was significantly affected by small intestine site and pig nested within diet ($P < 0.0001$), diet ($P < 0.05$) and a trend for a diet by site interaction

was observed (P = 0.09). The ratio of villus height to crypt depth was consistently higher in piglets offered the BC diet compared to those offered the CON diet in all sites of the small intestine (P < 0.05), resulting in an overall increase in this variable of 31% in pigs consuming the BC diet (Table 4). The ratio of villus height to crypt depth was 22% greater in the proximal jejunum, 33% greater in the mid jejunum, and 42% greater in the distal ileum of piglets offered the BC diet, compared to those offered the CON diet. A decreasing ratio of villus height to crypt depth was observed from proximal to distal regions of the small intestine in pigs consuming the CON diet (P < 0.05), whereas in pigs consuming the BC diet, the ratio of villus height to crypt depth was similar in the proximal and mid jejunum, decreasing only in the distal ileum (P < 0.05). Pig nested within diet explained 26% of the total variation in the ratio of villus height to crypt depth. Irrespective of diet, the ratio of villus height to crypt depth was higher in the proximal and mid jejunum compared to the distal ileum (P < 0.05) (2.14, 2.09 and 1.72, respectively, SEM 0.03).

Table 4. Influence of dietary spray-dried bovine colostrum on the small intestine histology of weaner pigs.

	Dietary treatment*		
	CON	BC	SEM
Villus height†, μm			
Proximal jejunum	444[1a]	478[1b]	
Mid jejunum	397[2a]	465[1b]	7
Distal ileum	323[3a]	386[2b]	
Mean	388	443	25
Crypt depth, μm			
Proximal jejunum	234[1a]	208[1b]	
Mid jejunum	227[1a]	197[12b]	4
Distal ileum	232[1a]	194[2b]	
Mean	231[a]	200[b]	7
Villus height : crypt depth			
Proximal jejunum	1.93[1a]	2.35[1b]	
Mid jejunum	1.80[2a]	2.39[1b]	0.05
Distal ileum	1.42[3a]	2.02[2b]	
Mean	1.72[a]	2.26[b]	0.13
Epithelial cell height, μm			
Proximal jejunum	26.7	27.2	
Mid jejunum	27.2	28.2	0.4
Distal ileum	23.6	23.4	
Mean	25.9	26.3	0.6

* CON, control diet; BC, bovine colostrum diet.

† Least-square mean values with pooled standard error (SEM).

[a,b] Values with different superscripts within a row are significantly different (P < 0.05).

[1,2,3] Values with different superscripts within a column are significantly different (P < 0.05).

Epithelial cell height was significantly affected by small intestine site and pig nested within treatment ($P < 0.0001$), but not diet (Table 4) nor diet by site interaction ($P > 0.10$). Pig nested within treatment accounted for 12% of the total variation in epithelial cell height. Irrespective of diet, the ratio of villus height to crypt depth was higher in the proximal and mid jejunum compared to the distal ileum ($P < 0.05$) (27.0, 27.7 and 23.5 μm, respectively, SEM 0.3 μm).

The density of mid-jejunal lamina propria $CD4^+$ T lymphocytes (Figure 2i) was significantly affected by diet and litter of birth ($P < 0.05$). Consumption of the BC diet increased the density of $CD4^+$ T lymphocytes by 28% compared to that of piglets consuming the CON diet ($P < 0.05$; Table 5). Litter of birth accounted for 25% of the total variation in $CD4^+$ T lymphocyte density. The density of $CD8^+$ T lymphocytes (Figure 2ii) was also significantly affected by diet and litter of birth ($P < 0.01$), with consumption of the BC diet increasing $CD8^+$ T lymphocyte density by 37% compared to piglets consuming the CON diet ($P < 0.05$; Table 5). Litter of birth accounted for 18% of the total variation in $CD8^+$ T lymphocyte numbers. The ratio of $CD4^+$ to $CD8^+$ T lymphocytes was not affected by diet, nor litter of birth ($P > 0.10$).

Table 5. Influence of dietary spray-dried bovine colostrum on $CD4^+$ and $CD8^+$ T lymphocyte populations in mid-jejunal crypt lamina propria of weaner piglets.

	Dietary treatment[1]		
	CON	BC	SEM
$CD4^{+}$[2,3]	9.2[a]	11.8[b]	1.7
$CD8^+$	9.2[a]	12.6[b]	1.0
$CD4^+$:$CD8^+$	1.04	0.97	0.13

[1] CON, control diet; BC, bovine colostrum diet.
[2] Least-square mean values with pooled standard error (SEM).
[3] Number of positive T lymphocytes per 0.1 mm^2 crypt lamina propria.
[a,b] Least-square means with different superscripts are significantly different ($P < 0.05$).

3.3.4 Relationships between gut morphology, lymphocyte density, voluntary feed intake and weight gain

No significant correlation was observed between overall average daily feed intake and average villus height in the proximal or mid jejunum of piglets offered either experimental diet after weaning ($P > 0.05$). However, in the distal ileum a significant negative correlation was

observed between feed intake and small intestine villus height in piglets consuming the CON diet ($r = -0.93$, $P < 0.05$), whereas a trend for a positive correlation between these variables was observed in piglets consuming the BC diet ($r = 0.74$, $P = 0.09$). No relationship between average crypt depth and overall average daily feed intake was observed in any site of the small intestine within either dietary treatment ($P > 0.10$).

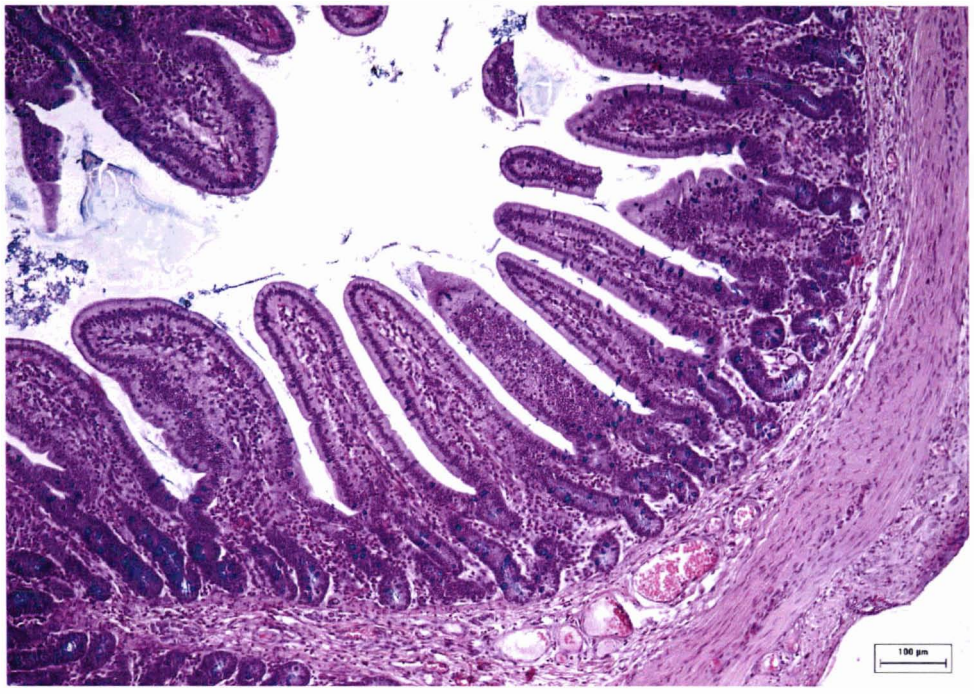
In piglets offered the CON diet, no relationship between overall average daily gain and villus height was observed in any of the small intestine sites ($P > 0.10$). Similarly, in piglets consuming the BC diet, no relationship between these variables was observed in the proximal jejunum and distal ileum, although a trend for a positive correlation was present in the mid-jejunum ($r = 0.77$, $P = 0.07$). No relationship between average crypt depth and overall average daily gain or intake was observed in any site of the small intestine within either dietary treatment ($P > 0.10$). In both treatments no relationship was observed between feed conversion ratio and villus height nor crypt depth, in any site of the small intestine ($P > 0.10$).

In piglets offered the CON diet, average density of $CD4^+$ and $CD8^+$ T lymphocytes and their ratio ($CD4^+$:$CD8^+$) showed no relationship with overall average daily gain, average daily feed intake and feed conversion ratio ($P > 0.10$). However in piglets offered the BC diet, the average density of $CD4^+$ T lymphocytes was positively correlated with overall average daily gain ($r = 0.94$, $P < 0.05$), but not average daily feed intake, nor feed conversion ratio ($P > 0.10$). Average density of $CD8^+$ T lymphocytes was positively correlated with average daily feed intake of piglets consuming the BC diet ($r = 0.85$, $P < 0.05$), but no other variable ($P > 0.10$). The ratio of $CD4^+$ to $CD8^+$ T lymphocytes in piglets offered the BC diet displayed a trend for a positive correlation with average daily gain ($r = 0.77$, $P = 0.08$), but no other variable ($P > 0.10$)

Independent of dietary treatment, $CD4^+$ T cell density was positively correlated with overall average daily gain ($r = 0.73$, $P < 0.05$), but not average daily feed intake nor feed conversion ratio ($P > 0.10$). $CD8^+$ T cell density showed a trend for positive correlations with both overall average daily gain ($r = 0.55$, $P = 0.06$), and overall average daily feed intake ($r = 0.55$, $P = 0.06$), but showed no relationship with feed conversion ratio ($P > 0.10$). The ratio of $CD4^+$ to $CD8^+$ T lymphocytes showed no relationship with any of the variables tested ($P > 0.10$). Average crypt depth was not positively correlated with $CD4^+$ nor $CD8^+$ T lymphocyte

density, nor their ratio ($P > 0.10$). Average villus height showed a trend to correlate with $CD8^+$ T lymphocyte density ($r = 0.57$, $P = 0.06$) but no other variable ($P > 0.10$).

(i)



(ii)

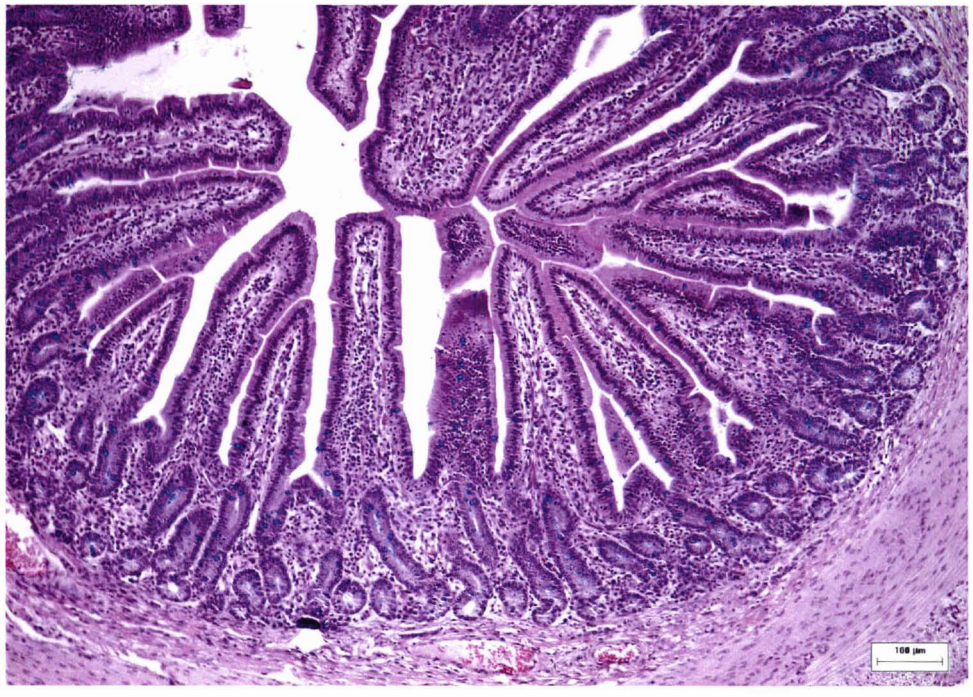
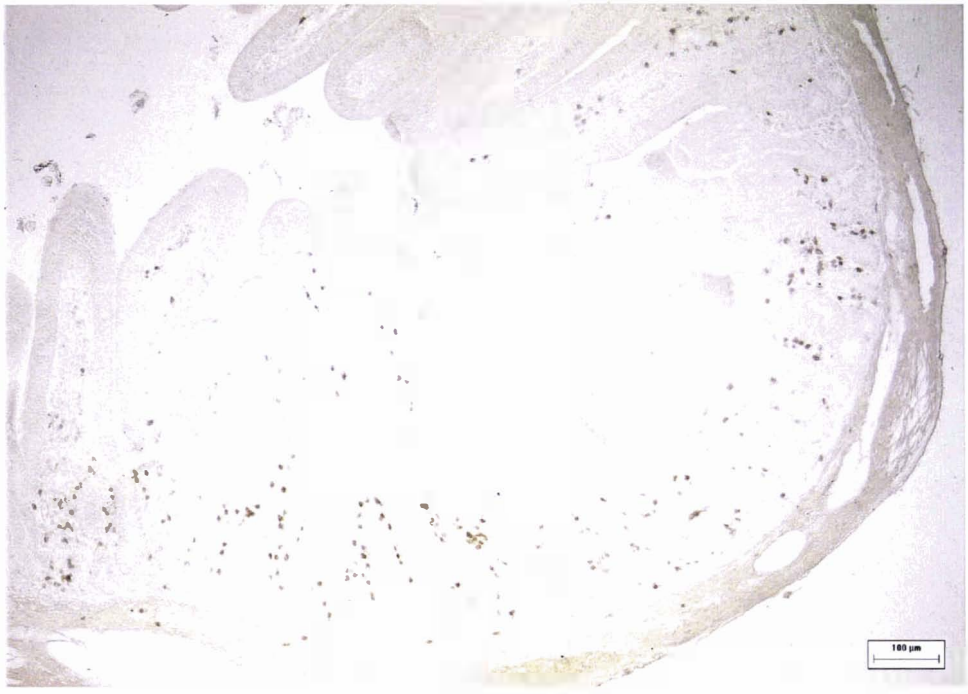


Figure 1. Transverse section of small intestine (haematoxylin and eosin, and alcian blue stain, 10x magnification) representative of the average morphology of a pig offered either control diet (i) or bovine colostrum (BC) diet after weaning (ii).

(i)



(ii)

Figure 2. Transverse section of the mid-jejunum of weaner pig intestine depicting presence and staining of $CD4^+$ (i) and $CD8^+$ (ii) T lymphocytes (brown/black cells; immunocytochemical stain, 10x magnification).

3.4 DISCUSSION

In this study, the addition of 5% spray-dried bovine colostrum to a weaner diet offered for two weeks after weaning altered the intestinal morphology and T lymphocyte density of weaner piglets, and reduced small intestine weight compared to piglets offered a diet containing no colostrum. However, production performance in the two weeks after weaning (as measured by growth rate, feed intake and feed conversion ratio) was unaffected by the inclusion of colostrum in the weaner diet.

Weaning is associated with villus atrophy and crypt hyperplasia which can reduce the absorptive area of the small intestine (Hampson, 1986) and the specific activities of brush border enzymes which may decrease the digestive capacity of the small intestine over the weaning period (Pluske et al., 1997). This is supported by studies in which mean villus height is positively correlated with weight gain after weaning, explaining a significant proportion of the total variation in the latter variable (Li et al., 1991; Pluske et al., 1995; Pluske et al., 1996a). However, since mean villus height displays a generally positive relationship with nutrient intake (McCracken et al., 1999; Pluske et al., 1996a), it can be difficult to separate the effect of gut morphology on weight gain from that of nutrient intake *per se* on this same variable.

In the present study, inclusion of spray-dried bovine colostrum to a weaner diet increased villus height by up to 19% in the proximal and mid-ileum and the distal jejunum, compared to piglets offered a diet devoid of colostrum. This improvement in villus height is similar to that observed after inclusion of spray-dried plasma in the diet of weaner pigs (Gatnau et al., 1995; Spencer et al., 1997; Touchette et al., 1997; Touchette et al., 1999a), although other experiments have shown no effect (Jiang et al., 2000; Touchette et al., 1999b; van Dijk et al., 2001, 2002). Conversely, consumption of the bovine colostrum diet reduced crypt depth in all areas of the small intestine, compared to piglets offered the control diet. The increase in crypt depth generally observed after weaning is indicative of an increase in epithelial cell mitosis, as epithelial cells recruited to the crypt epithelium cause it to elongate (Al-Mukhtar et al., 1982). The reduction in crypt depth observed in piglets offered the colostrum diet therefore suggests that the increase in villus height observed in these animals is due to a reduction in cell loss from the villus epithelium (villus atrophy), rather than an increase in epithelial mitotic activity.

No significant difference in feed intake was observed between piglets offered the two diets, however feed intake was positively correlated with mean villus height in the distal ileum of piglets offered the colostrum diet, whereas a negative correlation between these variables was observed in the distal ileum of piglets offered the control diet. The reasons for this difference are unclear, however different diets have been found to induce different relationships between feed intake and gut morphology. Such an example is provided by the study of Pluske et al. (1996b) in which a total dry matter intake was highly correlated with mean villus height in piglets offered a diet of ewes milk ($r = 0.65$, $P = 0.07$), whereas no relationship between these variables was observed in piglets offered a diet of ewes milk plus 20 g/l glutamine ($r = 0.14$, $P > 0.10$). The lack of a relationship between these variables suggests that dietary glutamine may affect small intestine structure independent of any effects of feed intake *per se*. The results of the present study suggest the reverse - that dietary spray-dried colostrum induced an intake-related improvement in small intestine structure.

In this study, piglets offered the diet containing bovine colostrum had significantly reduced small intestine weights compared to those offered the control diet which contained no colostrum. Similar results have recently been reported in which pigs weaned at 14 days of age and offered a diet containing 10% spray-dried porcine plasma had significantly lower small intestine weights by 16 days after weaning compared to pigs offered a diet containing extruded soy protein (Jiang et al., 2000). This was accompanied by reduced lamina propria cell density, which Jiang et al. (2000) suggested was indicative of a reduction in the immune cell recruitment and expansion that is associated with local intestinal inflammation.

The consumption of bovine colostrum before and after weaning has been associated with a reduction in proliferation of crypt lamina propria $CD4^+$ T lymphocyte within the first 24 hours of weaning (Pluske et al., 1999b), suggesting a reduction in the magnitude of the intestinal inflammatory response occurring at this time. The reduced proliferation resulted in a lower density of crypt lamina propria $CD4^+$ T lymphocytes at 24 hours after weaning, compared to piglets consuming a diet containing no colostrum. However, Pluske et al. (1999b) observed no effect of dietary bovine colostrum on lamina propria $CD8^+$ T lymphocyte density and $CD8^+$ T lymphocyte proliferation over weaning. In contrast to the results of Pluske et al. (1999b), the present study demonstrated an increase in both $CD4^+$ and $CD8^+$ T lymphocyte populations in piglets consuming a diet containing bovine colostrum, with no alteration in the ratio of $CD4^+$ to $CD8^+$ lymphocytes, as compared to piglets

consuming a diet containing no colostrum. Expansion of T lymphocyte subsets in the intestine is characteristic of local immune system activation, and has been observed at weaning (Vega-López et al., 1995; McCracken et al., 1999; Pluske et al., 1999b; Solano-Aguilar et al., 2001), during total parenteral nutrition (Ganessunker et al., 1999), and in soy-hypersensitivity reactions (Dréau et al., 1995), where it is usually accompanied by morphological restructuring of the small intestine. Indeed, Gannesunker et al. (1999) reported a negative correlation between T lymphocyte numbers and villus height in a piglet model of total parenteral nutrition, as did Spreeuwenberg et al. (2001) in weaner piglets. Activated T lymphocytes can produce an array of cytokines (Murtaugh, 1994; Wood and Seow, 1996) which bolster the inflammatory response and cause injury to the gut tissue (MacDonald, 1999), inducing crypt hyperplasia, villus atrophy and matrix metalloproteinases that degrade extracellular matrix proteins (MacDonald and Spencer, 1988; Ferreira et al., 1990; Pender et al., 1997; MacDondald et al., 1999; Monteleone et al., 1999). However, there is evidence that activated porcine mucosal T lymphocytes produce only low levels of the immunostimulant interleukin-2 (IL-2), whereas production of the immunosuppressive cytokines IL-4 and IL-10 is much greater (Bailey et al., 1994, 1998; Whary et al., 1995). This, along with other evidence based on the activity of T cells after activation (Stokes et al., 2001), and the activity of non-professional antigen-presenting cells in the lamina propria of the pig (Haverson et al., 1994, 1999, 2000; Stokes et al., 1996; Wilson et al., 1996), suggests a preferential induction of immunological tolerance and secretory immune responses in mucosal T lymphocytes, rather than an inflammatory, cellular immune response (King et al., 2003). The expansion of lamina propria T lymphocyte subsets in piglets consuming the bovine colostrum diet in the present experiment indicate induction of immunological tolerance to the numerous novel proteins present in bovine colostrum (Smith et al., 2002).

In the present study, $CD4^+$ T lymphocyte density showed no relationship with morphological indices in the small intestine, and $CD8^+$ T lymphocyte density was positively correlated with average villus height, which conflicts with the observations of Gannesunker et al. (1999) and Spreeuwenberg et al. (2001). The present study also demonstrated a positive correlation between $CD4^+$ T lymphocyte density and average daily gain, and $CD8^+$ T lymphocyte density was positively correlated with average daily feed intake and average daily gain. An immunosuppressive effect of the cytokines produced by activated lamina propria T lymphocytes may account for these observations by reducing the incidence of inflammatory cellular immune responses, and the associated homeorhetic response induced by

proinflammatory cytokines such as IL-1, IL-6 and TNF-α. These cytokines can reduce growth rate by inhibiting voluntary feed intake, altering the intermediary metabolism of fat, protein and carbohydrate, stimulating hepatic acute-phase protein synthesis, and inducing other physiological and behavioural effects (Kelley et al., 1994; Johnson, 1997).

It should be noted that pigs possess a population of double-positive T lymphocytes ($CD4^+CD8^+$) (Whary et al. 1995; Zuckermann and Gaskins, 1996; Zuckermann and Husmann, 1996; Zuckermann, 1999; Solano-Aguilar et al., 2001). The function of these double-positive T lymphocytes is currently unclear, and the immunocytochemical staining used in this and other studies (McCracken et al., 1999; Pluske et al., 1999b; Spreeuwenberg et al., 2001) will count this population within both $CD4^+$ and $CD8^+$ T lymphocyte subsets. However this is unlikely to constitute a significant source of error in young pigs, since the $CD4^+CD8^+$ T lymphocyte subset constitutes less than two percent of total peripheral blood lymphocytes in 7-day-old pigs, slowly rising to a substantial proportion (0.30-0.55) of the porcine peripheral T lymphocyte pool by 3 years of age (Zuckermann and Husmann, 1996).

In summary, the improvement in small intestine morphology and reduction in small intestine weight observed after consumption of the bovine colostrum diet suggests a reduction in small intestinal inflammation in these piglets. Although this was accompanied by an increase in mucosal T lymphocyte subsets, this may indicate the induction of mucosal tolerance to the novel dietary proteins present in bovine colostrum, which is supported by the various positive relationships between T lymphocyte density and villus height, feed intake and growth rate.

Unfortunately the present study offers no insight into the mechanism by which spray-dried bovine colostrum could reduce mucosal inflammation in the small intestine, nor the specific colostral components involved. Given the presence of immunoglobulins in colostrum and their reasonable resistance to proteolysis in the small intestine of the young pig (Morel et al., 1995), and also the adult human (Roos et al., 1995), it is possible that they afford passive protection of the intestinal mucosa through immune exclusion (Schollum et al., 1997). This is the most common hypothesis to account for the similar actions of spray-dried plasma, which has an immunoglobulin composition comparable to that of colostrum (Coffey and Cromwell, 1995). However bovine colostrum also contains numerous non-specific anti-microbial and anti-viral factors such as lactoferrin, lactoperoxidase, oligosaccharides and

glycoconjugates (Gopal and Gill, 2000; Schanbacher et al., 1997; Shah, 2000; Van Hooijdonk et al., 2000), as well as hormones that are capable of protecting the intestinal epithelium from injury (Berlanga et al., 2002; Playford et al., 1999), and stimulating mucosal repair (Blikslager et al., 1999; Rhoads et al., 1995; Zijlstra et al., 1994).

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Chapter 4

A COMPARISON OF THE EFFECTS OF DIETARY SPRAY-DRIED BOVINE COLOSTRUM AND ANIMAL PLASMA ON INDICES OF GROWTH PERFORMANCE AND INTESTINAL IMMUNITY IN WEANER PIGS.

4.1 INTRODUCTION

In pigs, weaning is commonly accompanied by reductions in voluntary feed intake and weight gain, (Pluske et al., 1995) and significant morphological restructuring of the small intestine. Generally, this restructuring is manifested as villus atrophy and crypt hyperplasia resulting in a decrease in villus height and an increase in crypt depth, and alterations in the specific activity of brush border enzymes, which may reduce the digestive and absorptive function of the small intestine after weaning (Pluske et al., 1997). Villus height has been shown to explain as much as 47% of the total variation in empty body weight gain after weaning (Pluske et al., 1995) and similar results have been reported by Li et al. (1991a) and Pluske et al. (1996a), implicating the status of gut architecture in the aetiology of the post-weaning 'growth check'.

One hypothesis developed to explain morphological restructuring after weaning is that it is caused by transient anorexia, which impairs epithelial barrier function allowing luminal antigens to enter the lamina propria, inducing an active immune response in the intestine (McCracken et al., 1995). A direct negative effect of mucosal inflammation on intestinal architecture is well-established (MacDonald and Spencer, 1988; MacDonald et al., 1999), and evidence that weaning in pigs is associated with leukocytic expansion characteristic of immune system activation in the small intestine also supports this hypothesis (Vega-López et al., 1995; McCracken et al., 1999; Pluske et al., 1999a; Solano-Aguilar et al., 2001). This is further supported by evidence linking low voluntary feed intake after weaning with increased epithelial permeability, mucosal inflammation and villus atrophy (McCracken et al., 1999; Spreeuwenberg et al., 2001).

The inclusion of low levels (2-8%) of spray-dried plasma in the diets of weaner pigs has been shown to improve voluntary feed intake and growth rate during the immediate post-weaning period (Coffey and Cromwell 2001; van Dijk et al., 2001a). However, the mechanism by which spray-dried plasma improves pig performance is currently unclear. It contains numerous compounds such as immunoglobulins and glycoproteins that are capable of antimicrobial actions (van Dijk et al., 2001a), and its effects can be replicated by dietary inclusion of the high molecular weight fraction alone (Gatnau et al., 1995; Pierce et al., 1995a), which is composed principally of immunoglobulins. These observations suggest an immunologically-based mechanism of action. This is further supported by the observation that dietary spray-dried plasma is most effective when pigs are housed under conditions that

provide an immune challenge, whereas in low-pathogen conditions its effects are minimal or absent (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995; Stahly et al., 1995; Touchette et al., 1996; Bergstrom et al., 1997; Bregendahl et al., 1998; Chirra et al., 1999). Coffey and Cromwell (1995) therefore proposed that the antimicrobial components of spray-dried plasma may bind luminal antigen, reducing or preventing interaction between pathogens and the intestinal mucosa, reducing the incidence of infection and the negative effect of infection on epithelial barrier function. This is supported by evidence that spray-dried plasma can reduce faecal excretion of haemolytic *E. coli* (Deprez et al., 1990, 1996; Nollett et al., 1999). However, the effect of spray-dried plasma on intestinal morphology is variable, with some experiments demonstrating positive effects (Gatnau et al., 1995, Spencer et al., 1997; Touchette et al., 1997, 1999a) and others no effect (Touchette et al., 1999b; Jiang et al., 2000; van Dijk et al., 2001b, 2002).

Spray-dried bovine colostrum has a similar composition of immunoglobulins to spray-dried plasma, and also contains other compounds capable of antimicrobial and antiviral actions, such as lactoferrin, lactoperoxidase, oligosaccharides and glycoconjugates (Schanbacher et al., 1997; Gopal and Gill, 2000; Shah, 2000; Van Hooijdonk et al., 2000). Similar to spray-dried plasma, dietary inclusion of spray-dried bovine colostrum has been shown to improve feed intake and growth rate during the post-weaning period (Pluske et al., 1999b; Dunshea et al., 2002). Given the similarity between these two products, the same immunological mechanism of action may be considered to underlie the effects of each. Supporting this notion, dietary spray-dried bovine colostrum has been shown to reduce lamina propria T lymphocyte proliferation when fed before and after weaning (Pluske et al., 1999a), and the inclusion of 5% spray-dried bovine colostrum in diets for early weaned piglets was found to increase small intestine villus height and also reduce small intestine weight after 14 days (Chapter 3, this thesis). A similar reduction in small intestine weight has also been reported in plasma-fed pigs (Jiang et al., 2000).

This study was undertaken to evaluate the effects of dietary spray-dried plasma from two species (bovine and porcine) alongside those of spray-dried bovine colostrum in pigs weaned at 21 days of age, focussing on the effect of these ingredients on small intestine histology, morphology and indices of intestinal inflammation.

4.2 MATERIALS AND METHODS

4.2.1 Animals and conduct of the trial

Experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Recommendations and Minimum Standards for the Care and Use of Animals for Scientific Purposes (New Zealand Animal Welfare Advisory Committee, 1995).

The experiment used 40, 21-day-old mixed-sex piglets (PIC 231 x Camborough 22; 6.65 ± 0.14 kg), which were obtained at weaning from a commercial piggery. Piglets were blocked by litter of birth and weight, and randomly allocated to receive one of four dietary treatments for 7 days, or euthanased immediately to provide baseline data. The treatments were as follows: 1) baseline (BASE), in which pigs were euthanased, and post-mortem measurements obtained, at 21 days of age; 2) control diet (CON); 3) spray-dried bovine colostrum diet (BC); 4) spray-dried porcine plasma diet (PP); 5) spray-dried bovine plasma diet (BP). The experimental diets were offered *ad libitum* from day 1-7 of the experiment. Body weight was recorded at days 0 and 7, and feed intake was recorded daily. Piglets were housed individually in stainless steel cages which permitted no contact between animals, and were separated from waste products by perforated flooring. Airflow to the facility was controlled, and room temperature was maintained at 26°C, with a heat lamp above each cage providing a local temperature of ~30°C. Due to availability of pigs, two replicates of this design were performed, allowing a total of 8 pigs per treatment.

4.2.2 Experimental diets

The diets were based on wheat and skim milk powder (Table 1), and were formulated to contain 15 MJ DE/kg, 1.4% apparent digestible lysine, and to meet or exceed National Research Council (1998) recommendations for all nutrients. Test proteins (bovine colostrum, bovine plasma, and porcine plasma) were included at a concentration of 7.5% in diets BC, BP and PP, respectively. Test proteins replaced skim milk powder on an isolysine basis, lactose was used to balance the diets for lactose content, and diets were fed in meal form.

Table 1. Percentage composition and calculated analysis of the experimental diets.

	Experimental diets[1]			
Ingredient, %	CON	BC	BP	PP
Wheat	67.2	61.7	64.2	68.0
Fishmeal	3.8	2.6	3.7	2.1
Skim milk powder	25.0	12.7	11.9	13.2
Bovine colostrum[2]	0.0	7.5	0.0	0.0
Bovine plasma[3]	0.0	0.0	7.5	0.0
Porcine plasma[4]	0.0	0.0	0.0	7.5
Soybean oil	0.5	2.5	1.8	0.7
L-Lysine	0.5	0.6	0.5	0.6
D, L-Methionine	0.3	0.4	0.4	0.4
L-Threonine	0.7	0.7	0.6	0.7
Dicalcium phosphate	1.5	5.0	2.5	0.5
Salt	0.2	0.3	0.0	0.0
Vitamin and mineral premix[5]	0.3	0.3	0.3	0.3
Calculated Analysis				
DE[6], MJ/kg	15.0	15.0	15.0	15.0
Crude protein, %	21.0	21.0	21.0	21.0
Digestible lysine, %	1.4	1.4	1.4	1.4
Digestible Methionine + Cysteine, %	1.1	1.1	1.1	1.1
Lactose, %	12.5	12.6	12.6	12.6
Sodium, %	0.24	0.23	0.38	0.56

[1] CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma.
[2] Immulac (Specialty Ingredients Group, Fonterra, Hautapu, New Zealand).
[3] AP820 (Proliant Corp., Ames, Iowa, USA).
[4] U70 (Harimex BV, Loenen, The Netherlands).
[5] Vitastart (Vitec Nutrition Ltd, Auckland, New Zealand). Supplied per kilogram diet: Mn, 45 mg; Zn, 120 mg; Cu, 125 mg; Co, 0.5 mg; I, 1 mg; Fe, 100 mg; Se, 300 μg; Vitamin A, 15 000 IU; Vitamin D_3, 2000 IU; Vitamin E, 70mg; Vitamin K, 2.5 mg; Vitamin B_1, 2 mg; Vitamin B_2, 3 mg; Vitamin B_6, 2 mg; Vitamin B_{12}, 30 μg; Calcium Pantothenate, 20 mg; Niacin, 20 mg; Biotin, 100 μg; Folic Acid, 500 μg; Choline 150 mg.
[6] DE, digestible energy.

4.2.3 Chemical analysis

Representative samples of the test proteins were analysed for dry matter, crude protein and crude fat content using standard procedures (AOAC, 1990). Sodium, potassium, calcium and phosphorus content was determined by inductive coupled plasma emission spectrometry (In-house method, Analytical laboratory, AgResearch Grasslands, Palmerston North, New Zealand). Gross energy was determined using an adiabatic bomb calorimeter (Gallenkamp Autobomb, UK) standardised with benzoic acid. Amino acids were determined by hydrolysing duplicate samples with HCl (containing phenol) for 24 h at 110 ± 2 ^{0}C in glass tubes sealed under vacuum. Amino acids were detected on a Waters ion exchange HPLC

system, and the chromatograms were integrated using dedicated software (Maxima 820, Waters, Millipore, Milford, MA) with the amino acids identified and quantified using a standard amino acid solution (Pierce, Rockford, IL). Cysteine and methionine were analysed as cysteic acid and methionine sulphone by oxidation with performic acid for 16 h at 0 ^{0}C and neutralisation with hydrobromic acid prior to hydrolysis. Immunoglobulin G (IgG) concentrations were determined in bovine colostrum and bovine plasma using protein-G affinity high performance liquid chromatography (Pharmacia), followed by UV detection at 280nm.

4.2.4 Post-mortem procedure

Piglets from the BASE treatment were euthanased on day 0 of the experiment, and piglets from the CON, BC, BP and PP treatments were euthanased on day 7 of the experiment. Prior to euthanasia, pigs were anaesthetised by inhalation of halothane, and a venous blood sample collected in a heparinised vaccutainer for measurement of plasma urea nitrogen. Piglets were then euthanased with an intracardial injection of sodium pentobarbitone (125 mg/kg live-weight) and post-mortem measurements taken. The abdomen was opened immediately, from the sternum to the pubis, and the entire gastrointestinal tract removed. A pair of scissors was used to disconnect the small intestine at the gastric pylorus and the ileo-caecal valve, and the intestine was clamped. The small intestine was laid out on a stainless-steel tray and a section at proportionally 25, 50 and 75% along the intestine was clamped with haemostats, excised, and placed immediately into a plastic container with Bouin's fluid (24% formalin, 5% glacial acetic acid, 71% picric acid). After fixation for 24 hours, the Bouin's fluid was replaced with 70% ethanol. The spleen and liver were removed and weighed. The stomach and large intestine were weighed upon removal with their contained digesta, then emptied, washed with water, blotted dry, and weighed again. Total processing time, from killing to obtaining gut samples, was about 10 minutes. Sites at 0.25, 0.5 and 0.75 are referred to by their approximate position in the small intestine, i.e. proximal jejunum, mid-jejunum and distal ileum, respectively.

4.2.5 Histology and immunocytochemistry

After fixation, ring-shaped lengths of small intestine from all three sites were excised, dehydrated and embedded in paraffin wax. From each of these, 4 transverse sections (6 μm) were cut, stained with haematoxylin and eosin and alcian blue, and examined under a light

microscope. Measurements of villus height and crypt depth were taken from sections where the plane of section ran vertically from the tip of each villus to the base of an adjacent crypt. For each section the image analysis software Sigma Scan (Jandel Scientific, San Rafael, CA), and a light microscope were used to measure 10 of the tallest, well oriented villi from villus tip to crypt mouth, and 10 associated crypts from crypt mouth to base. Measurement of average epithelial cell height on the 10 villi was also performed, by taking 6 measurements of epithelial cell height at even distances along both sides of the villus length. Goblet cells were identified in the epithelium as blue staining cells, which were counted in 10 villi and 10 crypts of each section, and expressed as number of cells per 100 μm of villus or crypt epithelium.

Mid jejunal lamina propria $CD4^+$ and $CD8^+$ T lymphocytes were identified by immunocytochemistry and enumerated as described previously (Chapter 3, this thesis).

4.2.6 Analysis of plasma urea concentrations.

Plasma was harvested from whole blood samples and stored at -70°C until analysis. Plasma urea nitrogen was measured using an endpoint enzymatic assay (Roche, Summerville, N.J., U.S.A.).

4.2.7 Statistical analysis

The data were subjected to analysis of variance (ANOVA) by the General Linear Models procedures of SAS (2000) using piglet as the experimental unit. Organ weights were expressed as a percentage of the empty body weight. In all models, the effect of sex was tested, found to be non-significant, and was therefore removed. The effect of replicate was tested in all models, and found to be non-significant for all variables measured, with the exception of average daily feed intake and growth rate, and cumulative feed intake. Replicate was therefore included in the analysis of these latter variables only.

The statistical model used in the analysis of average daily growth rate, feed intake, organ weight, plasma urea nitrogen concentration and lamina propria T lymphocyte density was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

Where:

y_{ijk} = observation from the k^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment.

μ = the population mean.

α_i = the fixed effect of the i^{th} dietary treatment.

β_j = the random effect of the j^{th} litter of birth.

ε_{ijk}= residual error of the k^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment.

To discern the effect of time on cumulative daily feed intake, repeated measure analysis was performed using the following model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \pi_l(\alpha_i, \beta_j) + \chi_k + \chi_k\alpha_i + \chi_k\beta_j + \varepsilon_{ijkl}$$

Where:

y_{ijkl} = observation from the l^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment and the k^{th} time period.

μ = the population mean.

α_i = the fixed effect of the i^{th} dietary treatment.

β_j = the random effect of the j^{th} litter of birth.

$\pi_l(\alpha_i, \beta_j)$ = random effect of the l^{th} pig within the i^{th} dietary treatment and the j^{th} litter of birth.

χ_k = the fixed effect of the k^{th} time.

$\chi_k\alpha_i$ = the interaction between the k^{th} time and the i^{th} dietary treatment.

$\chi_k\beta_j$ = the interaction between the k^{th} time period and the j^{th} litter of birth.

ε_{ijkl}= residual error of the l^{th} pig from the j^{th} litter of birth, within the i^{th} dietary treatment and the k^{th} time period.

The statistical model used in the analysis of small intestine histology was:

$$y_{(i)jk} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + \alpha_i\gamma_k + \varepsilon_{(i)jk}$$

Where:

$y_{(i)jk}$ = observation from the k^{th} site from the j^{th} pig nested within the i^{th} experimental treatment.

μ = the population mean.

α_i = the fixed effect of the j^{th} experimental treatment.

$\beta_{j(i)}$ = the random effect of the j^{th} pig nested within the i^{th} experimental treatment

γ_k = the fixed effect of the k^{th} site in the small intestine.

$\alpha_i\gamma_k$ = the interaction between the i^{th} experimental treatment and the k^{th} site of the small intestine.

$\varepsilon_{(i)jk}$= residual error of the k^{th} site from the j^{th} pig nested within the i^{th} experimental treatment.

Where a significant treatment effect was observed, Fisher's least significant difference test was performed to determine significant differences between least-square means of treatment groups. Level of significance was pre-set at $P < 0.05$, and trends were identified at $P < 0.10$. Pearson correlation analysis was performed to evaluate a possible correlation between feed intake and histological variables, and among histological variables where appropriate. Data are presented as least-square means with the associated pooled standard error of the mean (SEM).

4.3 RESULTS

4.3.1 Composition of test proteins

Analysed composition of the test proteins is presented (Table 2).

Table 2. Compositional analysis of the test proteins, on an as-fed basis.

	Bovine colostrum[1]	Bovine plasma[2]	Porcine plasma[3]
Gross energy, MJ/kg	20.6	19.5	19.1
Crude protein, %	76.6	73.6	68.4
Crude fat, %	0.89	0.09	0.15
Amino Acids, %			
Alanine	2.8	3.9	3.7
Arginine	2.9	3.8	3.8
Aspartic acid	5.9	6.9	6.2
Cystine	0.8	1.9	1.9
Glutamic acid	14.4	9.0	9.4
Glycine	1.8	2.5	2.3
Histidine	2.0	2.3	2.1
Isoleucine	3.4	1.8	2.3
Leucine	6.8	6.7	6.2
Lysine	5.8	6.1	5.5
Methionine	1.9	0.9	0.5
Phenylalanine	3.3	3.6	3.5
Proline	6.5	3.4	3.8
Serine	4.7	4.3	3.6
Threonine	3.9	4.3	3.7
Tyrosine	3.9	3.2	3.3
Valine	4.9	4.9	4.3
Minerals, %			
Sodium	0.11	3.50	5.87
Potassium	0.46	0.46	0.26
Calcium	1.29	0.10	0.85
Phosphorus	0.91	1.12	0.73
IgG, %	18.2	26.8	nm[4]

[1] Immulac™ (NZ Dairy, Specialty Ingredients Division, Hautapu, New Zealand).
[2] AP820 (Proliant Corp., Ames, Iowa, USA).
[3] U70 (Harimex B.V., Loenen, The Netherlands).
[4] Not measured.

4.3.2 Feed intake, growth rate and feed conversion ratio

Repeated measure analysis showed that daily cumulative feed intake (Figure 1) for the duration of the experiment was not affected by dietary treatment nor litter of birth ($P > 0.10$). However a significant effect of replicate was observed ($P < 0.05$), with pigs in the second replicate consuming more feed than those in the first replicate on every day of the experiment.

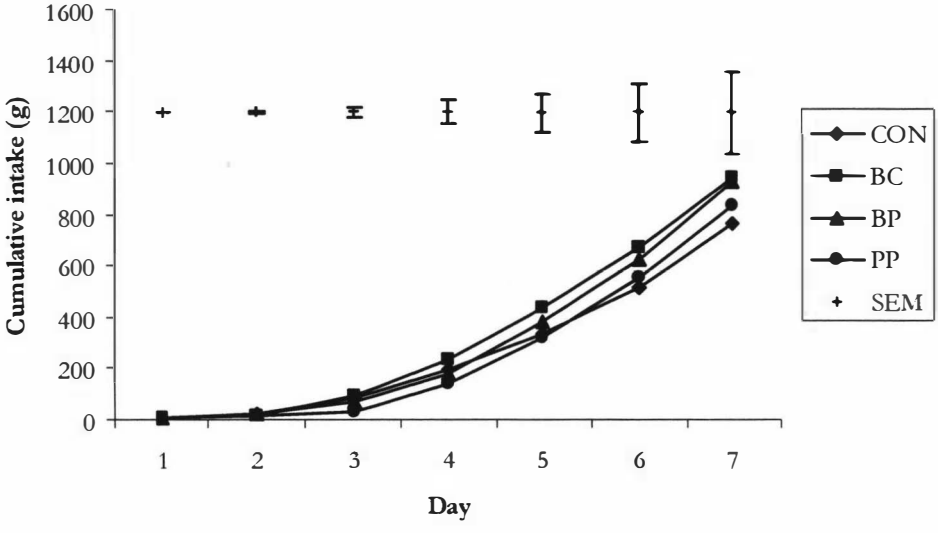

Figure 1. Daily cumulative feed intake of pigs offered one of four different diets for one week after weaning: control (CON), bovine colostrum (BC), bovine plasma (BP) and porcine plasma (PP).

Average daily feed intake for the 7 days of the experiment (Table 3) was not affected by dietary treatment nor litter of birth ($P > 0.10$), although replicate was found to have a significant effect, with pigs in the second replicate consuming more feed than those in the first ($P < 0.05$). Numerically, intake ranged between 9 and 23% higher in pigs offered a diet containing one of the test proteins, compared to those offered the control diet, however high variability between pigs rendered this difference non-significant.

Average daily gain over the 7 day duration of the experiment was unaffected by treatment and litter of birth ($P > 0.10$), however a trend for an effect of replicate was observed, with pigs in the second replicate growing faster than those in the first ($P = 0.09$). Average daily gain ranged from numerically 37-89% higher in pigs offered a diet containing one of the test

proteins compared to those offered the CON diet, although this was not significant due to high variability between pigs. Average feed conversion ratio for each treatment was numerically highest in piglets consuming the CON diet, followed by those consuming either the BC or PP diets, and was lowest in those consuming the BP diet.

Table 3. Average daily gain (ADG), feed intake (ADFI) and feed conversion ratio (FCR) of pigs offered one of four different diets for one week after weaning.

	Dietary treatment[1]				
	CON	BC	BP	PP	SEM
ADG[2], g	65	98	123	89	39
ADFI[2], g	109	134	132	119	23
FCR[3]	1.69	1.37	1.07	1.34	-

[1] CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma.
[2] Least square mean with associated pooled standard error of the mean (SEM).
[3] Single value calculated from total weight gain and total feed intake of all pigs within a treatment.

4.3.3 Empty body weight, organ weights and plasma urea nitrogen concentration

Table 4. Empty body weight and organ weights (expressed as a percentage of the empty body weight) of piglets killed either at weaning (BASE), or after consuming one of four different diets (CON, BC, BP, PP) for one week after weaning.

	Treatment[1]					
	BASE	CON	BC	BP	PP	SEM
Empty body weight, g	6423	6503	6458	6867	6462	339
Organs[2]						
Liver, %	2.1	2.4	2.5	2.7	2.7	0.2
Spleen, %	0.26	0.24	0.25	0.25	0.25	0.02
Stomach, %	0.37[a]	0.56[b]	0.54[b]	0.57[b]	0.54[b]	0.04
Small intestine, %	3.3	3.4	3.3	3.4	3.5	0.2
Large intestine, %	0.8	1.1	1.0	1.1	1.1	0.1
Plasma urea N, mmol L^{-1}	2.7	2.7	2.2	2.5	3.1	0.7

[1] BASE, baseline; CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma.
[2] Least-square mean values with pooled standard error of the mean (SEM).
[a,b] Least-square means with different superscripts are significantly different ($P < 0.05$).

A significant effect of treatment was observed for relative stomach weight only ($P < 0.05$), with pigs killed at weaning showing lighter stomach weights compared to pigs killed 1 week after weaning (Table 4). Litter of birth significantly affected empty body, liver and spleen weight ($P < 0.05$) accounting for 31, 30 and 34% of the total variation in these variables, respectively. The relative weights of the small intestine, stomach and large intestine were

significantly affected by replicate, with pigs in the second replicate showing higher weights than those in the first ($P < 0.05$). Neither treatment nor litter of birth had a significant effect on plasma urea nitrogen concentration ($P > 0.10$).

4.3.4 Histology

Villus height was significantly affected by treatment, pig nested within treatment, small intestine site and site by treatment interaction ($P < 0.0001$). Average villus height was 69-82% higher in piglets killed at weaning (BASE) compared to those killed one week after weaning (Table 5), but no difference in average villus height was observed between pigs offered the CON, BC, BP or PP diets. Pig nested within treatment accounted for 36% of the total variation in villus height. Independent of treatment, villus height was highest in the most proximal sampling site in the small intestine (proximal jejunum) ($P < 0.05$), with more distal sites (mid-jejunum and distal ileum) showing similar values (499, 472 and 471 μm, respectively, SEM 4.1 μm). A site by treatment interaction was observed, with BASE piglets displaying an increasing gradient of villus height from the proximal to distal small intestine, while piglets killed one week after weaning displayed a generally decreasing gradient. Among piglets killed one week after weaning, treatment effects were manifested at different sites of the small intestine. In the proximal jejunum, piglets consuming the BC diet showed a reduction in villus height compared to those consuming any other diet ($P < 0.05$), whereas no difference in villus height was observed in piglets offered the CON, BP and PP diets ($P > 0.10$). In the mid jejunum, villus height of piglets consuming the BP diet was increased compared to those consuming any other diet ($P < 0.05$). While no significant difference was observed between those offered the CON, BP and PP diets ($P > 0.10$), villus height tended to be lower in pigs consuming the PP diet compared to those offered the CON diet ($P = 0.07$). In the distal ileum, villus height of pigs consuming the BC and BP diets was increased compared to those consuming the CON diet ($P < 0.05$), while those offered the PP diet showed similar villus height to all pigs, regardless of diet ($P > 0.10$).

Table 5. Small intestine histology measured in piglets either at weaning (BASE), or after consuming one of four different diets (CON, BC, BP, PP) for one week after weaning.

	Treatment*					
	BASE	CON	BC	BP	PP	SEM
Villus height, μm†						
Proximal Jejunum	656^{1a}	469^{1b}	432^{1c}	478^{1b}	461^{1b}	
Mid Jejunum	741^{2a}	405^{2b}	395^{2b}	436^{2c}	381^{2b}	9‡
Distal Ileum	819^{3a}	365^{3b}	396^{2c}	398^{3c}	377^{2bc}	
Mean	739^{a}	413^{b}	407^{b}	438^{b}	406^{b}	45
Crypt depth, μm						
Proximal Jejunum	188^{1a}	201^{1b}	203^{1b}	204^{1b}	201^{1b}	
Mid Jejunum	169^{2a}	187^{2b}	186^{2b}	182^{2b}	182^{2b}	3
Distal Ileum	164^{2a}	191^{2b}	188^{2b}	175^{2b}	190^{3b}	
Mean	174	193	192	187	191	13
Villus height: crypt depth						
Proximal Jejunum	3.62^{1a}	2.44^{b}	2.18^{c}	2.42^{b}	2.39^{1b}	
Mid Jejunum	4.47^{2a}	2.36^{b}	2.23^{b}	2.49^{c}	2.25^{12b}	0.07
Distal Ileum	5.33^{3a}	2.25^{bc}	2.28^{b}	2.32^{b}	2.08^{2c}	
Mean	4.47^{a}	2.35^{b}	2.23^{b}	2.41^{b}	2.24^{b}	0.3
Epithelial cell height, μm						
Proximal Jejunum	19.3^{1a}	23.4^{1bc}	23.2^{1b}	24.2^{1c}	24.0^{1bc}	
Mid Jejunum	20.3^{2a}	22.8^{1b}	23.5^{1bc}	24.3^{1c}	23.3^{1b}	0.3
Distal Ileum	24.4^{3a}	21.5^{2b}	21.7^{2b}	21.7^{2b}	21.2^{2b}	
Mean	21.3	22.5	22.8	23.4	22.8	0.9
Villus goblet cell density$^{\Psi}$						
Proximal Jejunum	0.55^{1ab}	0.48^{1a}	0.60^{1b}	0.48^{1a}	0.56^{1ab}	
Mid Jejunum	0.52^{1a}	0.84^{2c}	0.82^{2c}	0.69^{2b}	0.61^{1ab}	0.04
Distal Ileum	0.82^{2ab}	0.77^{2a}	$0.86^{2\,ab}$	0.89^{3b}	0.82^{2ab}	
Mean	0.63	0.70	0.76	0.69	0.66	0.13
Crypt goblet cell density$^{\Psi}$						
Proximal Jejunum	1.91^{a}	1.28^{1c}	1.72^{1ab}	1.54^{1b}	1.65^{1b}	
Mid Jejunum	2.00^{a}	1.52^{2b}	2.12^{2a}	2.06^{2a}	2.02^{2a}	0.07
Distal Ileum	1.97^{a}	2.30^{3b}	2.30^{2b}	2.34^{3b}	2.56^{3c}	
Mean	1.96	1.70	2.05	1.98	2.07	0.16

* BASE, baseline; CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma.
† Least-square mean values with pooled standard error of the mean (SEM).
‡ Pooled standard error of the mean associated with all values within different sites and treatments.
Ψ Number of goblet cells per 100 μm epithelium.
^{a,b,c} Least-square means within a row with different superscripts are significantly different ($P < 0.05$).
^{1,2,3} Least square means within a column with different superscripts are significantly different ($P < 0.05$).

Crypt depth showed significant effects of pig nested within treatment and small intestine site ($P < 0.0001$), and a site by treatment interaction ($P = 0.01$), but not treatment alone ($P > 0.10$). Pig nested within treatment accounted for 61% of the total variation in crypt depth. Irrespective of treatment, crypt depth was highest in the proximal jejunum, compared to either the mid-jejunum or distal ileum ($P < 0.05$), which were similar ($P > 0.10$) (199, 181 and

182 μm, respectively, SEM 1.3 μm). A site by treatment interaction was observed, with crypt depth at all sites higher in piglets killed one week after weaning compared to those killed at weaning ($P < 0.05$; Table 5), while no difference in crypt depth was observed between piglets offered different post-weaning diets at any site of the small intestine ($P > 0.10$).

Ratio of villus height to crypt depth (VH:CD) was significantly affected by treatment, pig nested within treatment, site and a site by treatment interaction ($P < 0.0001$). Average VH:CD was 46-51% higher in piglets killed at weaning compared to those killed one week after weaning ($P < 0.05$; Table 5), however no difference in average VH:CD was observed between piglets offered different diets after weaning ($P > 0.10$). Pig nested within treatment accounted for 33% of the total variation in VH:CD. Independent of treatment, an increasing gradient of VH:CD from the proximal to the distal small intestine was observed, with VH:CD in the proximal jejunum, mid-jejunum and distal ileum all showing significant differences ($P < 0.05$) (2.61, 2.76 and 2.85 μm, respectively, SEM 0.03 μm). In all sites of the small intestine, VH:CD was higher in piglets killed at weaning, compared to those killed one week after weaning ($P < 0.05$). Among piglets killed one week after weaning, VH:CD in the proximal jejunum was lower in those offered the BC diet, compared to piglets offered any other diet ($P < 0.05$), which had similar values ($P > 0.10$). In the mid jejunum, VH:CD was higher in pigs offered the CON diet compared to that of pigs offered either the BC, BP or PP diets ($P < 0.05$), which were similar ($P > 0.10$). In the distal ileum, VH:CD was similar amongst piglets consuming the CON, BC and BP diets ($P > 0.10$), but lower in those consuming the PP diet ($P < 0.05$).

Epithelial cell height showed significant effects of pig nested within treatment, small intestine site and a site by treatment interaction ($P < 0.0001$), but not treatment alone ($P > 0.10$). Pig nested within treatment accounted for 38% of the total variation in epithelial cell height. Independent of treatment, epithelial cell height was similar in the proximal and mid jejunum ($P > 0.10$), which were both higher than the distal ileum ($P < 0.05$) (22.1, 22.8 and 22.8 μm, respectively, SEM 0.1 μm). Epithelial cell height was lower in the proximal and mid-jejunum and higher in the distal ileum of pigs killed at weaning, compared to those killed one week after weaning ($P < 0.05$; Table 5). Among piglets killed one week after weaning, epithelial cell height in the proximal jejunum of pigs consuming the CON diet was not different from that of pigs offered either the BC, BP or PP diets ($P > 0.10$). Mid jejunal epithelial cell height was higher in pigs consuming the BP diet compared to those offered the CON diet ($P <$

0.05). No difference in epithelial cell height in the distal ileum was observed among piglets consuming either diet ($P > 0.10$).

Villus goblet cell density was significantly affected by pig nested within treatment, site, and a site by treatment interaction ($P < 0.0001$), but not treatment alone ($P > 0.10$). Pig nested within treatment accounted for 51% of the total variation in villus goblet cell density. Independent of treatment, villus goblet cell density increased from the proximal jejunum to the mid jejunum and distal ileum, which were all significantly different ($P < 0.05$) (0.53, 0.70 and 0.83 cells/100μm epithelium, respectively, SEM 0.02). In the proximal jejunum and distal ileum, no difference in villus goblet cell density was observed between piglets killed at weaning and those killed one week after weaning ($P > 0.10$; Table 5). However, in the mid jejunum, goblet cell density was lower in pigs killed at weaning compared to those offered the CON, BC or BP diet for one week after weaning, and tended to be lower than those offered the PP diet ($P = 0.06$). Among piglets offered different diets for one week after weaning, villus goblet cell density in the proximal jejunum was higher in piglets consuming the BC diet compared to the CON diet ($P < 0.05$), whereas values for piglets offered the BP and PP diets did not differ from that of pigs offered the CON diet ($P > 0.10$). In the mid jejunum, villus goblet cell density was lower in pigs offered the BP and PP diets, compared to those offered either the CON or BC diet ($P < 0.05$), which were similar ($P > 0.10$). In the distal ileum, goblet cell density in pigs consuming the BP diet was higher than those offered the CON diet, and tended to be higher in pigs consuming the BC diet ($P = 0.07$).

Crypt goblet cell density was significantly affected by pig nested within treatment, site, and a site by treatment interaction ($P < 0.0001$), but not treatment alone ($P > 0.10$). Pig nested within treatment accounted for 26% of the total variation in villus goblet cell density. Irrespective of treatment, villus goblet cell density increased from the proximal jejunum to the mid jejunum and distal ileum, which were all significantly different ($P < 0.05$) (1.62, 1.94 and 2.29 cells/100μm epithelium, respectively, SEM 0.03). In the proximal jejunum, piglets killed at weaning displayed higher crypt goblet cell density compared to piglets offered the CON, BP or PP diet for one week after weaning ($P < 0.05$; Table 5), and tended to be higher than pigs consuming the BC diet ($P = 0.05$). In the mid jejunum, crypt goblet cell density of piglets killed at weaning was higher than those offered the CON diet for one week after weaning, but similar to those offered either the BC, BP or PP diet. In the distal ileum, crypt goblet cell density was lower in piglets killed at weaning, compared to those killed one week

after weaning ($P < 0.05$). Among piglets offered different diets for one week after weaning, proximal jejunal and mid jejunal crypt goblet cell density was lower in piglets consuming the CON diet, compared to those consuming either the BC, BP or PP diets ($P < 0.05$). In the distal ileum piglets offered the PP diet had a higher density of crypt goblet cells compared to piglets offered any other diet ($P < 0.05$).

4.3.5 Lamina propria T lymphocyte density

Table 6. Lamina propria $CD4^+$ and $CD8^+$ T lymphocyte density measured in piglets either at weaning (BASE), or after consuming one of four different diets (CON, BC, BP, PP) for one week after weaning.

	Treatment[1]					
	BASE	CON	BC	BP	PP	SEM
$CD4^{+}$ [2,3]	8.2^{a}	10.6^{c}	10.9^{c}	9.1^{ab}	10.5^{bc}	0.5
$CD8^{+}$	5.3^{a}	6.8^{bc}	6.2^{b}	6.1^{ab}	7.4^{c}	0.3
$CD4^{+}$:$CD8^{+}$	1.60	1.72	1.84	1.50	1.57	0.17

[1] BASE, baseline; CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma.
[2] Least-square mean values with pooled standard error of the mean (SEM).
[3] Number of positive T lymphocytes per $0.1mm^2$ of lamina propria.
[a,b,c] Least-square means within a row with different superscripts are significantly different ($P < 0.05$).

Mid jejunal lamina propria $CD4^+$ T lymphocyte density was significantly affected by litter of birth and treatment ($P < 0.001$). Litter of birth accounted for 20% of the total variation in $CD4^+$ T lymphocyte density. Compared to pigs killed at weaning, $CD4^+$ T lymphocyte density was 28-33% higher in pigs killed one week after weaning ($P < 0.05$), with the exception of those offered the BP diet, which were not significantly different (Table 6). Among pigs offered different diets after weaning, those consuming the BP diet had lower $CD4^+$ T lymphocyte density compared to those consuming the CON diet ($P < 0.05$), whereas those offered the BC and PP diets were not significantly different from CON values ($P > 0.10$). Mid jejunal lamina propria $CD8^+$ T lymphocyte density was significantly affected by both litter of birth and treatment ($P < 0.001$). Litter of birth accounted for 24% of the total variation in this variable. $CD8^+$ T lymphocyte density was 17-40% lower in piglets killed at weaning than those offered either the CON, BC or PP diets ($P < 0.05$), and tended to be lower than those offered the BP diet ($P = 0.06$). Among piglets killed one week after weaning, piglets consuming the PP diet had a higher density of $CD8^+$ T lymphocytes compared to pigs offered any other diet ($P < 0.05$), all of which were not significantly different ($P > 0.10$). The ratio of $CD4^+$ to $CD8^+$ T lymphocytes was not significantly

affected by litter of birth, nor treatment ($P > 0.10$). $CD4^+$ and $CD8^+$ T lymphocyte densities were not significantly correlated with villus height nor crypt depth ($P > 0.10$).

4.3.6 Correlations between feed intake, growth rate, and histological variables

Table 7. Correlation coefficients between villus height measured one week after weaning and average daily feed intake or average daily gain of pigs offered different diets after weaning.

	Dietary treatment[1]			
	CON	BC	BP	PP
Average daily feed intake				
Proximal jejunum	0.55	0.66*	0.60	0.60
Mid-jejunum	0.63*	0.66*	0.68*	0.71**
Distal ileum	0.75**	0.15	0.60	0.77**
Mean	0.74**	0.57	0.66*	0.75**
Average daily gain				
Proximal jejunum	0.67*	0.75**	0.67*	0.67*
Mid-jejunum	0.74**	0.72**	0.73**	0.81**
Distal ileum	0.72**	0.22	0.66*	0.88**
Mean	0.81**	0.66*	0.72**	0.85**

[1] CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma.
* $P < 0.10$; ** $P < 0.05$.

In pigs consuming the CON diet, positive correlations between average daily feed intake and average villus height were observed in the mid jejunum ($P < 0.10$) and distal ileum ($P < 0.05$), and the average of all sites ($P < 0.05$; Table 7). Pigs consuming the BC diet showed trends for a positive correlation between these variables in the proximal and mid jejunum only ($P < 0.10$), with no relationship observed in the distal ileum, nor in the average of all sites ($P > 0.10$). In pigs offered the BP diet, no relationship between villus height and average daily feed intake was observed in the proximal jejunum nor the distal ileum ($P > 0.10$), although a trend for a positive correlation between these variables was observed in the mid jejunum and the average of all sites ($P < 0.10$). In pigs offered the PP diet, positive correlations between villus height and average daily feed intake were observed in the mid jejunum and distal ileum, and the average of all sites ($P < 0.05$), but no relationship was observed in the proximal jejunum ($P > 0.10$). Regardless of diet and site in the intestine, crypt depth was not significantly correlated with average daily feed intake ($P > 0.10$; data not shown). No significant relationships were observed between average daily feed intake and $CD4^+$ nor $CD8^+$ T lymphocyte density. Ratio of $CD4^+$ to $CD8^+$ T lymphocytes displayed a trend for a

negative correlation with average daily feed intake in pigs offered the CON diet ($r = -0.66$, $P < 0.10$), whereas no significant relationship between these variables was observed in pigs offered any other diet ($P > 0.10$, data not shown).

In pigs offered the CON diet, positive correlations between average daily gain and average villus height were observed in all sites, and the average of all sites. In pigs consuming the BC diet, positive correlations between these variables were observed in the proximal and mid jejunum ($P < 0.05$), and the average of all sites ($P < 0.10$), but not in the distal ileum ($P > 0.10$). In pigs offered the BP and PP diets, positive correlations between average daily gain and average villus height were observed in all sites, and the average of all sites. Crypt depth was positively correlated with average daily gain in the distal ileum of pigs consuming the BP diet ($r = 0.77$, $P < 0.05$), however no other relationships between these variables were observed regardless of site and diet ($P > 0.10$; data not shown). $CD4^+$ and $CD8^+$ T lymphocyte density and $CD4^+$ to $CD8^+$ lymphocyte ratio were not significantly correlated with average daily gain in pigs offered either experimental diet ($P > 0.10$; data not shown).

4.4 DISCUSSION

In this experiment, the specialised dietary ingredients spray-dried bovine colostrum, bovine plasma and porcine plasma were included in weaning diets which were offered to 28-day-old pigs for one week after weaning. Inclusion of these ingredients had no effect on feed intake and growth rate, nor feed conversion ratio. The dietary proteins did, however, affect the histology and morphology of the small intestine, in varying ways. Pigs killed at weaning provided 'baseline' data, and demonstrated significant effects of weaning, particularly on intestinal morphology and histology.

Numerous experiments have demonstrated that weaning is associated with significant morphological restructuring of the small intestine, such as a reduction in villus height, an increase in villus complexity, and an increase in crypt depth (Homrich et al., 1973; Gay et al., 1976; Kenworthy, 1976; Hall et al., 1983; Smith, 1984; Hampson, 1986a,b; Miller et al., 1986; Cera et al., 1988; Dunsford et al., 1989; Hall and Byrne, 1989; Kelly et al., 1990; 1991a,b; Li et al., 1990, 1991a,b; Nabuurs et al., 1993a,b; Makkink et al., 1994; McCracken et al., 1995; Pluske et al., 1996a,b; McCracken et al., 1999; Spreeuwenberg et al., 2001; Marion et al., 2002). The results of the present study accord with these experiments, which demonstrated a 41-45% reduction in average villus height in the first week after weaning, but a more modest increase in crypt depth of 7-11% during this time. This study also demonstrated an increase in epithelial cell height after weaning, which appears not to have been reported elsewhere, and may indicate an increase in epithelial cell metabolic activity in weaned pigs.

An increase in the activation and cellularity of the gastrointestinal immune system has also been observed to occur after weaning, particularly with respect to lamina propria T lymphocyte populations (Vega-López et al., 1995; McCracken et al., 1999; Pluske et al., 1999a; Solano-Aguilar et al., 2001; Spreeuwenberg et al., 2001). The lamina propria $CD4^+$ T lymphocyte population has been shown to expand within 24 hours of weaning (Pluske et al., 1999a), and may increase further until at least 7 days after weaning (McCracken et al., 1999). $CD8^+$ T lymphocyte numbers have also been shown to increase after weaning (McCracken et al., 1999), and the net effect of these changes can be an alteration in the ratio of $CD4^+$ to $CD8^+$ T lymphocytes within 24 hours of weaning (Spreeuwenberg et al., 2001). However, it should be noted that not all studies demonstrate a significant effect of weaning on $CD4^+$ and/or $CD8^+$ T lymphocyte numbers in the small intestine, even though other populations

of T lymphocytes and leukocytes are affected (Vega-López et al., 1995; Pluske et al., 1999a; Spreeuwenberg et al. 2001). In the present study, both $CD4^+$ and $CD8^+$ T lymphocyte populations expanded within a week of weaning, indicating a weaning-related effect on the gastrointestinal immune system.

Crypt goblet cells are an integral component of the innate mucosal immune system in the intestine, which secrete mucin and trefoil peptides to form a viscoelastic gel that coats the mucosal surface protecting it from, and repairing, injury (Kindon et al., 1995; Deplanke and Gaskins, 2001). Crypt goblet cell density was similar in all sites of the intestine in pigs killed at weaning, whereas pigs killed one week after weaning generally displayed an increasing gradient of goblet cell density from the proximal to distal small intestine, with goblet cell density of pigs at weaning generally higher in more proximal areas and lower in the distal ileum, compared to pigs killed one week after weaning. The observation of this gradient accords with that of Spreeuwenberg et al. (2001), who reported a similar gradient of goblet cell density in pigs after weaning. The hyperplastic response of crypts to weaning was also observed to be greater in the distal ileum compared to more proximal areas, suggesting that increased mitotic activity in the distal intestine may be accompanied by an increase in epithelial cell differentiation into goblet cells in the crypts. Average crypt goblet cell density was numerically lower in pigs consuming the control diet, compared to those killed at weaning, due to significant reductions in this variable in both jejunal sites. This observation supports that of Dunsford et al. (1991), who demonstrated a negative effect of weaning on goblet cell density in the small intestine. This effect may be due to the characteristic post-weaning stress response (Wu et al., 2000), since stress is associated with goblet cell depletion (Hart and Kamm, 2002). In contrast to these findings, Spreeuwenberg et al. (2001) and McCracken et al. (1999) found no effect of weaning on goblet cell density in the small intestine, whereas McCracken et al. (1995) and Gu et al. (2002) observed increases in crypt goblet cell density after weaning. A dietary effect on goblet cell numbers after weaning was observed in the present study, with piglets consuming a diet containing test protein responding differently to those offered the control diet; this will be discussed later.

Aside from investigating weaning-related changes in small intestine morphology, this study attempted to evaluate the potential of spray-dried bovine colostrum, bovine plasma and porcine plasma to influence the response of pigs to weaning. The protein and amino acid composition of the spray-dried bovine plasma used in this study is similar to that reported by

Hansen et al. (1993), however the sodium concentration reported by Hansen et al. (1993) is considerably lower (0.99% vs. 3.5%), which likely reflects different processing methods during plasma production. The protein, amino acid and mineral composition of the porcine plasma used in this study is also similar to that reported by Hansen et al. (1993). Hansen et al. (1993) demonstrated a significantly higher concentration of sodium in porcine plasma compared to bovine plasma, which is confirmed by the findings of this study, in which the sodium concentration of porcine plasma was almost double that found in bovine plasma. The amino acid composition of the spray-dried bovine colostrum used in this study is similar to that reported for freeze-dried bovine colostrum by Dunshea et al. (2002), although the level of crude protein in the colostrum used in the present study was somewhat higher (76.6 vs. 58.5%). The IgG content of the spray-dried bovine plasma used in this study was higher than that of the bovine colostrum and also exceeded IgG levels in spray-dried porcine plasma reported by Pierce et al. (1995a,b) (22.5 and 17.9%, respectively).

The positive response of pigs to dietary inclusion of spray-dried plasma is generally greatest in the first week after weaning (van Dijk et al., 2001a), and hence was the time period used in this experiment. Despite numerical increases in feed intake and weight gain of pigs offered diets containing the test proteins, their dietary inclusion did not cause a significant improvement in these variables compared to pigs offered the control diet. The intakes of individual pigs in the present study showed a high degree of variation, which has also been reported by McCracken et al. (1995), who performed a similar experiment with pigs of the same age. McCracken et al. (1995) reported an average cumulative intake of less than 100g per pig in the first two days after weaning, whereas average cumulative intake did not exceed 100g in the present study until the third day after weaning.

The different experimental diets used in this experiment failed to influence the weight of either the liver, spleen, stomach or intestine one week after weaning. Spray-dried plasma has been shown in some studies to reduce heart weight within 4 days of weaning (Touchette et al., 1999b) and small intestine weight within 16 days of weaning (Jiang et al., 2000). Spray dried bovine colostrum has also been demonstrated to reduce small intestine weight within 14 days of weaning (Chapter 3, this thesis). Unfortunately heart weight was not measured in this study, and the relatively brief duration of the experiment may have been insufficient to induce an effect on small intestine weight. Jiang et al. (2000) also reported a reduction in plasma urea concentrations measured 16 days after weaning in pigs fed diets containing 10%

spray-dried plasma, whereas at 8 days after weaning no difference was observed. The present study did not demonstrate a diet-related effect on plasma urea concentrations, which may also be due to the relatively short duration of feeding.

Effects of the different diets on the morphology and histology of the small intestine were complex, due to the occurrence of different site by treatment interactions. Addition of spray-dried bovine colostrum to the diet reduced small intestine villus height in the proximal jejunum, but increased it in the distal ileum, whereas spray-dried bovine plasma increased villus height in the mid jejunum and distal ileum, and spray-dried porcine plasma had no effect on villus height in any region of the small intestine. Relating these results to those of other studies evaluating these products is somewhat difficult, since some have not measured or reported intestinal morphology in more than one section of the intestine (Gatnau et al., 1995; Touchette et al., 1997) and, of those that have, most have generally not demonstrated any effect of the test ingredient (in these cases spray-dried plasma) on intestinal morphology at any site of the small intestine (Jiang et al., 2000; Touchette et al., 1999b; van Dijk et al., 2001b, 2002). However, one study did report an interaction of dietary plasma with small intestine site, such that the ratio of villus height to crypt depth was increased in the jejunum and ileum, but not the duodenum, in pigs offered a diet containing 3.5% spray-dried plasma for 10 days after weaning at 18 days of age (Spencer et al., 1997). This effect was largely due to a significant increase in villus height, whereas crypt depth was unaffected by diet. These observations resemble those of pigs offered spray-dried bovine colostrum and bovine plasma in the present study, in which improvements in villus height were manifested in more distal regions of the small intestine, whereas crypt depth was unaffected by diet, and ratio of villus height to crypt depth was increased only in the mid jejunum of pigs consuming the bovine plasma diet, compared to pigs offered the control.

Level of feed intake has been shown to significantly affect small intestine morphology, with a generally positive relationship between these variables being observed (McCracken et al., 1995; Pluske et al., 1996a, 1997). These observations are confirmed by the present study, in which average daily feed intake was positively correlated with villus height in most areas of the small intestine regardless of diet. However, it is interesting to note that, although increasingly strong relationships between level of feed intake and villus height were observed from the proximal to distal small intestine of pigs offered the control and porcine plasma diets, pigs consuming the bovine colostrum and bovine plasma diets showed poorer

relationships between these variables in the most distal region of the intestine. This suggests an effect of these products independent of that of feed intake *per se* in the distal small intestine, which supports the interaction between diet and site of the small intestine observed in the present study and that of Spencer et al. (1997). One possible explanation for this phenomenon is that the activity of the active components is able to be expressed more readily in the distal small intestine. There is evidence that enteric infections caused by agents such as *Escherichia coli* and rotavirus preferentially induce mucosal damage in more distal regions of the small intestine, while more proximal regions may be left largely unscathed (Lai et al., 1991; Hall et al., 1989). It may therefore be possible that the more distal areas of the small intestine are more likely to benefit from any passive immunoprotection afforded by the immunoglobulins present in bovine colostrum and plasma. In this regard, it is noteworthy that reduction of antigen exposure through segregated early-weaned of pigs has been shown to increase villus height, and increase the ratio of villus height to crypt depth in the jejunum and ileum, but not the duodenum, compared to conventionally weaned pigs (Tang et al., 1999).

This hypothesis is not completely supported by the observed effect of the different experimental diets on lamina propria $CD4^+$ and $CD8^+$ T lymphocyte density. No consistent effect of the test proteins was observed with regard to T lymphocytes densities, although the number of $CD4^+$ T cells was reduced in pigs offered the bovine plasma diet, compared to those offered the control. Since level of feed intake after weaning has been shown to influence T lymphocyte populations (McCracken et al., 1995; Spreeuwenberg et al., 2001), it is possible that the reduced density of $CD4^+$ T cells observed in bovine plasma-fed pigs is due to their numerically higher intake during the post-weaning period. However, the fact that neither $CD4^+$ nor $CD8^+$ T lymphocyte density showed any relationship with average daily feed intake in the present study casts doubt on this explanation, suggesting an intake-independent effect of dietary bovine plasma on lamina propria $CD4^+$ T lymphocyte density. To the author's knowledge this is a novel finding, and provides further support to the hypothesis that immunoglobulin-containing dietary ingredients reduce immune stimulation by preventing interaction between luminal antigens and the intestinal mucosa through the provision of passive immune protection. However, the fact that, despite their similar immunoglobulin contents, neither bovine colostrum nor porcine plasma produced the same effect is problematic for this hypothesis. This hypothesis is further complicated by the confounding presence of other antimicrobial compounds in addition to immunoglobulins in

plasma and colostrum (Schanbacher et al., 1997; Gopal and Gill, 2000; Shah, 2000; Van Hooijdonk et al., 2000), which could contribute to, or be responsible for, an immunoprotective effect.

Aside from a passive immunoprotective role of immunoglobulins and other antimicrobial factors present in the test proteins, the results of the present study suggest a further mechanism by which the test proteins may improve the immune function of the pig. Consumption of the bovine colostrum and bovine plasma diets increased crypt goblet cell density in the proximal and mid jejunum, and consumption of the porcine plasma diet increased crypt goblet cell density in all regions of the small intestine, compared to pigs consuming the control diet. This may indicate an antigenic effect of the novel proteins present in the test ingredients, which induces a tolerogenic immune response in the animal. A central mechanism involved in the induction of oral tolerance is T lymphocyte mediated regulation or suppression of immune responsiveness. This involves T lymphocyte production of immunosuppressive cytokines such as transforming growth factor-β and interleukins 4 and 10 (Khoury et al. 1992; Chen et al., 1994), which may also induce a secretory immune response (Murray et al., 1987; Defrance et al., 1992; van Vlasselaer et al., 1992) that is often observed to accompany the development of immune tolerance (Challacombe and Tomasi, 1980). Activation of immune cells involved in the secretory immune response can influence goblet cell differentiation (Deplanke and Gaskins, 2001), which is increased during immune responses (Elwood and Garden, 1999). Alternatively, it is possible that the test proteins may have reduced the extent of the post-weaning stress response, and its suppressive effect on intestinal goblet cell numbers (Hart and Kamm, 2002), via an unknown mechanism.

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Chapter 5

THE EFFECT OF DIETARY SPRAY-DRIED BOVINE COLOSTRUM AND PLASMA ON THE RESPONSE OF PIGS TO ENTEROTOXIGENIC *ESCHERICHIA COLI* CHALLENGE AFTER WEANING.

5.1 INTRODUCTION

The inclusion of spray-dried plasma in the diets of weaner pigs, at levels ranging from 3-8%, generally results in an improvement in feed intake and growth rate, and sometimes an improvement in feed conversion ratio (Coffey and Cromwell, 2001; van Dijk et al. 2001), all of which can help to overcome the characteristic post-weaning 'growth check'.

The improvement in weaner pig feed intake and growth rate can be reproduced through dietary inclusion of the high molecular weight fraction of plasma alone, whereas the low and medium molecular weight fractions generally produce no effect (Gatnau et al., 1995; Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995). The high molecular weight fraction is composed principally of immunoglobulins, suggesting that these may be responsible for the performance benefits conferred by feeding plasma products. Dietary inclusion of spray-dried bovine colostrum, which contains comparable concentrations of immunoglobulins, can produce performance benefits similar to plasma products when included at levels of 5, 6 and 10% in weaner pig starter diets, supporting this hypothesis (Pluske et al., 1999a; Chapter 3, this volume).

Extensive data in the literature have shown that the performance-enhancing effects of dietary spray-dried plasma are more pronounced when pigs are housed under "practical conditions," which provide immune stimulus (i.e. on-farm or continuous-flow nurseries), compared to experimental conditions, in which antigenic stimulation is minimised (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995; Touchette et al., 1996; Bergstrom et al., 1997; Bregendahl et al., 1998; Chirra et al., 1999). Furthermore, it has been shown that dietary spray-dried plasma enhances growth rate and feed intake in pigs with a high degree of antigen exposure (caused by conventional weaning), but not those with a low degree of antigen exposure (caused by medicated early-weaning) (Stahly et al., 1995).

Taken together, the fact that the benefits of dietary spray-dried plasma appear to reside in the immunoglobulin-containing fraction, and that the effects of dietary spray-dried plasma are more pronounced when pigs are reared under conditions that provide a degree of bacterial challenge, has led to the formulation of an immune-mediated hypothesis to explain its mechanism of action. Although gut closure prevents absorption of immunoglobulins into the systemic circulation of weaner piglets, dietary immunoglobulins can exert a passive immunoprotective effect in the intestine, by binding antigens and reducing bacterial adhesion

to the intestinal mucosa (Porter, 1986; Kagnoff, 1993; Salmon, 1999). There are also other compounds present in plasma and colostrum that can provide antimicrobial and antiviral protection within the gut lumen, such as glycoproteins, lactoferrin, lactoperoxidase, and oligosaccharides (Sanchez et al., 1993; Schanbacher et al., 1997; Gopal and Gill, 2000; Shah, 2000; van Hooijdonk et al., 2000). A passive immunoprotective effect of these compounds may reduce the incidence and/or extent of intestinal infection over the weaning period, as suggested by Coffey and Cromwell (1995). Evidence supporting this hypothesis has been provided by Deprez et al. (1990, 1996) and Nollet et al. (1999), who demonstrated a suppressive effect of dietary spray-dried plasma on faecal excretion of haemolytic *E. coli* after weaning. However other studies have failed to confirm these results (Cain and Zimmerman, 1997; van Dijk et al., 2002).

Provision of passive immune protection may be especially beneficial during the immediate post-weaning period, which is characterised by a rapid expansion and maturation of the intestinal immune system (Vega-López et al., 1995; McCracken et al., 1999; Pluske et al., 1999b; Solano-Aguilar et al., 2001), which is induced, in part, by the removal of the passive protection provided by maternal immunoglobulins and antimicrobial factors present in sows milk (King et al., 2003). Another factor implicated as a cause of increased activity in the intestinal immune system after weaning is the low feed intake typical of the weaned piglet, which can compromise epithelial barrier function allowing luminal antigens into the underlying lamina, inducing an inflammatory response in the small intestine (McCracken et al. 1999; Spreeuwenberg et al., 2001). An active, inflammatory immune response is associated with significant restructuring of the intestinal mucosa, most notably characterised by villus atrophy (MacDonald and Spencer, 1988, MacDonald et al., 1999; McCracken et al. 1999), and the secretion of proinflammatory cytokines, which can reduce feed intake and alter the partitioning of nutrients to support immune processes at the expense of growth (Kelley et al., 1994; Johnson, 1997; Stahly, 2001). A passive protective effect of dietary immune factors during this period could therefore decrease the incidence of post-weaning inflammation by reducing the invasion of luminal antigens across the epithelial barrier. This has the potential to improve the structure and function of the intestine, improving piglet health and therefore feed intake and growth.

The objective of this study was therefore to test the effect of dietary spray-dried colostrum and plasma on the response of weaner pigs to an experimental infection challenge with

enterotoxigenic *E. coli*, with emphasis on indices of humoral and gastrointestinal immune status, intestinal histology and morphology, and piglet feed intake and growth.

5.2 MATERIALS AND METHODS

5.2.1 Pigs and conduct of the experiment

Experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Recommendations and Minimum Standards for the Care and Use of Animals for Scientific Purposes (New Zealand Animal Welfare Advisory Committee, 1995).

The experiment used 32, 21-day-old female pigs (Large White-Landrace-Duroc x Large White-Landrace-Duroc), 6.9 ± 0.04 kg live-weight, and was conducted in an isolated suite at the Massey University Animal Physiology Unit. Routine management of pigs prior to weaning included a subcutaneous injection of iron dextran (2ml), clipping of incisors and tail, and creep feeding from 14 to 21 days of age. On the day of weaning (21 days of age), 4 piglets from 4 litters were blocked according to litter of birth and live-weight and randomly allocated to one of four treatment groups. Due to availability of pigs, two replicates of this design were performed, allowing a total of 8 pigs per treatment.

Piglets were weaned onto one of three diets and, with the exception of those in the negative control group, received an oral bacterial challenge. The four treatments were: (1) control diet (CON-); (2) control diet with *E. coli* K88 challenge (CON+); (3) bovine plasma diet with *E. coli* K88 challenge (BP+); and (4) bovine colostrum with *E. coli* K88 challenge (BC+). After administration of the oral challenge (day 12), pigs were offered the experimental diets for a further 7 days, after which time they were euthanased (day 19), and post-mortem measurements taken. Faecal scores (1 = solid, 2 = pasty; 3 = liquid) were measured twice daily. Individual live-weights were recorded on days 1, 12 and 19. Rectal temperatures were recorded, and faecal samples obtained, on days 12, 14, 16 and 19. Blood samples were taken from the vena jugularis on days 12 and 19 for analysis of white blood cell differential counts. Individual feed intake was measured daily.

Piglets were housed individually in stainless steel cages that permitted visual, but not physical, contact between animals, and were separated from their waste products by perforated

galvanised-steel flooring. Unchallenged pigs, from the CON- treatment, were housed in a separate suite. Airflow to the facility was controlled, and room temperature was maintained at 30°C with a 12-h light/dark cycle. Pigs had *ad libitum* access to diets and water for the duration of the experiment.

5.2.2 Experimental diets

Control (CON), bovine plasma (BP) and bovine colostrum (BC) diets were based on maize, wheat and skim milk powder (Table 1) and formulated to meet or exceed National Research Council recommendations for major nutrients (National Research Council, 1998), with lysine as the first-limiting amino acid. Bovine colostrum and bovine plasma were included in diets BC and BP, respectively, at a level of 7.5%, replacing skim milk powder on an isolysine basis. Diets were balanced to a similar lactose content (12.5%) using crystalline lactose, and offered in meal form.

5.2.3 Bacteria and challenge procedure

The challenge strain used in this experiment was an *E. coli* O149:K88 isolated from a pig displaying clinical post-weaning diarrhoea. Strains of O serogroup 149 have a well-established link with post-weaning diarrhoea (Bertschringer, 1999; van Beers-Schreurs et al., 1992). The bacteria were grown in a brain-heart infusion broth at 37 °C for 24 hours. Bacteria were harvested by centrifugation, washed in 0.2 M phosphate buffered saline (PBS), and resuspended in PBS at a concentration of 1 x 10^9 colony forming units (CFU) per ml.

Piglets were initially starved overnight, and then offered the experimental diets for 12 days, to ensure a reasonable level of feed intake, before administration of the oral challenge. The oral challenge consisted of 1 x 10^9 CFU of *E. coli* suspended in 5ml of PBS. This was administered to the challenged pigs by syringe through a polyethylene tube held in the oral cavity. Unchallenged pigs were subjected to the same procedure and a sham dose of 5ml PBS was administered.

Table 1. Percentage composition and calculated analysis of the experimental diets.

	Experimental diets[1]		
Ingredient, %	CON	BC	BP
Wheat	45.57	36.20	34.70
Maize	20.00	30.00	30.00
Skim milk powder	25.00	12.60	11.80
Casein	6.48	4.62	5.57
Bovine colostrum[2]	-	7.5	-
Bovine plasma[3]	-	-	7.5
Lactose	-	5.65	6.60
L-Lysine	0.04	0.25	0.15
D, L-Methionine	0.01	0.11	0.09
L-Threonine	0.24	0.26	0.21
Soya bean oil	0.10	0.10	0.85
Dicalcium Phosphate	2.04	2.08	2.23
Salt	0.21	0.33	0.00
Sodium bicarbonate	0.41	0.46	0.02
Vitamin & Mineral Premix[4]	0.3	0.3	0.3
Calculated Analysis			
DE[5], MJ/kg	15.0	15.0	15.0
Crude protein, %	22.0	22.0	22.0
Crude fat, %	18.6	20.6	27.2
Digestible lysine, %	1.40	1.40	1.40
Digestible methionine + Cysteine, %	7.5	7.5	7.5
Lactose, %	12.5	12.5	12.5
Sodium, %	3.5	3.5	3.5

[1] CON, control; BC, Bovine colostrum; BP, Bovine plasma.
[2] Immulac (Fonterra, Specialty Ingredients Division, Hautapu, New Zealand).
[3] AP820 (Proliant Corp., Ames, Iowa, USA).
[4] Vitastart (Vitec Nutrition Ltd, Auckland, New Zealand). Supplied per kilogram diet: Mn, 45 mg; Zn, 120 mg; Cu, 125 mg; Co, 0.5 mg; I, 1 mg; Fe, 100 mg; Se, 300 µg; Vitamin A, 15 000 IU; Vitamin D_3, 2000 IU; Vitamin E, 70mg; Vitamin K, 2.5 mg; Vitamin B_1, 2 mg; Vitamin B_2, 3 mg; Vitamin B_6, 2 mg; Vitamin B_{12}, 30 µg; Calcium Pantothenate, 20 mg; Niacin, 20 mg; Biotin, 100 µg; Folic Acid, 500 µg; Choline 150 mg.
[5] DE, digestible energy.

5.2.4 Post-mortem procedure

On day 19 of the experiment all piglets were euthanased and post-mortem measurements taken. Piglets were anaesthetised by halothane inhalation, weighed, a 5ml blood sample was taken from the vena jugularis and a faecal sample was obtained. Piglets were then euthanased by an intracardial injection of sodium pentobarbitone (125 mg/kg). The abdomen was opened immediately, from the sternum to the pubis, and the entire gastrointestinal tract

removed. A pair of scissors was used to disconnect the small intestine at the gastric pylorus and the ileo-caecal valve, and the intestine was clamped. The small intestine was laid out on a stainless-steel tray and a section at proportionally 25, 50 and 75% along the intestine was clamped with haemostats, excised and placed immediately into a plastic container with Bouin's fluid (24% formalin, 5% glacial acetic acid, 71% picric acid). After fixation for 24 hours, the Bouin's fluid was replaced with 70% ethanol. The heart, liver, spleen and kidneys were removed and weighed. The stomach, caecum, and large intestine were weighed upon removal with their contained digesta, then emptied, washed with water, blotted dry, and weighed again. The piglet was weighed again, to obtain the carcass weight. Total processing time, from killing to obtaining gut samples, was about 10 minutes. Sites at 0.25, 0.5 and 0.75 are referred to by their approximate position in the small intestine, i.e. proximal jejunum, mid-jejunum and distal ileum, respectively.

5.2.5 Histology and immunocytochemistry

Intestinal samples were processed and stained, and histological measurements taken (villus height, crypt depth, epithelial cell height, goblet cell density in villus and crypt epithelium) as described previously (Chapter 3, this volume). Mid jejunal lamina propria $CD4^+$ and $CD8^+$ T lymphocytes were identified by immunocytochemistry and enumerated as described previously (Chapter 3, this volume).

5.2.6 Bacteriology

Faecal samples were stored at –70 °C until processed for determination of *E. coli* counts. To determine faecal *E. coli* counts, faecal samples were serially diluted in peptone physiological salt solution, and numbers of bacteria per gram of wet faeces were assessed by surface plating techniques on *E. coli*-specific agar plates. Colonies were counted after incubation for 24 hours at 37 °C. Randomly sampled colonies were tested for the presence of *E. coli* K88 by slide agglutination using *E.coli* K88 pilus antiserum (DS-213877; Medbio Ltd., Christchurch, New Zealand).

5.2.7 Analysis of blood samples

Whole blood samples were collected in EDTA vaccutainers, and leukocytes were counted by flow cytometry using the Avadia 120 Haematology System (Bayer Diagnostic Division, Tarrytown, NY). White blood cell parameters measured included: total number of

leukocytes, polymorphonuclear cells, mononuclear cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

5.2.8 Statistical analysis

The data were subjected to analysis of variance by the General Linear Models (GLM) procedures of SAS (SAS Institute, 2000) using pig as the experimental unit. Where appropriate, repeated measure analysis was also employed within the GLM procedure to discern the effect of time on variables such as daily feed intake, rectal temperature, faecal *E. coli* count and haematological parameters. Feed conversion ratio was calculated on a treatment basis, as total feed intake of pigs within a treatment divided by the corresponding total weight gain, and is presented for comparison only. Organ weights are expressed as a percentage of the empty body weight.

The general statistical model used in the analysis of growth rate and feed intake, average faecal score, empty body weight, carcass weight and organ weights was:

$$y_{ijk} = \mu + \beta_j + \alpha_i + \varepsilon_{ijk}$$

Where:

y_{ijk} = observation from the k^{th} pig from the j^{th} litter within the i^{th} treatment.

μ = the population mean.

β_j = the random effect of the j^{th} litter of birth.

α_i = the fixed effect of the i^{th} treatment.

ε_{ijk}= residual error of the k^{th} pig from the j^{th} litter within the i^{th} treatment.

To discern the effect of time on daily feed intake, haematological parameters, faecal *E. coli* count and rectal temperature, repeated measure analysis was performed using the following model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \pi_l(\alpha_i, \beta_j) + \chi_k + \chi_k\alpha_i + \chi_k\beta_j + \varepsilon_{ijkl}$$

Where:

y_{ijkl} = observation from the l^{th} pig from the j^{th} litter of birth, within the i^{th} treatment and the k^{th} time period.

μ = the population mean.

α_i = the fixed effect of the i^{th} treatment.

β_j = the random effect of the j^{th} litter of birth.

$\pi_l(\alpha_i, \beta_j)$ = random effect of the l^{th} pig within the i^{th} treatment and the j^{th} litter of birth.

χ_k = the fixed effect of the k^{th} time.

$\chi_k\alpha_i$ = the interaction between the k^{th} time and the i^{th} treatment.

$\chi_k\beta_j$ = the interaction between the k^{th} time period and the j^{th} litter of birth.

ε_{ijkl}= residual error of the l^{th} pig from the j^{th} litter of birth, within the i^{th} treatment and the k^{th} time period.

The statistical model used in the analysis of small intestine histology was:

$$y_{(i)jk} = \mu + \alpha_i + \beta_{j(i)} + \gamma_k + \alpha_i\gamma_k + \varepsilon_{(i)jk}$$

Where:

$y_{(i)jk}$ = observation from the k^{th} site from the j^{th} pig nested within the i^{th} treatment.

μ = the population mean.

α_i = the fixed effect of the i^{th} dietary treatment.

$\beta_{j(i)}$ = the random effect of the j^{th} pig nested within the i^{th} treatment

γ_k = the fixed effect of the k^{th} site in the small intestine.

$\alpha_i\gamma_k$ = the interaction between the i^{th} treatment and the k^{th} site of the small intestine.

$\varepsilon_{(i)jk}$= residual error of the k^{th} site from the j^{th} pig nested within the i^{th} treatment.

The effect of replicate was tested and found to be not significant in each model ($P > 0.05$), and was therefore removed. Where a significant treatment effect was observed, Fisher's least significant difference test was performed to determine significant differences between least-square means of treatment groups. Level of significance was pre-set at $P < 0.05$, and trends were identified at $P < 0.10$. Pearson correlation analysis was performed using the CORR procedure of SAS (SAS Institute, 2000), to evaluate a possible correlation between feed intake, growth rate and histological variables. Data are presented as least-square means with the associated pooled standard error of the mean (SEM).

5.3 RESULTS

5.3.1 Feed intake, growth rate and feed conversion ratio

Repeated measure analysis of daily feed intake demonstrated a significant effect of time ($P < 0.001$), with feed intake increasing throughout the experiment (data not shown). A time by litter effect was also observed ($P < 0.001$), but no time by treatment effect ($P > 0.05$).

Average daily gain and feed intake from day 1-12 (pre-challenge) was not significantly affected by litter of birth, nor treatment ($P > 0.05$). Feed conversion ratio from day 1-12 was similar amongst groups (Table 2). Average daily gain and average daily feed intake from day 12-19 (post-challenge) were not significantly affected litter of birth, nor treatment ($P > 0.05$). However, feed intake and weight gain during this period was numerically lower in all challenged groups, compared to the unchallenged group, while feed conversion ratio was similar amongst all groups. Overall, average daily gain (day 1-19) showed a trend for an effect of weaning weight only ($P = 0.07$), whereas overall average daily feed intake was not affected by weaning weight, litter of birth nor treatment ($P > 0.05$). Overall feed conversion ratio was similar amongst treatment groups.

Table 2. Growth rate, feed intake and feed conversion ratio of *E. coli* challenged and non-challenged pigs offered diets containing different protein sources for 19 days after weaning.

	Treatment[1]				
	CON-	CON+	BC+	BP+	SEM
Day 1-12					
Average daily gain[2], g	152	132	151	142	45
Average daily feed intake, g	183	143	168	167	41
Feed conversion ratio[3]	1.30	1.24	1.28	1.25	-
Day 12-19					
Average daily gain, g	475	430	423	356	38
Average daily feed intake, g	477	425	446	372	47
Feed conversion ratio	1.05	1.01	1.07	1.08	-
Day 1-19					
Average daily gain, g	278	248	257	225	38
Average daily feed intake, g	303	263	281	250	40
Feed conversion ratio	1.11	1.16	1.16	1.17	-

[1] CON-, Control diet, unchallenged; CON+, control diet, challenged; BP+, bovine plasma diet, challenged; BC+, bovine colostrum diet, challenged.

[2] Least square means with pooled standard error of the mean (SEM).

[3] Total weight gain of animals within treatment ÷ total feed intake.

5.3.2 Empty body, carcass, and organ weights.

Litter of birth and treatment had no significant effect on either empty body or carcass weights ($P > 0.05$). Empty body weights were 11.4, 10.4, 9.8 and 10.8 kg for CON-, CON+, BP+ and BC+, respectively (SEM 0.8 kg). Carcass weights were 9.3, 8.5, 7.9 and 8.9 kg for CON-, CON+, BP+ and BC+, respectively (SEM 0.6 kg). Litter of birth had a significant effect on the weight of all organs ($P < 0.05$) with the exception of the heart ($P > 0.10$) and the liver ($P = 0.06$). A significant effect of treatment was observed for large intestine weight ($P < 0.05$), with large intestine weight higher in the BP+ group compared to any other group ($P < 0.05$), amongst which large intestine weights were similar (1.64, 1.80, 2.01 and 1.80% of empty body weight, for CON-, CON+, BP+ and BC+, respectively; SEM 0.07%). Treatment had no significant effect on the weight of any other organ measured ($P > 0.05$; data not shown)

5.3.3 Blood parameters

Litter of birth significantly affected number of leukocytes ($P < 0.05$) but no other blood variable prior to administration of the oral challenge at day 12 (Table 3). The consumption of different diets for 12 days after weaning did not affect any blood parameters at day 12. One week after administration of the oral challenge (day 19), litter of birth was shown to significantly affect all blood parameters ($P < 0.05$), with the exception of percentage of monocytes ($P = 0.07$) and number of leukocytes ($P = 0.19$). Treatment had no significant effect on blood parameters at day 19, although a numerical increase in the number of blood leukocytes ranging from 23-30% was observed in challenged groups (CON+, BC+, BP+), compared to the unchallenged group. Also, the percentage of neutrophils was numerically higher in the CON+ and BP+ groups, compared to either the CON- or BC+ groups. Repeated measure analysis showed a significant effect of day of sampling on number of leukocytes, and percentage of eosinophils ($P < 0.05$), both of which increased over the sampling period. Trends for an effect of day of sampling were observed for percentage of neutrophils and lymphocytes ($P = 0.06$), which decreased and increased over the sampling period, respectively. A significant day by litter interaction was observed for the percentage of basophils ($P < 0.05$), and trends for a day by litter interaction were observed for number of leukocytes ($P = 0.07$), and percentage of eosinophils ($P = 0.08$). No day of sampling by treatment interaction was observed for any of the blood parameters measured ($P > 0.05$).

Table 3. White blood cell parameters of *E. coli* challenged and non-challenged pigs offered diets containing different protein sources for 19 days after weaning.

	Treatment[1]				
	CON-	CON+	BP+	BC+	SEM
Pre-challenge (Day 12)					
Leukocytes, number nl^{-1}	13.4	15.6	14.4	15.9	0.9
Neutrophils, %[2]	38.8	39.6	41.9	41.1	3.6
Eosinophils, %	1.3	1.5	0.9	0.7	0.3
Lymphocytes, %	51.8	51.0	50.3	50.1	3.4
Monocytes, %	4.3	3.6	3.0	4.3	0.6
Basophils, %	1.5	1.3	1.7	1.5	0.5
Post-challenge (Day 19)					
Leukocytes, number nl^{-1}	13.7	16.9	17.8	16.8	1.3
Neutrophils, %	33.0	39.1	39.6	31.3	3.3
Eosinophils, %	1.8	1.6	1.3	1.4	0.2
Lymphocytes, %	56.5	52.3	50.2	59.0	3.0
Monocytes, %	4.4	4.8	4.0	5.2	0.9
Basophils, %	1.4	1.4	1.3	1.5	0.3

[1] CON-, Control diet, unchallenged; CON+, control diet, challenged; BP+, bovine plasma diet, challenged; BC+, bovine colostrum diet, challenged; SEM, pooled standard error of the mean.
[2] Percentage of total number of leukocytes.

5.3.4 Faecal score, faecal *E. coli* count and rectal temperatures

Faecal score in the first 12 days after weaning showed no change, and remained at a level of 1 for pigs in all treatment groups. Average faecal score in the 7 days after administration of the *E coli* challenge displayed a significant effect of litter of birth ($P < 0.05$), which accounted for 49% of the total variation in faecal score during this time. Average faecal score after administration of the challenge was not significantly affected by treatment ($P = 0.21$), however examination of least square means showed that average faecal score of pigs in the CON+ group was significantly higher than that of pigs within the CON- group ($P < 0.05$). Average faecal score of the BC+ and BP+ groups was not significantly different from that of either CON- or CON+ groups ($P > 0.05$). Least-square mean faecal scores for treatment groups CON-, CON+, BC+ and BP+ were 1.00, 1.33, 1.11 and 1.20, respectively (SEM 0.11).

Faecal *E. coli* count was not affected by litter or treatment on any of the days sampled ($P > 0.05$; data not shown). Repeated measure analysis showed a significant effect of day of sampling on faecal *E. coli* count ($P < 0.05$), which decreased as the experiment progressed, and a trend for a day of sampling by litter interaction ($P = 0.09$). Average *E. coli* count was

negatively correlated with growth rate during day 1-12 ($r = -0.31$, $P = 0.08$) and day 12-19 ($r = -0.26$, $P = 0.10$).

Rectal temperature was also not affected by litter of birth nor treatment on any of the days tested ($P > 0.05$; data not shown). Repeated measure analysis showed that day of sampling had a significant effect on rectal temperature ($P < 0.01$), but no day by litter nor day by treatment interactions were observed ($P > 0.05$).

5.3.3 Histology and immunocytochemistry

Villus height was significantly affected by pig nested within treatment, small intestine site and a site by treatment interaction ($P < 0.001$). Average villus height was identical in CON- and CON+ groups (Table 4), but was numerically 7% and 12% lower in BC+ and BP+ groups, respectively, compared to those consuming the CON diet, however this difference was not significant ($P > 0.05$). Pig nested within treatment accounted for 47% of the total variation in villus height. Independent of treatment, villus height was similar in the more proximal sampling sites in the small intestine (proximal and mid-jejunum), which were lower in comparison to the distal ileum site ($P < 0.05$) (579, 574 and 628 μm, respectively, SEM 5.7 μm). A site by treatment interaction was observed, with treatment effects manifested at different sites of the small intestine. In the proximal and mid jejunum, piglets in CON- and CON+ groups displayed similar villus heights, which were higher than that of pigs in either the BC+ or BP+ groups ($P < 0.05$), which were similar. In the distal ileum, villus height of pigs in the CON- and CON+ groups was similar, whereas both the BC+ and BP+ groups had lower villus height compared to CON- ($P < 0.05$). Villus height in more proximal sites of the small intestine (proximal and mid-jejunum) was similar among CON-, CON+ and BC+ groups, with an increase observed in the distal ileum ($P < 0.05$), whereas villus height in the BP+ group was similar in all sites of the small intestine ($P > 0.05$). Average villus height was positively correlated with average daily feed intake from day 12-19 of the experiment in the CON- group ($r = 0.67$, $P = 0.07$), the CON+ group ($r = 0.95$, $P < 0.001$), and the BP+ group ($r = 0.77$, $P < 0.05$), but not the BC+ group ($P > 0.05$). Positive correlations between average villus height and average weight gain during day 12-19 of the experiment were observed in the CON+ group ($r = 0.87$, $P < 0.01$) and the BP+ group ($r = 0.78$, $P < 0.05$), but not the CON- nor BC+ groups ($P > 0.05$).

Table 4. Small intestine histology of *E. coli* challenged and non-challenged pigs offered diets containing different protein sources for 19 days after weaning.

	Treatment*				
	CON-	CON+	BC+	BP+	SEM
Villus height, μm†					
Proximal Jejunum	609^{1a}	618^{1a}	536^{1b}	555^{1b}	
Mid Jejunum	597^{1a}	604^{1a}	557^{1b}	537^{1b}	11‡
Distal Ileum	670^{2a}	652^{2ab}	637^{2b}	552^{1c}	
Mean	625	625	577	548	38
Crypt depth, μm					
Proximal Jejunum	274^{1a}	281^{1a}	258^{1b}	260^{1b}	
Mid Jejunum	251^{2a}	282^{1b}	249^{2a}	248^{2a}	3
Distal Ileum	248^{2b}	256^{2bc}	225^{3a}	258^{1c}	
Mean	258	273	244	255	10
Villus height: crypt depth					
Proximal Jejunum	2.30^{1a}	2.27^{1a}	2.20^{1a}	2.15^{1a}	
Mid Jejunum	2.44^{1c}	2.15^{1a}	2.29^{1bc}	2.20^{1ab}	0.06
Distal Ileum	2.74^{2c}	2.56^{2b}	2.88^{2c}	2.19^{1a}	
Mean	2.50	2.33	2.46	2.18	0.17
Epithelial cell height, μm					
Proximal Jejunum	24.5^{1a}	25.7^{1b}	25.7^{1b}	24.6^{1ab}	
Mid Jejunum	25.1^{1a}	25.6^{1a}	27.3^{2b}	27.2^{2b}	0.4
Distal Ileum	27.0^{2ab}	26.3^{1ab}	27.3^{2b}	26.0^{2a}	
Mean	25.5	25.9	26.7	25.9	0.9
Villus goblet cell density$^{\psi}$					
Proximal Jejunum	0.27^{1a}	0.35^{1ab}	0.28^{1a}	0.40^{1b}	
Mid Jejunum	0.38^{2a}	0.51^{2b}	0.41^{2a}	0.45^{1ab}	0.03
Distal Ileum	0.54^{3a}	0.52^{2a}	0.73^{3b}	0.57^{2a}	
Mean	0.40	0.46	0.47	0.47	0.08
Crypt goblet cell density$^{\psi}$					
Proximal jejunum	1.73^{1a}	2.00^{1b}	2.00^{1b}	2.10^{1b}	
Mid-jejunum	2.00^{2ab}	1.90^{1a}	2.17^{1b}	2.51^{2c}	0.07
Distal ileum	2.43^{3a}	2.27^{2a}	2.64^{2b}	2.48^{2ab}	
Mean	2.05	2.05	2.27	2.36	0.29

* CON-, Control diet, unchallenged; CON+, control diet, challenged; BP+, bovine plasma diet, challenged; BC+, bovine colostrum diet, challenged.

† Least-square mean values with pooled standard error of the mean (SEM).

‡ Pooled standard error of the mean associated with all values within different sites and treatments.

ψ Number of goblet cells per 100 μm epithelium.

^{a,b,c} Least-square means within a row with different superscripts are significantly different ($P < 0.05$).

^{1,2,3} Least square means within a column are significantly different ($P < 0.05$).

Crypt depth showed significant effects of pig nested within treatment, small intestine site, and a site by treatment interaction ($P < 0.001$), but not treatment alone ($P > 0.05$). Average crypt depth was numerically 6% higher in the CON+ group compared to the CON- group, and average crypt depth of BC+ and BP+ groups was 11 and 7% lower than that of the CON+ group, however these differences were not significant ($P > 0.05$; Table 4). Pig nested

within treatment accounted for 40% of the total variation in crypt depth. Irrespective of treatment, a decreasing gradient of crypt depth was observed from proximal to distal sites of the small intestine, which were all significantly different ($P < 0.05$) (268, 257 and 247 μm, respectively, SEM 1.5 μm). A site by treatment interaction was observed, with crypt depth in the proximal jejunum similar in CON- and CON+ groups, which were greater than the BC+ and BP+ groups ($P < 0.05$). In the mid jejunum, crypt depth was greatest in the CON- group compared to all other groups ($P < 0.05$), which were similar. In the distal ileum, crypt depth was similar between CON- and CON+ groups, but the BC+ group had higher, and the BP+ group lower, crypt depths compared to the CON- group ($P < 0.05$). Treatment groups displayed a generally decreasing gradient of crypt depth from the proximal to distal small intestine, with the exception of the BP+ group, in which the lower crypt depth was observed in the mid-jejunum, compared to both the proximal jejunum and distal ileum ($P < 0.05$), which were similar ($P > 0.05$).

Ratio of villus height to crypt depth (VH:CD) was significantly affected by pig nested within treatment, small intestine site and a site by treatment interaction ($P < 0.001$), but not treatment alone ($P > 0.05$). Pig nested within treatment accounted for 40% of the total variation in VH:CD. Irrespective of treatment, an increasing gradient of VH:CD from the proximal to the distal small intestine was observed, with VH:CD similar in the proximal and mid jejunum, which were both lower than that of the distal ileum ($P < 0.05$) (2.23, 2.27 and 2.59 μm, respectively, SEM 0.03 μm). In the proximal jejunum, VH:CD was similar among all groups ($P > 0.05$; Table 4). In the mid jejunum, VH:CD was lower in the CON+ and BP+ groups ($P < 0.05$), and tended to be lower in the BC+ group ($P = 0.05$), compared to unchallenged pigs. In the distal ileum, VH:CD was lower in the CON+ and BP+ groups compared to the CON- and BC+ groups ($P < 0.05$). In general, treatment groups displayed similar pattern of VH:CD in the small intestine, with similar values for proximal and mid jejunal sites ($P > 0.05$), which were both lower than that observed in the distal ileum ($P < 0.05$). However, the BP+ group is an exception, with no difference in VH:CD observed in any site of the small intestine ($P > 0.05$).

Epithelial cell height showed significant effects of pig nested within treatment, small intestine site and a site by treatment interaction ($P < 0.01$), but not treatment alone ($P > 0.05$). Pig nested within treatment accounted for 27% of the total variation in epithelial cell height. Independent of treatment, epithelial cell height was similar in the proximal and mid jejunum

($P > 0.05$), which were both lower than the distal ileum ($P < 0.05$) (25.1, 26.3 and 26.6 μm, respectively, SEM 0.2 μm). In the proximal jejunum, epithelial cell height of the CON- group was lower than that of the CON+ and BC+ groups ($P < 0.05$; Table 4), but similar to that of the BP+ group ($P > 0.05$). Mid jejunal epithelial cell height was higher in the BC+ and BP+ groups compared to either group consuming the CON diet ($P < 0.05$), which were similar ($P > 0.05$). Epithelial cell height in the distal ileum was similar among CON-, CON+, BC+ and BP+ groups, with the exception that epithelial cell height of the BC+ group was higher than that of the BC+ group. The generally increasing gradient of epithelial cell height from proximal to distal sites of the small intestine was present in all groups except CON+, which had similar values in all sites ($P > 0.05$).

Villus goblet cell density was significantly affected by pig nested within treatment, site, and a site by treatment interaction ($P < 0.0001$), but not treatment alone ($P > 0.05$). Although no significant effect of treatment was observed, average villus goblet cell density was numerically 15 to 17% greater in challenged groups (CON+, BC+, BP+), compared to those in the unchallenged group (CON-) (Table 4). Pig nested within treatment accounted for 37% of the total variation in villus goblet cell density. Independent of treatment, villus goblet cell density increased from the proximal jejunum to the mid jejunum and distal ileum, which were all significantly different ($P < 0.05$) (0.32, 0.44 and 0.59 cells/100μm epithelium, respectively, SEM 0.01). In the proximal jejunum, villus goblet cell density was similar among CON- CON+ and BC+ groups ($P > 0.05$), but generally higher in the BP+ group, which was significantly different from the CON- and BC+ groups. In the mid jejunum, goblet cell density was similar among the CON-, BC+ and BP+ groups, but higher in the CON+ group, which was significantly different from the CON- and BC+ groups. In the distal ileum, villus goblet cell density was similar in CON- CON+ and BP+ groups, which were lower than that of the BC+ group ($P < 0.05$). The increasing gradient of villus goblet cell density from proximal to distal areas of the small intestine was present in all treatment groups, although in the CON- group no difference in goblet cell density was observed in the mid jejunum and distal ileum ($P > 0.05$), whereas in the BP+ group, no difference in this variable was observed in the proximal and mid jejunum ($P > 0.05$).

Crypt goblet cell density was significantly affected by pig nested within treatment, site, and a site by treatment interaction ($P < 0.0001$), but not treatment alone ($P > 0.05$). Although not statistically significant, crypt goblet cell density was numerically 11 and 15% greater in BC+

and BP+ groups, respectively, compared to either groups consuming the CON diet (Table 4). Pig nested within treatment accounted for 58% of the total variation in villus goblet cell density. Irrespective of treatment, villus goblet cell density increased from the proximal jejunum to the mid jejunum and distal ileum, which were all significantly different ($P < 0.05$) (1.96, 2.14 and 2.46 cells/100μm epithelium, respectively, SEM 0.03). In the proximal jejunum, challenged groups (CON+, BC+, BP+) displayed similar crypt goblet cell density ($P > 0.05$), which was greater than that of the unchallenged group (CON-) ($P < 0.05$). In the mid jejunum, crypt goblet cell density was similar in the CON- and CON+ groups, with generally higher density observed in the BP+ and BC+ groups. In the distal ileum, similar crypt goblet cell density was observed in the CON-, CON+ and BP+ groups, but density was higher in the BC+ group compared to either group consuming the CON diet ($P < 0.05$). The increasing gradient of crypt goblet cell density from the proximal to distal small intestine was most obvious in the CON- group, where this variable was observed to increase along all sites of the small intestine ($P < 0.05$). Among challenged groups, an increase in crypt goblet cell density was only observed in the distal ileum within CON+ and BC+ groups ($P < 0.05$), whereas in the BP+ group, this occurred more proximally, with both the mid jejunal an distal ileal sites displaying higher goblet cell density compared to the proximal jejunum ($P < 0.05$).

Due to poor and variable staining, counting of $CD4^+$ and $CD8^+$ T lymphocytes was not possible on many intestinal samples. For each treatment, the number of samples that stained correctly and were used for observation of T lymphocyte density were as follows: CON-, n = 2; CON+, n = 1; BC+, n = 5; BP+, n = 5. Mean lamina propria $CD4^+$ T lymphocyte density was 9, 15, 16 and 13 cells per $0.1mm^2$ of lamina propria for treatments CON-, CON+, BC+ and BP+, respectively. Mean lamina propria $CD8^+$ T lymphocyte density was 5, 13, 13 and 10 cells per $0.1mm^2$ lamina propria for treatments CON-, CON+, BC+ and BP+, respectively. Mean ratio of $CD4^+$ to $CD8^+$ T lymphocytes was 1.84, 1.15, 1.32 and 1.23 for treatments CON-, CON+, BC+ and BP+, respectively. Due to low and variable sample size, statistical analysis of these data was not possible. However, numerical increases in both $CD4^+$ and $CD8^+$ T lymphocyte density were observed in all challenged groups (CON+, BC+, BP+), compared to the unchallenged group (CON-). Challenged groups also displayed a numerically reduced ratio of $CD4^+$ to $CD8^+$ T lymphocytes, compared to the unchallenged group.

5.4 DISCUSSION

In this experiment, specific indicators of pig performance, immunocompetence and immune challenge were measured over a 19-day post-weaning period in pigs offered diets containing either skim milk powder, spray-dried bovine colostrum or spray-dried bovine plasma. An oral challenge of *E. coli* K88 was administered 12 days after weaning, to provide an immune challenge approximating that encountered by pigs housed in a conventional farming situation. These situations, which increase the exposure of weaner piglets to immune-stimulating antigens, have been shown to increase the beneficial effects of dietary spray-dried plasma, compared to more sanitary experimental conditions (Gatnau and Zimmerman, 1991; Coffey and Cromwell, 1995; Stahly et al., 1995; Touchette et al., 1996; Bergstrom et al., 1997; Bregendahl et al., 1998; Chirra et al., 1999).

The challenge strain of *E. coli* used in this experiment has a well-established association with post-weaning diarrhoea, which is causally linked to the post-weaning syndrome observed in weaner piglets (Bertschringer, 1999; van Beers-Schreurs et al., 1992). Pathogenic *E. coli* infection is often associated with a reduction in the level of voluntary feed intake (van Beers-Schreurs et al., 1992; van Dijk et al., 2002), and in the present study a mild numerical depression of feed intake was observed in challenged groups. The absence of an effect of pathogenic *E. coli* challenge on rectal temperatures in the present experiment accords with the observations of van Beers-Schreurs et al. (1992) and van Dijk et al. (2002). No incidences of post-weaning diarrhoea were observed in unchallenged pigs, whereas faecal scoring indicated the presence of post-weaning diarrhoea in all challenged groups after administration of the *E. coli* challenge. However, the incidence of diarrhoea throughout challenged groups was variable, with some pigs showing no observable signs of scouring after administration of the oral challenge. This is not uncommon after *E. coli* challenge, where the occurrence of diarrhoea does not necessarily accompany colonisation (Hampson, 1994; Pluske et al., 1997; Nabuurs, 1998), and often stressors such as moderate chronic cold stress are required to induce clinical symptoms such as diarrhoea (Wathes et al., 1989; van Beers-Schreurs et al., 1992; Hampson, 1994; Nabuurs, 1998). The challenge model used in this experiment did not include any additional stressors over and above those that normally accompany weaning, and did not use any animal pre-treatments to increase the sensitivity of challenged pigs to pathogenic *E. coli* infection which have been employed in other models (van Dijk et al., 2002). This may offer an explanation of the somewhat variable clinical response of pigs in

the present study, and the absence of any significant alteration in haematological variables after administration of the *E. coli* challenge. Also, the fact that adhesion of *E. coli* K88 to the epithelial mucosa of the small intestine is mediated by intestinal receptors specific for K88 fimbrae means that some pigs are genetically resistant to infection by this strain of *E. coli* (Sellwood et al., 1975; Sarmiento et al., 1988; Jin and Zhao, 2000). There is also evidence that $K88^+$ *E. coli* is more likely to cause diarrhoea around 4 days after weaning, whereas $K88^-$ strains tend to cause diarrhoea between 7 and 10 days after weaning (Fahy et al., 1987), which may be due to alterations in the number and/or status of the associated epithelial receptors after weaning (Hampson, 1994). The influence of these receptors may account for the mild clinical response of pigs in the present experiment, and likely contributes to the fact that simple oral dosing with haemolytic *E. coli* can often fail to induce disease (Hampson, 1994; Nabuurs, 1998).

In this study, the consumption of a diet containing 7.5% of either spray-dried bovine plasma or colostrum did not significantly affect faecal shedding of *E. coli*. This is in contrast to recent work showing decreased faecal excretion of haemolytic *E. coli* in pigs consuming diets containing spray-dried plasma (Deprez et al., 1990, 1996; Nollett et al., 1999), regardless of the presence of specific antibodies against this strain in the plasma. However, several studies have shown no effect of spray-dried plasma on the qualitative presence of haemolytic *E. coli* throughout the intestine (Chirra et al., 1999), nor on faecal shedding of haemolytic *E. coli* after weaning (Cain and Zimmerman, 1997; Bosi et al., 2001; van Dijk et al., 2002). However, Bosi et al. (2001) also observed reduced titres of IgA antibody specific to the challenge strain (K88) in both plasma and saliva of plasma-fed pigs, and van Dijk et al. (2002) observed improvements in both condition score and voluntary feed intake, and a tendency for improved faecal score, as a result of inclusion of 8% spray-dried plasma in the weaning diet of pigs challenged with haemolytic *E. coli*. Similar results were found in the present study, where the extent of post-weaning diarrhoea in challenged pigs offered the control diet (CON+ group) was significantly higher than unchallenged pigs, whereas diarrhoea scores of challenged pigs offered either the bovine plasma (BP+) or bovine colostrum (BC+) diet was not significantly different from that of unchallenged pigs. These findings suggest that beneficial effects of dietary spray-dried plasma may be manifested despite the absence of any influence of plasma on intestinal proliferation of pathogenic bacteria.

In the present study, the effects of *E. coli* challenge on histology and morphology of the small intestine were investigated. Comparing groups consuming the control diet, challenged pigs showed no alteration in villus height, but an increase in crypt depth in the mid jejunum and reductions in the VH:CD ratio were observed in the mid jejunum and distal ileum, suggesting increased epithelial cell mitosis in these areas. Also, villus and crypt goblet cell densities were increased in the proximal and mid jejunum, respectively, indicating the induction of goblet cell differentiation in these areas of the intestine as a result of the *E. coli* challenge. Some evidence of an increase in $CD4^+$ and $CD8^+$ T lymphocytes proliferation and a reduction in their ratio ($CD4^+$:$CD8^+$) was observed in the mid jejunum of challenged pigs, and it is unfortunate that more observations were not possible, which would have enabled an appropriate quantification and statistical comparison of the densities of these T lymphocyte populations. Some evidence of alterations in intestinal morphology due to *E. coli* challenge were therefore variously observed throughout the small intestine. This accords with observations of Nabuurs et al. (1993), who reported that that pigs from herds with a history of post-weaning diarrhoea had deeper crypts than pigs from a specific-pathogen-free farm. The fact that villus height of pigs consuming the control diet was not affected is supported by the notion that enterotoxigenic *E. coli* infection is not generally thought to have a significant effect on small intestine morphology (Hampson, 1994; Isaacson, 1998), as demonstrated by Touchette et al. (1999).

However, in the present study adverse effects of the *E. coli* challenge were observed in pigs consuming either the bovine colostrum or bovine plasma diets, who displayed villus atrophy throughout the small intestine, and increases in crypt and villus goblet cell density in various sites of the small intestine, suggestive of enterotoxin-induced intestinal damage, and an associated inflammatory response. This is supported by the numerical increase in lamina propria $CD4^+$ and $CD8^+$ T lymphocytes observed in mid jejunal sections, which is associated with mucosal inflammation and T lymphocyte-mediated intestinal restructuring (King et al., 2003). These observations accord with those of Rose et al. (1987) and Whipp et al. (1986, 1987) who demonstrated that villus atrophy can be induced by the heat stable enterotoxin 'a' in pig small intestine. Similarly, Vogelweid and Elmore (1983) observed epithelial ulceration and increases in goblet cell expression in jejunal loops of pig small intestine infused with *E. coli* endotoxin, and Nabuurs et al. (1994) observed a decrease in the absorptive capacity of the small intestine in enterotoxigenic *E. coli* infected pigs. The heat-labile enterotoxin 'b' has also been shown to be a powerful mucosal immunogen, causing activation of intestinal $CD4^+$ T

cells, and induction of T cell proinflammatory cytokine mRNA expression, including interferon (IFN)-γ, and interleukins (IL) –2, 4, 5 and 6 (Nakagawa et al., 1996).

The fact the *E. coli* challenge only caused adverse alterations in the small intestine architecture and histology of pigs consuming the bovine colostrum or bovine plasma diets is supported by evidence demonstrating that pigs consuming dietary spray-dried plasma after weaning have decreased basal immune system activation, which causes them to be hyper-responsive to intraperitoneal lipopolysaccharide (LPS) challenge (Carroll et al., 2002; Touchette et al., 2002). Consumption of spray-dried plasma after weaning has been shown to reduce mRNA expression of the proinflammatory cytokines tumour necrosis factor (TNF)-α and IL-1β in the adrenal gland, spleen, hypothalamus, pituitary gland and liver, and reduce mRNA expression of IL-6 in the pituitary gland and spleen (Touchette et al., 2002). Furthermore, spray-dried plasma-fed pigs display reduced activation of the hypothalamic–pituitary-adrenal (HPA) axis, with diminished mRNA expression of hypothalamic corticotropin-releasing hormone (CRH), pituitary gland CRH receptor and adrenal gland adrenocorticotrophin-releasing hormone receptor (Carroll et al., 2002). In response to LPS-challenge, Touchette et al. (2002) observed villus atrophy in pigs consuming a diet containing 7% spray-dried plasma, whereas the intestinal morphology of pigs offered a diet devoid of plasma was unaffected. LPS challenge was also accompanied by a greater increase in serum TNF-α and IFN-γ in plasma-fed pigs (Touchette et al., 2002), and increased activation of the hypothalamic pituitary axis (Carroll et al., 2002). Carroll et al. (2002) and Touchette et al. (2002) both hypothesised that this hyper-responsiveness is due to a protective effect of dietary spray-dried plasma in the intestine, which reduces the level of antigenic insult received by pigs during the post-weaning period, inducing a state of comparative immunological 'naïveté'. This has been observed in mice, where animals 'primed' with Complete Freund's Adjuvant respond to LPS challenge with a greater release of TNF-α and IL-6, compared to 'naïve' animals, which did not receive adjuvant (DeForge et al., 1994). Similarly, Erroi et al. (1993) and Handid et al. (1995) demonstrated that repeated exposure to LPS induced systemic hypo-responsiveness to LPS challenge, compared to mice which were LPS 'naïve' prior to the challenge.

However, compared to the present study, the level of challenge provided by LPS was much greater in the experiments of Touchette (2002) and Carroll (2002), and the mode of entry of the challenge was not via the intestine. One experiment that followed a similar method to

the present study was that of Touchette et al. (1999), in which weaner pigs were offered a diet containing either 0 or 7% spray-dried plasma for 7 days before administering an oral challenge containing a strain of *E. coli* expressing $F17^+$ fimbrae. The plasma-fed pigs displayed HPA axis hyper-responsiveness to the challenge, as measured by serum ACTH, however no effect of the *E. coli* challenge on gut morphology was observed, which conflicts with the results of the present study (Touchette et al., 1999). This may be due to the fact that Touchette et al. (1999) observed intestinal morphology only 10 hours after administration of the oral challenge, whereas, in the present experiment, morphology was observed 7 days after administration of the challenge. Also, the *E. coli* challenge employed by Touchette et al. (1999) may have been insufficient to induce expression of the proinflammatory cytokines observed in the experiment of Touchette et al. (2002). These cytokines are integral to the coordination of mucosal destruction associated with gastrointestinal immune challenge (King et al., 2003), and were suggested to be involved in the induction of villus atrophy after LPS challenge in the experiment of Touchette et al. (2002). Unfortunately, expression of these cytokines was also not measured in the present study. Also, in the present study, the feeding period prior to administration of the challenge was almost double that used in the experiment of Touchette et al. (1999), which may have increased the immunological naïveté of the plasma and colostrum-fed pigs.

In this experiment, similar responses to the *E. coli* challenge were observed in both plasma and colostrum fed pigs, which suggests that spray-dried bovine colostrum may be a potential alternative to spray-dried bovine plasma, and that they may share a common mode of action. This may be due to the fact that both contain immunoglobulins, and other antimicrobial factors, such as glycoproteins (van Dijk et al., 2001), which may reduce the antigenic stimulation of the intestine through immune exclusion. This notion supports the hypothesis of immunological naïveté induced by plasma and colostrum ingestion. However, the fact that in the present study the *E. coli* challenge induced adverse alterations in intestinal morphology suggests that the active compounds present in colostrum and plasma may not provide sufficient passive immune protection during significant immune challenge. Also, as concluded by Touchette et al. (2002) and Carroll et al. (2002), these results suggest that, while spray-dried plasma may provide immune protection after weaning, the decrease in immune system activation may increase the negative consequences of major subsequent immunological challenges.

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Chapter 6

THE INFLUENCE OF WEANER-STARTER DIETS CONTAINING SPRAY-DRIED BOVINE COLOSTRUM AND PLASMA ON THE GROWTH PERFORMANCE AND FEED INTAKE OF PIGS FROM WEANING TO SLAUGHTER.

6.1 INTRODUCTION

Spray-dried animal plasma is used extensively in the American pig industry, where it is included in "segregated-early-weaning" and "Phase 1" weaning diets as a replacement for other highly digestible, usually milk-based, protein sources. Spray-dried plasma is especially valuable as a dietary ingredient in early-weaning production systems, where the provision of highly palatable and digestible diets, formulated to contain high concentrations of protein and energy, is necessary to maintain an adequate level of feed intake and growth rate after weaning. To this end, spray-dried plasma is extremely palatable (Ermer et al., 1994), high in protein (~80%), and highly digestible (Chae et al., 1999).

The popularity of spray-dried animal plasma is largely due to the fact that its inclusion in starter diets, at levels generally ranging from 3 to 8%, typically produces an increase in feed intake and growth rate (Gatnau and Zimmerman, 1990; Sohn et al., 1991; Hansen et al., 1993; Kats et al., 1994; de Rodas et al., 1995; Chae et al., 1999), and sometimes an improvement in feed conversion ratio (Jiang et al., 2000), all of which help to overcome the characteristic post-weaning 'growth check'.

The improvement in weaner pig feed intake and growth rate can be reproduced through dietary inclusion of the high molecular weight fraction of plasma alone, whereas the low and medium molecular weight fractions generally produce no effect (Gatnau et al., 1995; Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995). The high molecular weight fraction is composed principally of immunoglobulins, suggesting that these may be responsible for the performance benefits conferred by feeding plasma products. Dietary inclusion of spray-dried bovine colostrum powder, which contains comparable concentrations of immunoglobulins, produces performance benefits similar to plasma products when included at levels of 5 and 10% in weaner pig starter diets, supporting this hypothesis (Pluske et al., 1999a).

This study is therefore designed to directly compare spray-dried colostrum with spray-dried plasma as ingredients for use in weaner pig diets. The hypothesis tested in this experiment is that inclusion of either spray-dried bovine plasma or bovine colostrum as replacements for skim milk powder in a starter diet for weaner pigs will improve performance during the feeding period, which may in turn improve subsequent growth through to market weight.

6.2 MATERIALS AND METHODS

6.2.1 Pigs and conduct of the experiment

Experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Recommendations and Minimum Standards for the Care and Use of Animals for Scientific Purposes (New Zealand Animal Welfare Advisory Committee, 1995).

The experiment used 292 pigs (PIC 231 x Camborough 22), weaned at 28 ± 0.1 days of age, 7.4 ± 0.09 kg live weight, and was conducted on a 200 sow commercial unit. Routine management of pigs prior to weaning included a subcutaneous injection of 2ml iron dextran (providing 200 mg Fe), clipping of incisors and tail, and creep feeding from 14 to 28 days of age. On the day of weaning, piglets from 10 litters were blocked according to litter of birth and live weight and randomly allocated to one of three size classes (small, medium and large). Each size class was divided into two pens of pigs, providing six pens of pigs in total per weaning. Three experimental diets were allocated to the pens within each size class, allowing the use of two diets per class. Due to availability of pigs, three replicates of this design were used, allowing a total of 6 pens of pigs per experimental diet, two within each size class (Figures 1 and 2).

Piglets were offered one of three 'starter' diets *ad libitum* for 7 days after weaning (28-35 days of age): (1) Wheat-skim milk based starter (Control, CON); (2) Control diet + 6% bovine plasma (BP); (3) Control diet + 6% bovine colostrum (BC). After this time, all pigs were fed identical diets: Control diet (as fed from 28-35 days of age) from 35-42 days of age; Weaner diet from 42-70 days of age; Grower diet from 70-98 days of age; Finisher diet from 98 days of age to market weight (~140 days of age).

Pigs were individually weighed at weaning (28 days of age), one week post-weaning (35 days of age), two weeks post-weaning (42 days of age), at transfer from 'weaner' to 'grower' accommodation (70 days of age), at transfer from 'grower' to 'finisher' accommodation (98 days of age), and at sale (~140 days of age). Age at sale was recorded to calculate the number of days taken for pigs to reach a common slaughter weight of 85 kg (days to slaughter). Weekly feed intake was recorded on a pen basis for two weeks post-weaning, allowing weekly feed conversion ratio to be calculated on a pen basis for this period.

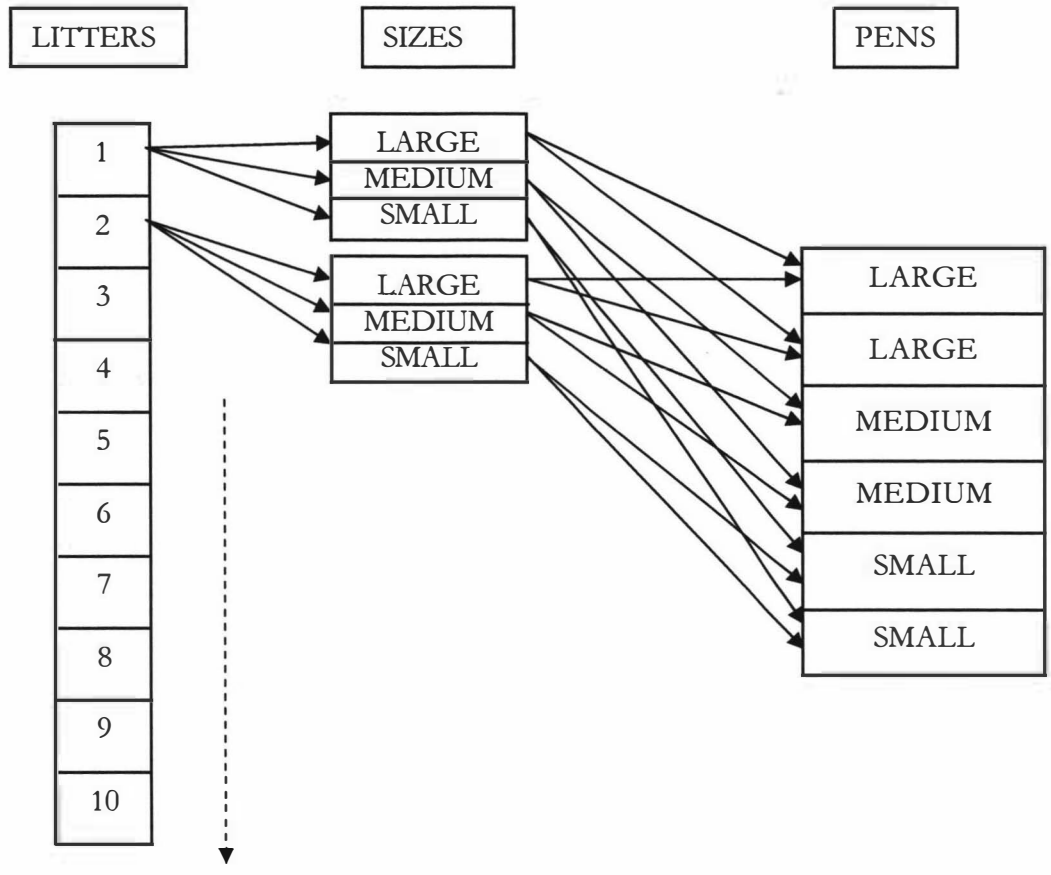

Figure 1. Experimental allocation of animals within a replicate, including division of each litter into 3 size classes, and their allocation to 6 pens according to size.

Replicate 1	Replicate 2	Replicate 3
BP LARGE	BC LARGE	CON LARGE
CON LARGE	BP LARGE	BC LARGE
BP MEDIUM	BC MEDIUM	CON MEDIUM
BC MEDIUM	CON MEDIUM	BP MEDIUM
CON SMALL	BP SMALL	BC SMALL
BC SMALL	CON SMALL	BP SMALL

Figure 2. Experimental allocation of treatment groups in 18 pens within 3 replicates, producing a semi-unbalanced design with each dietary treatment represented 6 times, twice within each size class.

6.2.2 Experimental diets

All experimental starter diets (CON, BC and BP) were based on wheat and skim milk powder (Table 1) and formulated to meet or exceed National Research Council (1998) recommendations for major nutrients, with lysine as the first-limiting amino acid. Bovine colostrum and bovine plasma were included in diets BC and BP, respectively, at a level of 6%, replacing skim milk powder on an isolysine basis. Diets were balanced to a similar lactose content (12%) using crystalline lactose, and fed in meal form.

The composition of the test protein products spray-dried bovine colostrum (Immulac; Specialty Ingredients Group, Fonterra, Hautapu, New Zealand) and spray-dried bovine plasma (AP820; Proliant Corp., Ames, Iowa, United States of America) have been presented previously (Chapter 4, this volume).

The weaner diet (WD) was based on wheat and soybean meal, the grower diet (GD) was based on barley, wheat and soybean meal, and the finisher diet was based on barley, wheat and broll. All diets were formulated to meet or exceed National Research Council (1998) recommendations for major nutrients.

Table 1. Percentage composition and calculated analysis of the experimental diets.

	Experimental diets[1]					
Ingredient, %	CON	BC	BP	WD	GD	FD
Wheat	53.42	51.88	52.34	64.85	55.00	30.00
Barley	-	-	-	-	23.80	48.41
Dried skim milk	25.00	16.10	12.65	8.00	-	-
Bovine plasma[2]	-	-	6.00	-	-	-
Bovine colostrum[3]	-	6.00	-	-	-	-
Lactose	-	4.37	6.15	-	-	-
Poultry meal	2.50	2.50	2.50	10.00	8.00	4.00
Fishmeal	6.24	6.24	6.25	5.00	-	-
Full-fat soybean meal	10.00	9.98	10.00	8.00	-	-
Meat and bone meal	-	-	-	1.30	4.00	4.00
Soybean meal	-	-	-	-	8.00	2
Broll	-	-	-	-	-	10.00
Ring dried blood meal	-	-	-	2.00	-	-
L-Lysine	0.42	0.42	0.43	0.20	0.39	0.37
D, L-Methionine	0.20	0.18	0.28	-	-	-
L-Threonine	0.22	0.16	0.16	0.05	0.07	0.07
Limestone	0.25	0.19	0.06	-	0.10	0.25
Dicalcium Phosphate	-	0.24	0.63	-	-	-
Salt	0.30	0.39	-	0.20	0.35	0.35
Soya bean oil	0.10	-	1.2	-	-	-
Tetramutin[4]	0.15	0.15	0.15	-	-	-
Zinc oxide	0.30	0.30	0.30	-	-	0.30
Ultrawean[5]	0.40	0.40	0.40	-	-	-
Tylan[6]	-	-	-	0.10	0.04	-
Vit. & Min. Premix	0.50	0.50	0.50	0.30	0.25	0.25
Calculated Analysis						
DE[7], MJ/kg	15.0	15.0	15.0	14.6	13.4	12.8
Crude protein, %	24.1	23.6	25.4	25.3	20.0	15.8
Crude fat, %	3.9	3.6	4.8	4.5	2.7	2.1
Total lysine, %	1.77	1.77	1.77	1.5	1.20	1.00
Sodium, %	0.15	0.15	0.29	0.22	0.21	0.24
Lactose, %	12.5	12.5	12.5	4	0	0

[1] CON, Control; BP, bovine plasma; BC, bovine colostrum; WD, weaner diet; GD, grower diet; FD, finisher diet.
[2] AP820 (Proliant Corp., Ames, Iowa, USA).
[3] Immulac (Specialty Ingredients Division, Fonterra, Hautapu, New Zealand).
[4] Tetramutin (Novartis Animal Health Ltd., Auckland, New Zealand).
[5] Ultrawean (Ridley AgriProducts Pty. Ltd., Australia).
[6] Tylan (Elanco Animal Health Ltd., Wiri, New Zealand).
[7] DE, digestible energy.

6.2.3 Statistical analysis

The data were subjected to analysis of variance (ANOVA) by the General Linear Models procedures of SAS (SAS Institute, 2000) using pen as the experimental unit for feed intake and feed conversion ratio, and pig as the experimental unit for growth performance.

The statistical model used in the analysis of feed intake and feed conversion ratio was:

$$y_{ij} = \mu + \alpha_i + \beta(\chi_{ij}) + \beta(\chi_{ij})^* \alpha_i + \varepsilon_{ij}$$

Where:

y_{ij} = observation from the j^{th} pen within the i^{th} dietary treatment.

μ = the population mean.

α_i = the fixed effect of the i^{th} dietary treatment.

$\beta(\chi_{ij})$ = average weaning weight covariate χ, specific to the j^{th} pen within the i^{th} dietary treatment multiplied by the slope β.

$\beta(\chi_{ij})^*\alpha_i$ = interaction of the average weaning weight covariate χ, with the i^{th} dietary treatment. This is included to test the homogeneity of the slope β between dietary treatments.

ε_{ij}= residual error of the j^{th} pen within the i^{th} dietary treatment.

The statistical model used in the analysis of live weight and average daily gain was:

$$y_{ijkl} = \mu + \beta_j + \alpha_i + \gamma_k + \eta(\chi_{ijkl}) + \eta(\chi_{ijkl})^* \alpha_i + \varepsilon_{ijkl}$$

Where:

y_{ijkl} = observation from the l^{th} pig from the k^{th} sex and the j^{th} litter within the i^{th} dietary treatment.

μ = the population mean.

β_j = the random effect of the j^{th} litter of birth.

α_i = the fixed effect of the i^{th} dietary treatment.

γ_k = the fixed effect of the k^{th} sex.

$\eta(\chi_{ijkl})$ = average weaning weight covariate χ, multiplied by η.

$\eta(\chi_{ijkl})*\alpha_i$ = interaction of the average weaning weight covariate χ, with the i^{th} treatment. This is included to test the homogeneity of the slope η between dietary treatments.

ε_{ijkl}= residual error of the l^{th} pig from the k^{th} sex and the j^{th} litter within the i^{th} dietary treatment.

In both models the effect of replicate was tested, found to be non-significant, and was therefore removed from the linear model. Where a significant treatment effect was observed, Fisher's least significant difference test (LSD) was performed to determine significant differences between least-square means of dietary treatment groups. Pearson correlation and regression analysis was performed to evaluate relationships between variables, where appropriate. Level of significance was pre-set at $P < 0.05$, and trends were identified at $P < 0.10$. Data are presented as least-square means with the associated pooled standard error of the mean (SEM).

6.3 RESULTS

6.3.1 Feed intake, growth rate, feed conversion ratio and live weight from 28-35 days of age

Table 2. Performance of piglets fed diets containing different protein sources during first week (28-35 days of age) after weaning, followed by a common diet for the second week.

	Dietary treatment[1]				P-values[2]		
	CON	BP	BC	SEM	WW	DIET	DIETxWW
28-35 days							
FI[3], kg/d	0.216[a]	0.248[ab]	0.265[b]	0.01	0.11	0.04	ns[4]
FCR	1.12	1.16	1.12	0.05	0.42	0.04	0.03
35-42 days							
FI, kg/d	0.487	0.486	0.508	0.03	0.01	0.83	ns
FCR	1.22	1.22	1.17	0.06	0.08	0.84	ns

[1] CON, control; BP, bovine plasma; BC, bovine colostrum.
[2] WW, weaning weight; DIET, dietary treatment offered during the week after weaning; DIET*WW, dietary treatment by weaning weight interaction; SEM, pooled standard error of the mean.
3 FI, average daily feed intake; FCR, feed conversion ratio.
[4] ns, variable was tested, found to be non-significant, and was therefore removed from the statistical model.
[a,b] Least-square means with different superscripts are significantly different ($P < 0.05$).

A significant effect of treatment on feed intake between 28 and 35 days of age was observed ($P < 0.05$; Table 2), with piglets offered the BC diet consuming, on average, 22.7% more feed than those offered the control diet ($P < 0.05$). Piglets offered the BP diet showed a trend for

improved intake, consuming 14.8% more feed, on average, than those offered the control diet ($P < 0.10$). No significant effect of weaning weight or diet by weaning weight interaction was observed for feed intake ($P > 0.10$).

Average daily gain in the first week post-weaning (Table 3) was significantly affected by litter of birth, which explained 21.3% of the variation in weight gain ($P < 0.05$), and sex, with boars growing, on average, 19.9% faster than gilts ($P < 0.05$). A trend for an effect of diet was observed ($P < 0.10$), with piglets offered either the BP or BC diet growing 4.2 and 17% faster , respectively, than piglets offered the control diet. A trend for an effect of diet by weaning weight interaction was also observed ($P < 0.10$). Illustrating this interaction (Figure 3), the regression coefficients for pigs offered the CON (0.012 ± 0.009, $P > 0.10$) and BC (-0.005 ± 0.008, $P > 0.10$) diets show no significant relationship between weaning weight and average daily gain, whereas pigs offered the BP diet showed a significant negative relationship between weaning weight and average daily gain (-0.023 ± 0.002, $P < 0.05$). Weaning weight *per se* had no significant effect on average daily gain during this period ($P > 0.10$).

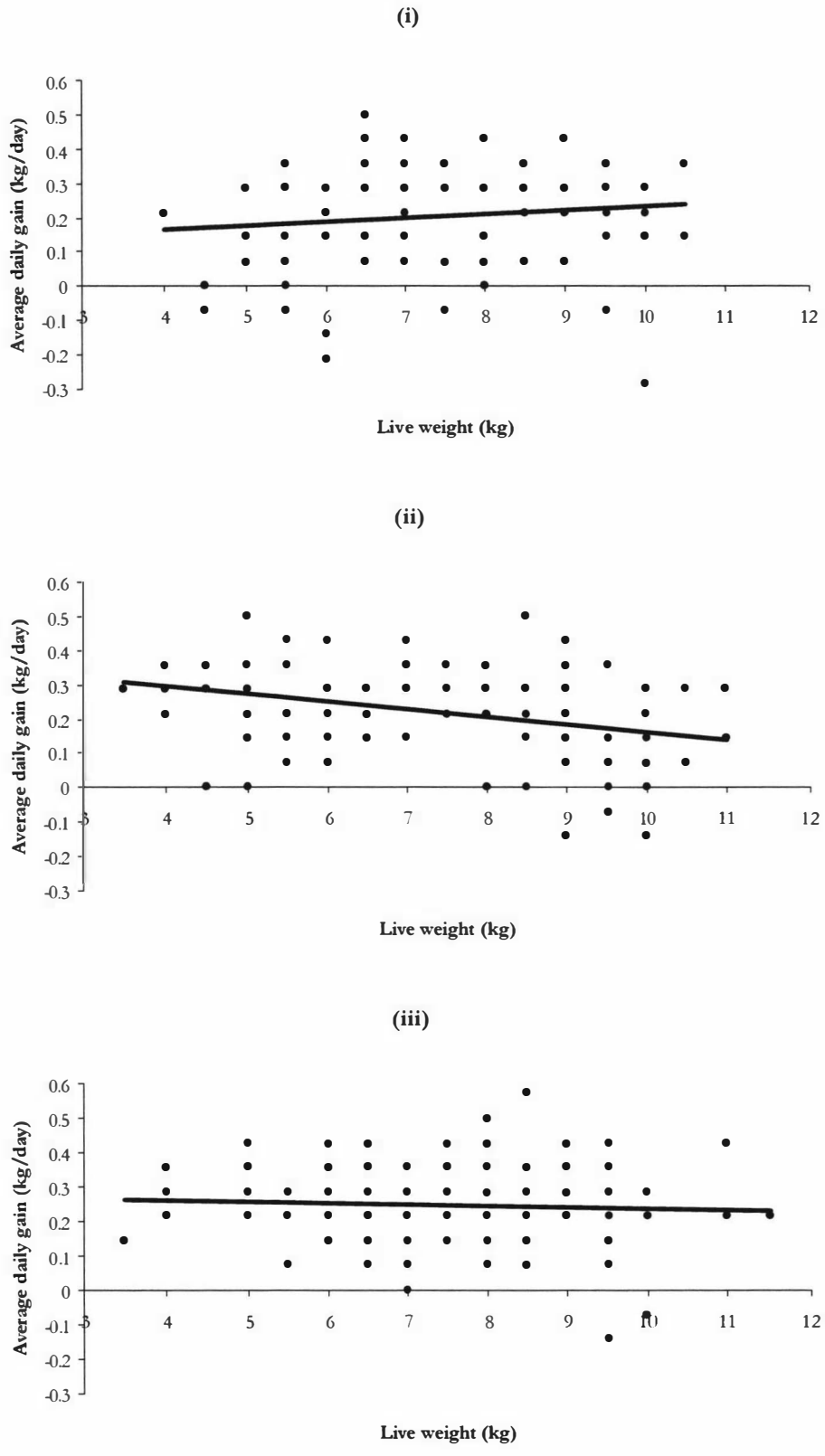

Figure 3. The relationship between weaning weight and average daily gain in the week after weaning for pigs offered diets containing skim milk powder (i; Control), 6% bovine plasma (ii), or 6% bovine colostrum (iii) for the first week after weaning.

A significant effect of treatment on feed conversion ratio (FCR) was found ($P < 0.05$; Table 2), although no significant differences in FCR were found between pigs offered the different dietary treatments ($P > 0.10$). FCR was unaffected by weaning weight *per se* ($P > 0.10$), although a significant effect of weaning weight by diet interaction was observed ($P < 0.05$, Figure 4). Regression coefficients characterising the relationship between weaning weight and FCR in piglets offered the control diet demonstrated a negative trend (-0.075 ± 0.038, $P < 0.10$), whereas piglets offered the BC diet showed no significant relationship (0.052 ± 0.039, $P > 0.10$), and piglets offered the BP diet displayed a relationship with a positive trend (0.077 ± 0.036, $P < 0.10$).

Live weight at 28 days of age (weaning weight) was significantly affected by litter, which explained 18.9% of the total variation in weaning weight ($P < 0.05$, Table 4). Weaning weight was not different between sexes, or among treatment groups ($P > 0.10$). Live weight at 35 days of age was significantly affected by weaning weight, litter of birth and sex ($P < 0.05$). Live weight at 35 days of age showed a strong positive correlation with weaning weight ($r = 0.86$, $P < 0.05$), litter of birth explained 6.1% of the total variation in live weight ($P < 0.05$), and boars were, on average, 3.4% heavier than gilts ($P < 0.05$). A trend for an effect of diet by weaning weight interaction was observed ($P < 0.10$), characterised by regression coefficients demonstrating a strong positive relationship between these variables in piglets offered the control diet (1.087 ± 0.062, $P < 0.05$), which was weaker in piglets offered either the BC (0.097 ± 0.057, $P < 0.05$) or BP diet (0.084 ± 0.050, $P < 0.05$).

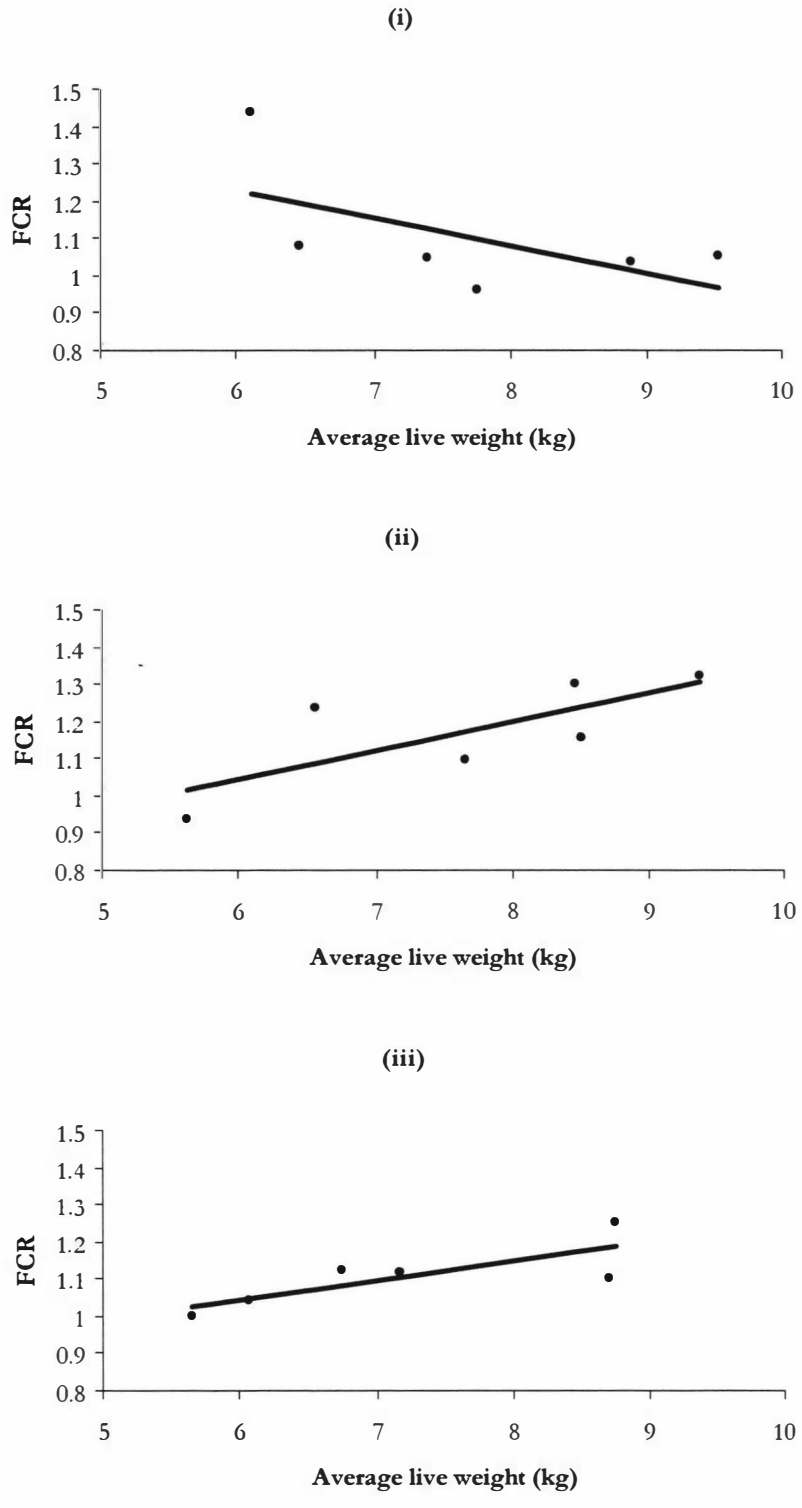

Figure 4. The relationship between weaning weight and feed conversion ratio during the week after weaning for pigs offered diets containing skim milk powder (i; control), 6% bovine plasma (ii), or 6% bovine colostrum (iii) for the first week after weaning.

Table 3. Growth performance (kg/day) of pigs, fed diets containing different protein sources during week 1 after weaning, followed by common diets, over three production phases.

	Dietary treatment[1]			Sex			P-value[2]				
Age[3] (days)	CON	BP	BC	Boar	Gilt	SEM	WW	LITTER	SEX	DIET	DIETxWW
28-35	0.212	0.221	0.248	0.247[a]	0.206[b]	0.013	0.12	0.0001	0.008	0.08	0.07
35-42	0.413	0.411	0.428	0.424	0.410	0.013	0.0007	0.0001	0.36	0.51	0.43
42-70	0.669[a]	0.644[b]	0.651[ab]	0.664[a]	0.645[b]	0.007	0.0001	0.0003	0.03	0.049	0.02
70-98	0.856	0.871	0.880	0.885[a]	0.853[b]	0.009	0.0001	0.0002	0.005	0.12	0.21
98-140	0.774	0.788	0.788	0.800[a]	0.767[b]	0.014	0.12	0.01	0.04	0.18	0.14
28-140	0.707	0.710	0.716	0.724[a]	0.697[b]	0.006	0.0001	0.0001	0.0004	0.08	0.06
Days to 85kg	139.5	139.0	138.4	137[a]	140[b]	1.05	0.0001	0.003	0.03	0.18	0.15

[1] CON, control; BP, bovine plasma; BC, bovine colostrum.

[2] WW, weaning weight; LITTER, litter of birth; SEX, pig gender; DIET, diet offered during the week after weaning; DIET*WW, treatment by weaning weight interaction; SEM, pooled standard error of the mean.

[3] Weaner, 28-70 days of age; Grower, 70-98 days of age; Finisher, 98 days of age - slaughter (approximately 140 days of age).

[a,b] Least-square means with different superscripts are significantly different ($P < 0.05$).

Table 4. Live weight (kg) of piglets fed diets containing different protein sources during week 1 after weaning, followed by a common diet for week 2.

	Dietary treatment[1]			Sex			P-value[2]				
Age[3] (days)	CON	BP	BC	Boar	Gilt	SEM	WW	LITTER	SEX	DIET	DIETxWW
28	7.3	7.4	7.4	7.4	7.3	0.2	-	0.0001	0.84	0.97	-
35	8.9	9.0	9.2	9.2[a]	8.9[b]	0.09	0.0001	0.0001	0.004	0.13	0.07
42	11.8	11.9	12.2	12.1[a]	11.7[b]	0.04	0.0001	0.0001	0.008	0.15	0.05
70	31.9	31.2	31.7	32.1[a]	31.1[b]	0.31	0.0001	0.0001	0.004	0.25	0.01
98	54.1	53.8	54.6	55.1[a]	53.3[b]	0.44	0.0001	0.0001	0.0003	0.51	0.02
~140	85.1	86.4	85.6	86.7[a]	84.7[b]	0.67	0.0001	0.0008	0.01	0.36	0.45

[1] CON, control; BP, bovine plasma; BC, bovine colostrum.

[2] WW, weaning weight; LITTER, litter of birth; SEX, pig gender; DIET, diet offered during the week after weaning; DIET*WW, treatment by weaning weight interaction; SEM, pooled standard error of the mean.

[3] 28 days of age = weaning; 70 days of age = end of weaner phase; 98 days of age = and of grower phase; ~140 days of age = approximate age at slaughter.

[a,b] Least-square means with different superscripts are significantly different ($P < 0.05$).

6.3.2 Feed intake, growth rate, feed conversion ratio and live weight from 35-42 days of age

From 35-42 days of age pigs from all treatments were offered the control diet. Feed intake during this period was positively correlated with weaning weight ($r = 0.59$, $P < 0.05$). Diet had no effect on feed intake during this time ($P > 0.10$; Table 2).

Average daily gain showed a significant effect of weaning weight ($P < 0.05$; Table 3) with a positive correlation between the two variables ($r = 0.30$, $P < 0.05$). Litter of birth also had a significant effect, accounting for 24.9% of the total variation in average daily gain during this period ($P < 0.05$). No effects of sex, diet, or diet by weaning weight interaction were evident during this period ($P > 0.10$).

FCR was not affected by diet (Table 2), however a trend for an effect of weaning weight was found, with FCR increasing proportional to weaning weight, as described by the regression coefficient (0.05 ± 0.03, $P < 0.10$).

Live weight at 42 days of age was significantly affected by weaning weight, litter of birth and sex ($P < 0.05$; Table 4). Live weight was positively correlated with weaning weight ($r = 0.78$, $P < 0.05$), litter of birth accounted for 12.7% of the variation in live weight ($P < 0.05$), and boars were 3.4% heavier than gilts ($P < 0.05$). A trend for the diet by weaning weight interaction to affect live weight at 42 days of age was observed ($P = 0.05$). This interaction is likely a flow-on effect from the initial diet by weaning weight interaction for weight gain and FCR from 28-35 days of age. The live weight of piglets at 42 days of age was positively correlated with weaning weight in all treatments, although the regression coefficient was lower in piglets that received the BP diet (0.96 ± 0.08, $P < 0.05$) compared to those that received the BC (1.13 ± 0.09, $P < 0.05$) or control diet (1.39 ± 0.10, $P < 0.05$) in the week after weaning.

6.3.3 Average daily gain and live weight from 42-70 days of age

Average daily gain from 42-70 days of age showed significant effects of weaning weight, litter of birth and sex ($P < 0.05$; Table 3). Weaning weight was positively correlated with average daily gain ($r = 0.44$, $P < 0.05$), litter of birth explained 15.5% of the variation in average daily gain ($P < 0.05$), and boars grew on average 2.9% faster than gilts ($P < 0.05$). Diet offered in

the week after weaning significantly affected growth rate from 42-70 days of age ($P < 0.05$), with piglets offered the BP diet growing 3.7% slower than piglets offered the control diet ($P < 0.05$). A significant diet by weaning weight interaction was also observed for average daily gain, where the regression coefficient for pigs offered the BP diet (0.012 ± 0.004, $P < 0.05$) was lower than that of pigs offered either the BC (0.027 ± 0.005, $P < 0.05$) or control diet (0.31 ± 0.005, $P < 0.05$).

Live weight at 70 days of age showed significant effects of weaning weight, litter, sex and a diet by weaning weight interaction ($P < 0.05$; Table 4). Live weight at 70 days of age was positively correlated with weaning weight ($r = 0.67$, $P < 0.05$), litter of birth accounted for 14.6% of the variation in live weight ($P < 0.05$), and boars were on average 3.2% heavier than gilts ($P < 0.05$). A significant diet by weaning weight interaction was observed ($P < 0.05$), where the regression coefficient for pigs offered the BP diet (1.32 ± 0.18, $P < 0.05$) was lower than that of pigs offered either the BC (1.94 ± 0.21, $P < 0.05$) or control diet (2.33 ± 0.22, $P < 0.05$).

6.3.4 Average daily gain and live weight from 70-98 days of age

Average daily gain from 70-98 days of age was significantly affected by weaning weight, litter of birth, and sex ($P < 0.05$; Table 3). Weaning weight showed a positive correlation with average daily gain ($r = 0.39$, $P < 0.05$), and litter of birth explained 17.0% of the total variation in average daily gain ($P < 0.05$). No effect of diet nor diet by weaning weight interaction was observed ($P > 0.10$).

Live weight at 98 days of age was significantly affected by weaning weight, litter of birth, sex, and a diet by weaning weight interaction ($P < 0.05$; Table 4). Weaning weight was positively correlated with live weight ($r = 0.64$, $P < 0.05$), litter of birth explained 15.4% of the total variation in live weight ($P < 0.05$), and boars were, on average, 3.4% heavier than gilts ($P < 0.05$) at this time. A significant diet by weaning weight interaction was observed for live weight at 98 days of age, where the regression coefficient for pigs offered the BP diet (1.76 ± 0.26, $P < 0.05$) was lower than that of pigs offered either the BC (2.53 ± 0.29, $P < 0.05$) or control diet (3.15 ± 0.32, $P < 0.05$).

6.3.5 Average daily gain and live weight from 98-140 days of age

Average daily gain from 98-140 days of age was significantly affected by litter of birth, which accounted for 16.1% of the variation in average daily gain ($P < 0.05$; Table 3), and sex, with boars growing 4.3% faster than gilts ($P < 0.05$). Weaning weight, diet, and diet by weaning weight interaction had no effect on growth rate ($P > 0.10$) during this period.

Live weight at 140 days showed significant effects of weaning weight, litter and sex ($P < 0.05$; Table 4). Weaning weight was positively correlated with live weight at 140 days of age ($r = 0.35$, $P < 0.05$), litter of birth explained 16.9% of the total variation in live weight at this time ($P < 0.05$), and boars were, on average, 2.4% heavier than gilts ($P < 0.05$). No effect of diet nor diet by weaning weight interaction was observed ($P > 0.10$).

6.3.6 Overall average daily gain and number of days taken to reach slaughter weight

Average daily gain (from 28-140 days of age) was significantly affected by weaning weight, litter of birth, and sex ($P < 0.05$; Table 3). Average daily gain was positively correlated with weaning weight ($r = 0.31$, $P < 0.05$), litter of birth accounted for 18.9% of the total variation in average daily gain during this period ($P < 0.05$), and boars grew, on average, 3.9% faster than gilts ($P < 0.05$). A trend for an effect of diet was found ($P < 0.10$), although average daily gain of pigs in the different treatment groups was not significantly different ($P > 0.10$). A trend for an effect of diet by weaning weight interaction was also found ($P < 0.10$; Figure 5), with significantly positive regression coefficients for pigs offered either the BC (0.017 ± 0.004, $P < 0.05$) or control diet (0.018 ± 0.004), whereas the coefficient for pigs offered the BP diet was lower, and not significantly different from zero (0.007 ± 0.004, $P = 0.07$).

The number of days taken to reach a slaughter weight of 85kg was significantly affected by weaning weight, litter of birth, and sex ($P < 0.05$; Table 4). Days to slaughter was negatively correlated with weaning weight ($r = -0.43$, $P < 0.05$), and the regression coefficient of the relationship (-3.0 ± 0.4) indicates that a 1 kg increase in average weaning weight reduces the average number of days to slaughter by 3 days. Litter of birth explained 14.1% of the total variation in days to slaughter ($P < 0.05$), and boars reached the slaughter weight of 85 kg 2 days, or 2.4% faster than gilts ($P < 0.05$). Diet offered during the week after weaning did not affect the number of days taken to reach a slaughter weight of 85kg, and no diet by weaning weight interaction was observed ($P > 0.10$).

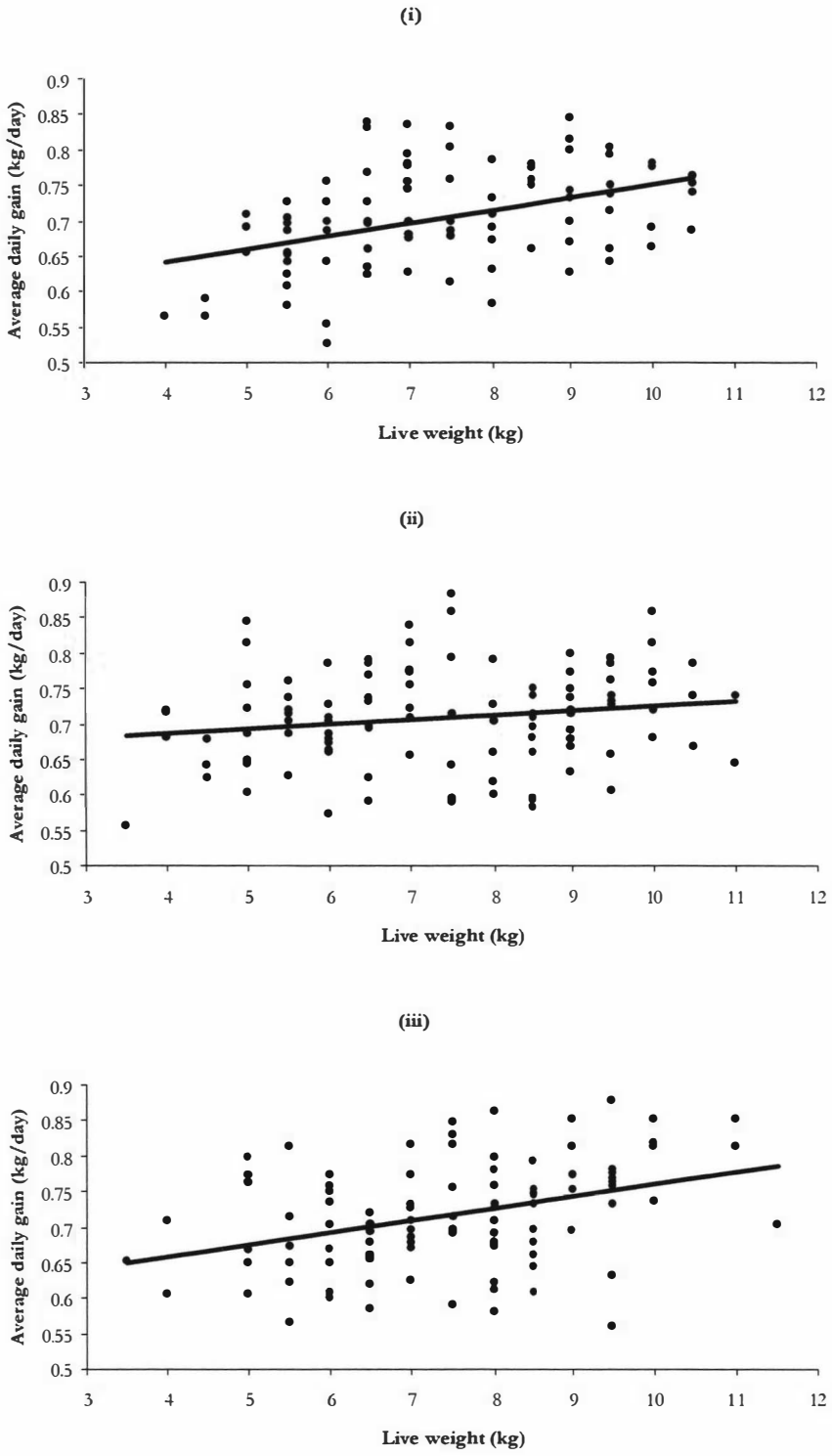

Figure 5. The relationship between weaning weight and total growth from weaning to slaughter for pigs offered diets containing skim milk powder (i; control), 6% bovine plasma (ii) or 6% bovine colostrum (iii) for the first week after weaning.

6.4 DISCUSSION

The data presented in this paper validate the use of spray-dried plasma and spray-dried bovine colostrum as alternative protein sources to dried skim milk in weaner starter diets. These results also show that the inclusion of spray-dried bovine plasma or spray-dried bovine colostrum in a starter diet can positively influence piglet feed intake and growth rate during the feeding period.

The fact that pigs of different weaning weights responded differently to the inclusion of spray-dried plasma compared to skim milk powder is a finding that appears not to have been reported elsewhere. This effect was evident during the first week post-weaning in pigs offered the diet containing spray-dried plasma, where lighter weaners grew faster than their heavier counterparts, leading to a significantly negative relationship between weaning weight and average daily gain in the week after weaning. This was in contrast to the pigs offered the diets containing skim milk powder and bovine colostrum, which showed no significant relationship between weaning weight and average daily gain in the week after weaning.

A recent review of the use of spray-dried plasma (van Dijk et al., 2001) demonstrated a negative linear relationship between average daily gain of control piglets and the percentage change in average daily gain of piglets fed a diet containing spray-dried animal plasma; piglets that generally perform poorly after weaning will exhibit a greater improvement in growth after addition of plasma to the diet compared to piglets which generally perform well after weaning. This relationship was derived through a comparison of the results of separate experiments, and may not be applicable to individual animals within an experiment, however, it offers an interesting parallel to the growth responses observed in the week after weaning in the present study.

In the first week after weaning, no treatment by weaning weight interaction was observed for feed intake. However, there was a significant interaction between treatment and weaning weight for FCR in the first week after weaning, which shows that the FCR of groups offered the bovine plasma diet tended to worsen as average weaning weight of the group increased, whereas the FCR of groups offered the control diet tended to improve with increasing weaning weight (Figure 4). Thus, it appears that the negative relationship between weaning weight and growth rate of pigs offered the bovine plasma diet is likely to have been mediated largely through a reduction in efficiency of gain as weaning weight increases. Physiological

basis for this occurrence is unclear, however this is an interesting finding that appears not to have been reported elsewhere. Similarly, it is interesting to note that there was no relationship between weaning weight and FCR in the first week after weaning in pigs offered the bovine colostrum diet, which is again different from pigs offered the control diet.

The interaction between weaning weight and average daily gain observed during the first week after weaning appears to have had flow-on effects on growth rate until 70 days of age, after which time no interaction is evident. The effect on live weight carries on further, however, only losing significance at time of slaughter (~140 days).

Average daily gain in the second week after weaning (35-42 days of age) showed no difference between treatment groups, however gain during the period from 42-70 days of age was significantly lower in pigs offered the bovine plasma diet during the week after weaning, compared to those offered the control diet. The common diets offered during both these periods contained no bovine plasma (control diet from 35-42 days of age, and standard weaner diet from 42-70 days of age). A similar reduction in performance has been noted in pigs which are transferred from diets containing spray-dried plasma to those containing none, which can ameliorate any positive effects of dietary spray-dried plasma on weight gain during the immediate post-weaning period (Touchette et al., 1998). This effect has led to the use of a 'transition' diet (or diets), which contain(s) a lower level of plasma than the initial diet, before pigs are offered a diet devoid of spray-dried plasma, which reduces any growth check during diet changes. The use of these 'phase' diets also improves the economic utilisation of feed by reducing the use of high levels of plasma in diets to periods of maximum benefit, such as immediately after weaning (Dritz et al., 1993).

The use of spray-dried bovine colostrum in diets for the young pig has been examined in several experiments. There is evidence that bovine colostrum may reduce intestinal inflammation over the weaning transition (Pluske et al., 1999b), and can improve intestinal villus height when offered as a supplement during the suckling period (King et al., 1999). When included in weaner diets at levels of 5 and 10%, bovine colostrum was found to increase piglet feed intake and growth rate during the first 10 days after weaning, and reduce the number of days taken to reach market weight by 2.9-4.2 days compared to control values (Pluske et al., 1999a). Recently, Dunshea et al. (2002) included freeze-dried porcine plasma and bovine colostrum in weaner diets, noting no beneficial effects in the first week after

weaning, compared to pigs offered a diet containing dried skim milk. However, pigs consuming diets containing bovine colostrum and porcine plasma tended to grow faster than those consuming diets containing dried skim milk in the second week after weaning. Dunshea et al. (2002) observed similar performance between pigs offered dietary bovine colostrum and porcine plasma, which is supported by the results of the present study, which used bovine plasma. Therefore, the results of Dunshea et al. (2002) and the present study both support the conclusion that dietary colostrum is a viable alternative to the use of spray-dried plasma in weaning diets.

In the present study, neither spray-dried colostrum nor plasma produced any significant differences in average growth performance compared to pigs offered the control diet. However, the growth rate of control pigs in the two weeks after weaning was relatively high, which has been shown to reduce the magnitude of the growth response when spray-dried plasma is included in the feed (van Dijk et al., 2001). Furthermore, the effectiveness of plasma is influenced by environmental factors such as level of hygiene (Coffey and Cromwell, 1995) and degree of pig antigen exposure (Stahly et al., 1995). The effect of spray-dried plasma is more pronounced in a continuous-flow on-farm nursery, rather than an off-site all-in-all-out experimental nursery (Coffey and Cromwell, 1995). The present study used an on-farm nursery for this reason, although all-in-all-out management was used, and the nursery was pressure-washed and disinfected prior to use. The high level of hygiene may explain the lack of a significant growth response in this case.

The results of this study also reinforce the significance of such factors as weaning weight, sex, and litter of birth in determining piglet growth and live weight during the period from weaning to slaughter. The effect of weaning weight on post-weaning performance is well documented, with heavier pigs growing faster than their lighter counterparts (Sloat et al., 1985; Tokach et al., 1992; Dunshea et al., 1997; Wolter and Ellis, 2001). It has also been shown that an increase in weaning weight of 1 kg reduces the time taken to reach slaughter weight by 2.5-3.2 days (Mahan and Lepine, 1991; Tokach et al., 1992; Mahan, 1993; Kavanagh et al., 1997), which accords with the results of the present study. Sexual dimorphism is also well-described, with boars generally growing faster than gilts during the post-weaning period (Giles et al., 1981; McPhee, 1981), and reaching slaughter weight 2.5-5.8 days faster than gilts (Kennedy, 1984), which are consistent with the findings of the present study.

An interesting aspect of sexual dimorphism has recently been reported by Dunshea (2001), who observed increased growth in gilts compared to boars in the first 7-21 days after weaning, and gilts also performed better than boars after transitions in accommodation, which suggests that gilts respond to stress associated with weaning and transition better than boars. In the present study, boars grew faster than gilts in the week after weaning, but the sex effect disappears in the second week after weaning, and occurs consistently thereafter. The absence of a sex effect in the second week after weaning may be due to sex-related factors such as those described by Dunshea (2001).

The effect of litter of birth (which is comprised of both genetic and maternal effects) was highly significant throughout the life of the pigs used in this study. Its effect on preweaning growth may be inferred by the fact that litter of birth accounted for 18.9% of the variation in weaning weight, and during the post-weaning period litter of birth accounted for 15.5 to 24.9% of the variation in average daily gain, 12.7 to 16.9% of the variation in live weight, and 14.1% of the variation in the number of days taken to reach slaughter weight. The significant effect of litter of birth observed in this study accords with data from Castell et al. (1985), and Kennedy (1984). Kennedy (1984) observed large variances between litters, particularly for the number of days taken for pigs to reach 90 kg, which indicated substantial maternal or common environmental effects on littermates. Similarly, Castell et al. (1985) reported a significant litter effect on age to 90 kg, as well as post-weaning growth and various carcass measurements. These statistics serve to emphasise the importance of this effect in determining pig performance, and the need for experiments of this nature to take this factor into account in the allocation of pigs amongst treatment groups.

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Chapter 7

EVALUATION OF SPRAY-DRIED BOVINE COLOSTRUM AND ANIMAL PLASMA AS PROTEIN SOURCES IN BROILER DIETS.

7.1 INTRODUCTION

Spray-dried animal plasma has been evaluated extensively in pig diets, where it is included in weaning diets as a replacement for other, usually milk-based, protein sources. When included in weaning diets at concentrations generally ranging from 30 to 80 g kg^{-1}, spray-dried plasma typically produces an increase in feed intake and growth rate, and often an improvement in feed conversion ratio, all of which help to overcome the characteristic post-weaning 'growth check' (Coffey and Cromwell, 2001; van Dijk et al., 2001). These effects are replicated with the inclusion of the high molecular weight fraction of plasma, while the low and medium weight fractions generally produce no effect (Gatnau et al., 1995; Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995). The high molecular weight fraction is composed principally of immunoglobulins, suggesting that these may be responsible for the performance benefits conferred by feeding plasma products. Previous work from our laboratory (Pluske et al., 1999a; King et al., 2001) has shown that spray-dried bovine colostrum powder, which contains similar concentrations of immunoglobulins, produces performance benefits comparable to plasma products when included in pig starter diets.

To the authors' knowledge, there have been no published reports evaluating the use of spray-dried animal plasma products in poultry diets. The fact that, unlike pigs, broilers do not experience weaning and the accompanying psychological and nutritional stresses may be responsible for this apparent lack of interest. The present study was therefore designed to evaluate the feeding value of spray-dried bovine colostrum (BC), bovine plasma (BP) and porcine plasma (PP) as protein sources in broiler diets.

7.2 MATERIALS AND METHODS

Experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Recommendations and Minimum Standards for the Care and Use of Animals for Scientific Purposes (New Zealand Animal Welfare Advisory Committee, 1995).

7.2.1 Dietary treatments

A maize-soybean meal diet (Table 1), formulated to meet or exceed National Research Council (1994) recommendations for major nutrients for broiler starters, served as the control (CON). The experimental diets were formulated to contain 5% BC, BP or PP by replacing soybean meal. The diets were formulated using analysed values (Chapter 4, this thesis) to contain similar concentrations of lysine and sulphur-containing amino acids. Because of the high concentrations of sodium and potassium in BP and PP, dietary electrolyte balance (DEB) was calculated for each diet using the method of Mongin (1981), and sodium bicarbonate was used to increase the DEB in CON and BC diets. After mixing, the diets were cold pelleted (60 °C).

7.2.2 Birds and conduct of the trial

Experimental procedures were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Code of Practice for the Care and Use of Animals for Scientific Purposes.

Day-old male broiler (Ross) chicks, obtained from a commercial hatchery, were wing-tagged, blocked according to live weight, and randomly assigned to 20 pens (8 chicks/pen) in electrically heated, raised wire-floored starting batteries in an environmentally controlled room. The four dietary treatments were each randomly assigned to 5 pens containing 8 chicks each, and diets were fed from day 1 to 14. On day 15, the treatment diets were removed and replaced with a common grower/finisher diet, which was offered until day 35. The grower/finisher diet was based on maize and soybean meal and formulated to meet or exceed National Research Council (1994) recommendations for major nutrients for growing broilers.

Feed and water were supplied *ad libitum* and fluorescent illumination was provided for 24 hours per day. Individual body weights and pen feed intake were recorded at weekly intervals. On day 8, excreta from each pen were scored for liquidity on a scale of 1 to 5 (1 representing normally formed excreta and 5 representing very liquid excreta). On day 35, two median birds were selected per pen and fasted for a period of 4 hours. The birds were weighed, then killed by cervical dislocation, followed by exsanguination. After the removal

of feathers, viscera, shanks and neck, the weights of the eviscerated hot carcass, heart, liver, spleen, pancreas and small intestine were recorded.

Table 1. Percentage composition and calculated analysis of the diets for broiler starters.

	Experimental diet[1]			
Ingredient, %	CON	BC	BP	PP
Maize	61.76	62.94	63.36	63.31
Soybean meal	33.60	27.00	27.00	27.00
Bovine colostrum[2]	-	5.00	-	-
Bovine plasma[3]	-	-	5.00	-
Porcine plasma[4]	-	-	-	5.00
Soybean oil	1.60	1.30	1.30	1.30
Salt	0.25	0.25	-	-
L-Lysine	0.20	0.04	0.01	0.04
D, L-methionine	0.36	0.27	0.26	0.28
L-Threonine	0.06	-	-	-
Limestone	1.22	1.05	1.21	1.21
Dicalcium phosphate	1.55	1.65	1.56	1.56
Sodium bicarbonate	0.20	0.20	-	-
Trace mineral premix[5]	0.25	0.25	0.25	0.25
Vitamin premix[6]	0.05	0.05	0.05	0.05
Calculated Analysis				
AME, kcal/kg	2990	2990	2990	2990
Crude protein, %	21.2	22.7	22.6	22.4
Calcium, %	0.90	0.90	0.90	0.90
Available phosphorus, %	0.40	0.40	0.40	0.40
Methionine + cystine, %	0.90	0.90	0.90	0.90
Lysine, %	1.15	1.15	1.15	1.15
Sodium, %	0.15	0.15	0.29	0.29
Potassium, %	0.74	0.74	0.67	0.67
Chloride, %	0.18	0.18	0.17	0.17
DEB[7], mEq/kg	310	300	350	350

[1] CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma.
[2] Immulac™ (NZ Dairy, Specialty Ingredients Division, Hautapu, New Zealand).
[3] AP820 (Proliant Corp., Ames, Iowa, USA).
[4] U70 (Harimex B.V., Loenen, The Netherlands).
[5] Poultry mineral 4 (Tegel Feeds Ltd., Auckland, New Zealand). Supplied per kilogram diet: Mn, 125 mg; Zn, 60 mg; Cu, 3 mg; Mo, 0.5 mg; Co, 0.3 mg; I, 1 mg; Fe, 25 mg; Se, 200 µg; choline chloride, 638 mg.
[6] Broiler starter vitamin (Tegel Feeds Ltd., Auckland, New Zealand). Supplied per kilogram diet: trans-retinol, 3.33 mg; cholecalciferol, 60 µg; dl-∝-tocopheryl acetate, 60 mg; menadione, 4 mg; thiamine, 3.0 mg; riboflavin, 12 mg; calcium pantothenate, 12.8 mg; niacin, 35 mg; pyridoxine, 10 mg; folic acid, 5.2 mg; cyanocobalamin, 0.017 mg; biotin, 0.2 mg; antioxidant, 100 mg.
[7] DEB, dietary electrolyte balance.

7.2.3 Intestinal histology

Each test diet was also fed to a pen of cohort birds (8 birds/ pen) from day 1 to day 14 post-hatching to obtain samples for measurement of gut morphology and histology. On day 14, all birds were euthanased by intracardial injection of sodium pentobarbitone. The gastrointestinal tract was immediately removed and a 5 cm sample was excised from the apex of the duodenal loop. The intestinal samples were flushed with ice-cold saline and immediately placed in Bouin's fluid (24% formalin, 5% glacial acetic acid and 71% picric acid) for fixation. The samples were transferred into 70% ethanol after fixation for 24 hrs.

Morphological measurements were carried out according to the method of Pluske et al. (1996). After fixation, duodenal sections were excised, dehydrated and embedded in paraffin wax. From each of these a section of 6 μm thickness was cut, stained with haematoxylin and eosin and alcian blue, and examined under a light microscope. For each section the image analysis software Sigma Scan (Jandel Scientific, San Rafael, CA, USA), and a light microscope were used to measure 10 of the tallest, well oriented villi from villus tip to crypt mouth, and 10 associated crypts from crypt mouth to base. Goblet cells were identified in the epithelium as blue staining cells, which were counted in 10 villi and 10 crypts of each section, and expressed as number of cells per 100 μm of villus or crypt epithelium.

7.2.5 Statistical analysis

The data were subjected to analysis of variance using the General Linear Models procedures of SAS (SAS, 2000) using pen as the experimental unit for feed intake and feed conversion ratio, and individual bird as the experimental unit for weight gain, organ weight and gut morphology data. In the analysis of individual growth performance, a linear model, which included starter diet as a fixed effect and initial live weight as a covariate, was fitted to the data. For the analysis of feed intake, feed conversion ratio and histology, a linear model including diet as a fixed effect, was fitted to the data. Orthogonal contrast analysis was used to compare immunoglobulin-containing diets (bovine colostrum, bovine plasma and porcine plasma) with the diet which contains no immunoglobulins (control), to compare the colostrum-containing diet with those that contain plasma (bovine plasma, porcine plasma), and to compare the bovine plasma diet to the porcine plasma diet. Organ weights are expressed as a percentage of the empty body weight. Differences between the least-square

means were identified using Fisher's Least Significant Difference test; the level of significance was pre-set at $P < 0.05$ and trends were identified at $P < 0.10$.

7.3 RESULTS

7.3.1 Broiler performance

Mortality during the trial was low (6%) and was not related to dietary treatments.

Dietary treatments had no effect ($P > 0.10$) on the weight gains and feed intake from day 1-14 of the experiment, but influenced feed/gain during this period (Table 2). Compared to those fed the CON diet, feed/gain from day 1-14 was lower ($P < 0.05$) in broilers offered the diet containing BC and a trend ($P = 0.06$) for lower feed/gain was observed in broilers fed diets containing BP and PP. Orthogonal contrast analysis demonstrated that inclusion of immunoglobulin-containing protein in starter diets improved feed/gain during the feeding period (day 1-14) compared to birds offered a diet devoid of immunoglobulins. A trend was observed for bovine colostrum to be more effective at improving feed/gain than both types of plasma combined ($P < 0.10$).

Dietary treatments offered from day 1-14 of the experiment had no effect ($P > 0.10$) on weight gain, feed intake or feed/gain ratio during days 14-35, when all birds were fed a common diet. Similarly, over the entire 35-day trial period, performance parameters were unaffected ($P > 0.10$) by dietary protein source.

Excreta score, taken on day 8 of the experiment, was significantly ($P < 0.05$) affected by starter diet. Inclusion of porcine plasma in the starter diet increased ($P < 0.05$) the excreta scores compared to that of birds offered any other diet (1.8, 1.6, 1.6 and 3.4 for CON, BC, BP and PP, respectively, SEM 0.2).

7.3.2 Carcass and organ weight data

Dietary treatments offered from day 1-14 of the experiment had no effect on carcass recovery ($P > 0.10$), but influenced dressed weight, which was higher in birds offered the BC and BP diets, compared to those offered either the CON or PP diets (Table 3). Orthogonal contrast analysis showed that inclusion of immunoglobulin-containing proteins in the starter diet significantly increased dressed weight compared to birds offered a diet devoid of

immunoglobulins ($P < 0.05$), and also that bovine plasma was more effective in this regard than porcine plasma ($P < 0.05$).

Dietary treatments had no effect ($P > 0.10$) on the relative weights of liver, spleen, heart and small intestine (Table 3). However, the relative pancreas weights in birds offered either the BP or PP diets were lower ($P < 0.05$) compared to those in birds offered the CON or BC diets. Orthogonal contrast analysis showed that inclusion of immunoglobulin-containing proteins in the starter diet tended to reduce spleen weight compared to birds consuming a diet devoid of immunoglobulins ($P < 0.10$), but that bovine colostrum was less effective in this regard than both types of plasma combined ($P < 0.05$).

7.3.3 Histological measurements

Dietary treatments significantly ($P < 0.05$) affected duodenal villus height measured at 14 days of age (Table 4). Inclusion of BC in the starter diet had no effect ($P > 0.10$) on villus height, whereas inclusion of PP in the diet increased ($P < 0.05$) villus height by 5.7%, compared to birds offered the CON diet. Villus height in birds offered the BP diet was 4.6% greater ($P = 0.05$) than that of birds offered the CON diet. Orthogonal contrast analysis demonstrated no significant effect of immunoglobulin-containing proteins on villus height, however bovine colostrum reduced villus height compared to birds offered a diet containing either type of plasma ($P < 0.05$).

Crypt depth in birds offered the PP diet was greater ($P < 0.05$) than those offered the CON diet (Table 4). Similarly, crypt depth in birds offered the BC diet tended ($P = 0.08$) to be greater than those offered the CON diet. Orthogonal contrast analysis showed that the inclusion of immunoglobulin-containing protein in the starter diet increased crypt depth compared to birds offered a diet devoid of immunoglobulins ($P < 0.05$).

Ratio of villus height to crypt depth in birds offered the BC diet was lower ($P < 0.05$) than those offered the BP diet, and tended to be lower than birds offered either the PP or CON diets ($P = 0.05$ and 0.06, respectively; Table 4). This was reflected in the contrast analysis, which showed that bovine colostrum reduced the ratio of villus height to crypt depth compared to both types of plasma combined.

The density of crypt goblet cells was influenced by dietary treatments offered from 1-14 days of age ($P < 0.05$; Table 4). The density of crypt goblet cells was reduced ($P < 0.05$) by 13.9% in birds offered the BC diet, compared to those offered the CON diet. Crypt goblet cell density did not differ ($P > 0.10$) among birds offered the CON, BP or PP diets. Orthogonal contrast analysis demonstrated that bovine colostrum significantly reduced crypt goblet cell density compared to both types of plasma combined ($P < 0.05$), and showed a trend for bovine plasma to reduce crypt goblet cell density compared to porcine plasma ($P < 0.10$).

Table 2. Influence of protein source on the growth performance of broilers fed maize-soybean meal starter diets from 1-35 days of age.

	Dietary treatment[1]					Orthogonal contrast analysis		
	CON	BC	BP	PP	SEM	Ig vs. no Ig	Colostrum vs. plasma	Bovine plasma vs. Porcine plasma
Weight gain (g/bird)[2]								
Day 1-14	419	435	427	416	8	0.46	0.17	0.32
Day 14-35	1683	1737	1729	1706	32	0.26	0.62	0.62
Day 1-35	2100	2170	2161	2121	36	0.23	0.51	0.43
Feed intake (g/bird)[3]								
Day 1-14	517	518	517	501	9	0.59	0.40	0.21
Day 14-35	2925	2981	2992	2959	54	0.41	0.94	0.67
Day 1-35	3442	3459	3484	3442	66	0.61	0.96	0.66
Feed/gain (g/g)[3]								
Day 1-14	1.232[a]	1.193[b]	1.210[ab]	1.210[ab]	0.008	0.006	0.08	1.0
Day 14-35	1.754	1.7352	1.751	1.746	0.015	0.53	0.41	0.85
Day 1-35	1.647	1.619	1.639	1.638	0.012	0.31	0.21	0.95

[1] CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma; SEM, pooled standard error of the mean.

[2] Least-square mean of 48 birds per treatment.

[3] Least-square mean of 6 cages per treatment.

[a,b] Least square means with different superscripts are significantly different ($P < 0.05$).

Table 3. Influence of protein source on carcass recovery, dressed weight and relative organ weights of broilers fed maize-soybean meal starter diets from 1-35 days of age[1].

	Experimental diet[2]					Orthogonal contrast analysis		
	CON	BC	BP	PP	SEM	Ig vs. no Ig	Colostrum vs. plasma	Bovine plasma vs. Porcine plasma
Carcass recovery, g/ 100 g body weight	71.8	72.4	72.4	71.7	0.5	0.40	0.51	0.23
Dressed weight, g	1413[a]	1489[b]	1489[b]	1422[a]	21	0.03	0.21	0.03
Organs, %[3]								
Liver	2.38	2.22	2.24	2.36	0.07	0.21	0.38	0.24
Spleen	0.12	0.12	0.11	0.12	0.01	0.60	0.93	0.61
Heart	0.64	0.60	0.61	0.65	0.02	0.30	0.23	0.10
Pancreas	0.20[a]	0.21[a]	0.18[b]	0.17[b]	0.01	0.097	0.046	0.78
Small intestine	2.46	2.40	2.43	2.42	0.08	0.62	0.76	0.95

[1] Least-square mean of 12 birds per treatment.
[2] CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma; SEM, pooled standard error of the mean.
[3] Expressed as a percentage of the empty body weight.
[a,b] Least square means with different superscripts are significantly different ($P < 0.05$).

Table 4. Morphological and histological variables measured in the duodenum of broilers fed maize-soybean meal starter diets containing different sources of protein from 1-14 days of age.

	Experimental diet[1]					Orthogonal contrast analysis		
	CON	BC	BP	PP	SEM	Ig vs. no Ig	Colostrum vs. plasma	Bovine plasma vs. Porcine plasma
Villus height, μm	1325^{ab}	1286^{a}	1386^{bc}	1400^{c}	22	0.20	< 0.0001	0.66
Crypt depth, μm	199^{b}	207^{ab}	204^{ab}	211^{a}	3	0.02	0.76	0.14
Villus height: crypt depth	6.7	6.4	6.9	6.7	0.1	0.68	0.01	0.50
Villus goblet cells[2]	4.3	4.1	4.3	3.7	0.2	0.15	0.58	0.02
Crypt goblet cells[2]	14.4^{b}	12.4^{a}	13.2^{ab}	14.6^{b}	0.5	0.12	0.03	0.07

[1] CON, control; BC, bovine colostrum; BP, bovine plasma; PP, porcine plasma; SEM, pooled standard error of the mean.

[2] Number of goblet cells per 100μm epithelium.

^{a,b} Least square means with different superscripts are significantly different ($P < 0.05$).

7.4 DISCUSSION

This study evaluated the use of spray-dried bovine plasma, porcine plasma and bovine colostrum in broiler starter diets. Spray-dried bovine and porcine plasma are commonly used as sources of highly digestible and palatable protein in the pig industry, where their inclusion in weaner-starter diets improves pig performance over the problematic weaning period. Spray-dried bovine colostrum has been shown to produce similar benefits when included in weaner-starter diets, improving growth rate, feed intake and feed conversion ratio in the first 10 days after weaning (Pluske et al., 1999a). Similarly Dunshea et al. (2002) reported that growth rate and feed intake of early-weaned pigs was improved by inclusion of either freeze-dried bovine colostrum or porcine plasma. Recently, King et al. (2001) demonstrated that dietary bovine colostrum may be more effective at stimulating voluntary feed intake after weaning than bovine plasma.

In the present study, inclusion of the test proteins had no effect on the feed intake and weight gain of broilers, but did improve the efficiency of feed conversion during the period of feeding (1-14 days of age), compared to those offered the control diet. This effect was significant only with diets containing bovine colostrum, while a trend was observed with bovine plasma and porcine plasma diets. These results differ from those generally reported in studies with weaner pigs. In a summary of 48 published experiments where piglets were offered diets containing spray-dried plasma for 14-28 days after weaning, 89% showed positive responses in terms of feed intake and growth rate, while only 53% showed a positive response in terms of feed/gain (Coffey and Cromwell, 2001). In this review, the average improvements (expressed as a percentage difference between the performance of pigs offered dietary spray-dried plasma and those offered a plasma-free control diet) in daily gain, feed intake and feed efficiency were found to be 25, 23 and 4%, respectively. Similar findings have also been reported by van Dijk et al. (2001).

The effect of dietary porcine plasma may have been altered by that diets high DEB relative to the other diets, resulting from the high sodium concentration in the porcine plasma sample used. This is likely to have caused the increase in excreta score observed in this treatment group at day 8 of the experiment. Interestingly, however, this appears not to have affected feed/gain during 1-14 days of age.

From experimental work with pigs, it appears that the beneficial effects of dietary spray-dried plasma are manifested most strongly when animals are housed in conditions which provide an immune challenge. This interaction between dietary spray-dried plasma and the housing environment has been demonstrated by comparing the marginal improvement in feed intake, weight gain and feed efficiency when plasma is included in the diet of pigs housed in a highly sanitised experimental facility and in a less-sanitary conventional farming situation (Bergstrom et al., 1997; Bregendahl et al., 1998; Chirra et al., 1999; Coffey and Cromwell, 1995; Gatnau and Zimmerman, 1991; Stahly et al., 1995; Touchette et al., 1996). In a study by Coffey and Cromwell (1995), a 247% and 700% greater improvement in feed intake and growth rate, respectively, was observed in pigs housed in a commercial environment compared to those housed in an experimental facility. Similarly Stahly et al. (1995) observed that dietary spray-dried plasma produced a greater improvement in feed intake and weight gain in high antigen-exposure pigs compared to those which received low antigen exposure. Similar results have been observed in chickens, where the growth-promoting activity of in-feed antibiotics has been shown to be expressed to a greater degree in an unsanitary environment that provided chronic immune stress, but not in a clean environment (Roura et al., 1992). The present study used clean, raised wire cages, which separated the birds from their excreta, and presumably provided minimal antigenic stimulation. It is possible that if the study had been conducted in an environment that is more conducive to immune stimulation, such as the commercial floor-pen situation, a more pronounced response to dietary plasma or colostrum may have been observed.

There is considerable evidence that small intestine histology and morphology are extremely susceptible to perturbation by alterations in feed intake (Pluske et al., 1997; King et al., 2003), so experimental situations in which individual feed intake is not measured (such as the present study) tend to confound the effect on gut histology of dietary components, with the effect of intake of the experimental diets. Nevertheless, despite the fact that direct causal relations are not easily distinguishable in these situations, the results obtained are nevertheless ultimately attributable to effects of the experimental diet, which in this case are due to dietary inclusion of spray-dried bovine or porcine plasma or spray-dried bovine colostrum.

Orthogonal contrast analysis demonstrated no consistent effect of the test proteins on small intestine morphology in the present study, with the exception of duodenal villus height and crypt depth. Compared to birds offered the control diet, duodenal villus height was greater in

birds offered the starter diet containing porcine plasma, and tended to be greater in birds offered the bovine plasma starter diet. Contrast analysis demonstrated a significant increase in crypt depth caused by the immunoglobulin-containing diets, despite there being no significant individual effect of the diets on this variable. Increased crypt depth is generally indicative of increased epithelial cell mitosis; as epithelial cells are added to the intestinal mucosa, they cause an elongation of the crypt area (Al-Mukhtar et al., 1982). These results therefore suggest a mitotic effect of the test proteins on epithelial cells, which may also be responsible for the increase in villus height observed in birds fed the plasma diets. In this regard, it should be noted that, aside from their immunoglobulin content, the test proteins also contain an array of growth factors (Antoniades, 1977; Playford et al., 2000) including insulin-like growth factor-I (de Rodas et al., 1995), which can increase villus height after oral administration (Burrin et al., 1996; Houle et al., 1997).

Inclusion of bovine colostrum in the starter diet reduced the density of crypt goblet cells compared to birds offered the control diet. Goblet cell differentiation is responsive to alterations in proinflammatory cytokine expression in the gastrointestinal mucosa, and increases in response to antigenic stimulation and subsequent mucosal inflammation (Elwood and Garden, 1999; King et al., 2003). A reduction in goblet cell differentiation may therefore be indicative of reduced antigenic stimulation of the intestinal mucosa, leading to a reduction in intestinal inflammation. Colostrum contains an array of antibacterial and antiviral factors which are capable of performing both specific and non-specific passive immunological defence functions in the intestinal lumen. These include immunoglobulins, lactoferrin, lactoperoxidase, oligosaccharides and glycoconjugates (Gopal and Gill, 2000; Salmon, 1999; van der Strate et al., 2001; van Hooijdonk et al., 2000; Wagstrom et al., 2000). Such a passive protective effect of bovine colostrum within the intestinal lumen could account for the observed reduction in crypt goblet cell density. In pigs, dietary bovine colostrum has been shown to reduce proliferation of inflammatory T cell numbers in the intestine over the weaning period (Pluske et al., 1999b), indicating potential anti-inflammatory properties of bovine colostrum, which may be due to passive immune protection. Given that activation of the immune system involves repartitioning of available nutrients away from growth and towards immune function (Johnson, 1997; Kelley et al., 1994; Stahly, 2001), a protective effect of colostral immune factors also offers a potential explanation for the observed improvement in feed conversion ratio when birds were offered the diet containing bovine colostrum.

The present study demonstrates that high quality spray-dried proteins such as bovine and porcine plasma and bovine colostrum are suitable sources of protein for use in starter diets for broiler chickens. The preliminary findings presented herein suggest that these proteins may offer added benefits, possibly due to immunological and/or hormonal activity within the intestinal lumen. However, further investigations are warranted in situations which provide a greater immune stimulus to evaluate this notion.

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SECTION III

General Discussion

8.1 INTRODUCTION

This study investigated the effects of dietary bovine colostrum, bovine plasma and porcine plasma on indices of intestinal health and performance in pigs after weaning. In particular, it compared the effects of dietary plasma, which is a common dietary ingredient in some pig-producing countries, to those of dietary bovine colostrum, which contains similar components to plasma. This elucidated some of the effects of both products, which were assessed in relation to current hypotheses of the mechanism of action of dietary spray-dried plasma. The potential integration of bovine colostrum into these hypotheses was also considered. Furthermore, the potential use of dietary plasma and colostrum in the poultry industry was investigated, through inclusion of these products in diets for broiler chickens. This study also observed weaning-related changes in intestinal immunity and morphology, which are of particular significance to pig science.

8.2 WEANING

Weaning is generally associated with low voluntary feed intake, poor weight gain or weight loss, and alterations in the morphology and immunology of the small intestine (Chapter 1; Pluske et al., 1995, 1997). Morphological restructuring during this time generally takes the form of villus atrophy, and crypt hyperplasia, which may reduce the absorptive area of the small intestine (Pluske et al., 1997). The data presented in Chapter 4 accord with these observations, demonstrating a 41-45% reduction in villus height and an increase of 7-11% in crypt depth one week after weaning, regardless of the weaning diet consumed during that time. Additionally, an increase in epithelial cell height was also observed after weaning, which may be due to increased metabolic activity within epithelial cells (Birtles, 2003; personal communication). To the author's knowledge this is a novel finding, and requires further observations under a variety of weaning situations for corroboration.

The data of Chapter 4 also provide further evidence of leukocytic expansion in the lamina propria of the small intestine after weaning, with a 17-40% increase in $CD8^+$ T lymphocytes and a 28-33% increase in $CD4^+$ T lymphocytes occurring within 7 days of weaning. This supports similar observations of T lymphocyte expansion in the week after weaning by Pluske et al. (1999a) and McCracken et al. (1999). However, some studies have shown

expansion of $CD4^+$ and $CD8^+$ T cells to occur more gradually after weaning (Spreeuwenberg et al., 2001; Solano-Aguilar et al., 2001; Vega-López et al., 1995).

There are two main hypotheses which attempt to account for the observed increase in immunological activity in the intestine after weaning: (1) that transient anorexia after weaning disrupts normal epithelial barrier function, allowing luminal antigens into the underlying lamina propria stimulating immune activity, and (2) that immune activation after weaning results from hypersensitivity to dietary antigens derived from unrefined soy protein (Chapter 1). The experimental diets used in Chapter 4 were devoid of unrefined soy proteins, with the exception of soybean oil, which does not contain the major soy storage proteins glycinin and β-conglycinin that are implicated in soy allergies (Chapter 1). The fact that expansion of lamina propria T lymphocyte subsets was observed in Chapter 4, despite the absence of soy proteins in the diet, supports the "luminal nutrition" hypothesis, but does not falsify the "soy hypersensitivity" hypothesis. Further support for the luminal nutrition hypothesis is provided by the generally positive correlations between measures of voluntary feed intake and intestinal villus height, observed in the other experiments of this study (Chapters 3, 4, 5). However, occasionally some poor and negative correlations between these variables were observed in these experiments suggesting that other factors besides the level of feed intake are involved in determining mucosal architecture. Similarly variable relationships between these variables have been observed in other experiments (Spreeuwenberg et al., 2001; Pluske et al., 1996). Also, it should be noted that simple correlations between variables does not necessarily imply a causative relationship. For example, it may be speculated that the small intestine primarily responds to endogenous stimuli associated with weaning, such as the expression of cytokines and hormones, which simultaneously induce both mucosal destruction and depression of voluntary feed intake in a related fashion (Chapter 1). This is supported by the fact that cytokines associated with disease states (Kelley et al., 1994; Johnson, 1997; Stahly, 2001) are also implicated in mucosal restructuring (Chapter 1; Touchette et al., 2002).

8.3 SPRAY-DRIED COLOSTRUM AND PLASMA

8.3.1 Influence on feed intake and growth rate

In every experiment of this study, voluntary feed intake and growth rate have been observed, however a significant effect of either spray-dried colostrum or plasma has only been observed in one (Chapter 6). In this experiment, voluntary feed intake increased in the week

after weaning by 22.7 and 14.8% respectively, in pigs offered a diet containing 6% bovine colostrum or bovine plasma, compared to pigs offered a diet containing dried skim milk. This represented a significant increase for pigs consuming the bovine colostrum diet ($P < 0.05$), whereas the increase of pigs consuming the bovine plasma diet was a trend ($P < 0.10$). Piglet growth rate was similar among treatment groups in this experiment, although numerical increases in growth rate were observed in groups consuming either the bovine plasma or bovine colostrum (Chapter 6) There are several possible reasons why improvements in these variables were only observed in one experiment. First, the beneficial effects of dietary spray-dried plasma are generally more pronounced when pigs are housed in environments that are likely to provide immune stimulation (Chapter 2). The experiments of Chapters 3 and 4 were performed in a housing situation in which possible disease vectors were eliminated, or reduced as much as possible, through thorough cleaning and disinfecting of the equipment, and individual housing of pigs. Antigenic stimulation in these experiments may therefore be considered to be low compared to the performance experiment which was conducted on a commercial pig farm (Chapter 6). This factor is likely to reduce the potential benefits of dietary inclusion of spray-dried plasma in the experimental setting used in Chapters 3 and 4. It should be noted, however, that the commercial pig farm employed several techniques to reduce the pathogen load experienced by weaner pigs, such as pressure-washing and disinfecting the rooms prior to use, and using all-in all-out housing rather than continuous-flow (which allows contact between old and young animals). It is therefore likely that a greater response to dietary inclusion of spray-dried plasma may be observed in commercial farming situations that do not employ the aforementioned hygiene practices.

Interestingly, in Chapter 6, a numerically greater performance benefit was observed after dietary inclusion of spray-dried colostrum, compared to inclusion of plasma. This indicates that bovine colostrum is a potential substitute for bovine plasma in weaning diets in the pig industry. A similar conclusion was reached by Dunshea et al. (2002) who compared freeze-dried bovine colostrum and porcine plasma. Also, Pluske et al (1999b) demonstrated a dose-dependent improvement in the feed intake and growth rate as spray-dried colostrum was included in weaning diets at levels of 5 and 10%, although there was no plasma-containing diet for comparison. Given the similarity in pig response to dietary inclusion of spray-dried plasma and colostrum, and the similar composition of the two products, it is possible that they share a common mode of action. It is therefore possible that the benefits of dietary spray-dried colostrum are increased when animals are housed in an environment that

provides greater immune stimulus, as is the case for plasma. This may explain the growth and feed intake benefits conferred by dietary colostrum in Chapter 6, whereas no significant benefits were observed in Chapters 3 and 4, respectively.

However, in Chapter 5 an experimental setting was used, and a bacterial challenge introduced to increase the immune stimulus of the experimental subjects. In this experiment, despite the fact that signs of intestinal inflammation were present in the intestine of *E. coli*-challenged pigs, no significant improvement in feed intake nor growth rate was demonstrated. In a similar experiment, Touchette et al. (1999) administered an oral pathogenic *E. coli* challenge to weaner pigs who were then allocated to receive either a control diet containing no spray-dried plasma or a diet containing 7% plasma. Touchette et al. (1999) demonstrated no effect of dietary spray-dried plasma on the growth of challenged pigs. Also, Touchette et al. (2002) and Carroll et al. (2002) observed no beneficial effect of dietary spray-dried plasma on growth performance of pigs challenged with intraperitoneal lipopolysaccharide, despite observing signs of immune stimulation due to the challenge. These results, and those presented in Chapter 5, may indicate that the challenges, while sufficient to induce immune activation, were not significant enough to alter pig growth, as concluded by Touchette et al. (2002) and Carroll et al. (2002). This is supported by the observation, in Chapter 5, that the oral *E. coli* challenge induced signs of a local inflammatory response in the intestine, while haematological parameters were not influenced, indicating no significant effect of the challenge on humoral immune status. A further explanation is that variability in the voluntary feed intake and growth rate of pigs after weaning necessitates the use of greater numbers of animals in these experiments in order to demonstrate significant differences in these variables due to dietary treatments. In Chapters 3, 4 and 5 individual feed intakes were variable throughout the feeding periods, which is characteristic of the abrupt weaning process in pigs (Chapter 1; Bruininx et al., 2001; McCracken et al., 1999).

Chapter 7 investigated the potential application of spray-dried plasma and colostrum powder to the broiler industry. To the author's knowledge there are no published data on this subject, to which the results of Chapter 7 may be compared. The results of this experiment demonstrated the potential of products such as spray-dried bovine colostrum, bovine plasma and porcine plasma to improve feed conversion ratio of broilers during a feeding period from 1-14 days of age. However, no effect of the test proteins on feed intake or weight gain was observed. This suggests that chickens may not respond to dietary plasma and colostrum

products in the same manner as pigs, which most commonly demonstrate improved feed intake and weight gain in response to their inclusion in weaning diets, while evidence for a consistent effect of plasma on pig feed conversion ratio is equivocal (Chapter 1). The experiment of Chapter 7 was conducted in clean wire-floored cages which minimised the contact of chickens with their excreta. This is likely to have reduced the immune stimulation of the chickens used in this experiment, compared to a similar experiment using a floor-penning situation, in which excreta is not separated from the general environment. The improvement in feed conversion ratio observed in chickens consuming the test proteins may therefore be increased in a floor-penning situation.

8.3.2 Influence on indices of intestinal health

The influence of the test proteins on indices of intestinal structure, function and immunity in pigs were emphasised in Chapters 3, 4 and 5. The effect of dietary spray-dried plasma on villus height and crypt depth have been investigated in numerous experiments (Chapter 2), and generally show variable results, with no consistent effect of dietary plasma emerging. This is mirrored in the observations of villus height and crypt depth in Chapters 3, 4 and 5, which show no consistent effect of the test proteins on these variables among the different experiments. For example, in Chapter 3, inclusion of 5% bovine colostrum in the diet of early-weaned pigs resulted in improvements in villus height of up to 19% compared to pigs offered the control diet, whereas in Chapter 4 dietary inclusion of 7.5% bovine colostrum reduced villus height by 10% in the proximal small intestine, and increased it by 8% in the distal small intestine, compared to pigs offered the control diet. This difference in response may be caused by the difference in the age of the animals at weaning (14 vs. 21 days of age in Chapters 3 and 4, respectively), suggesting that dietary bovine colostrum is more beneficial in younger or lighter pigs. This is supported by the results of Chapter 6, in which lighter pigs appeared to benefit more from dietary bovine colostrum and plasma than their heavier counterparts. The observed difference may also be due to the longer feeding period in Chapter 3 (14 vs. 7 days post-weaning in Experiments 3 and 4, respectively), which increased the duration of piglet exposure to bovine colostrum.

Villus height and crypt depth may be used as indirect indices of intestinal inflammation (Chapter 1), however to the author's knowledge, there are no published data measuring the effect of spray-dried plasma and colostrum on intestinal health and inflammation after weaning more directly, using analysis of intestinal $CD4^+$ and $CD8^+$ T lymphocyte subsets and

goblet cell expression. There is therefore a paucity of data with which to compare the results of the present experiments. However, comparisons may be drawn between the results of Chapters 3, 4 and 5. As demonstrated in the review of Chapter 2, the effect of the test proteins on indices of intestinal immunity, morphology and function was somewhat variable. In Chapter 3, consumption of bovine colostrum increased intestinal $CD4^+$ and $CD8^+$ T lymphocyte density by 28 and 37%, respectively, compared to piglets offered the control diet, whereas in Chapter 4, consumption of colostrum had no effect on these T lymphocyte subsets. As mentioned previously, this may be due to the difference in age of the experimental animals or the reduced feeding period employed in Chapter 4.

Due to the experimental design of Chapter 5, in which administration of an *E. coli* challenge and consumption of diets containing bovine plasma and colostrum were superimposed, examination of the interaction between dietary treatment and oral *E. coli* challenge is not possible. In this experiment, a 2 x 3 factorial design using the three diets (control, bovine colostrum and bovine plasma) and challenged or unchallenged pigs within each diet, would have elucidated a possible interaction between these variables, and allowed comparisons to be drawn between the histological data of Chapters 4 and 5.

In Chapter 7, the effect of dietary bovine colostrum, bovine plasma and porcine plasma on intestinal morphology and histology in broiler chickens was investigated. To the author's knowledge, there is no published data of this kind in chickens, to which these results may be compared. Overall, the inclusion of the test proteins increased small intestine crypt depth, which suggests an increase in epithelial cell mitosis in animals consuming these products. Consumption of porcine plasma significantly increased both crypt depth and villus height, which suggests that an increase in epithelial cell mitosis increased the length of intestinal villi, as discussed in Chapter 7. This was not observed in pigs offered a diet containing porcine plasma diet for one week after weaning (Chapter 4), and may be caused by the difference in feeding period (7 vs. 14 days in Chapters 4 and 7, respectively), or a difference in the response of the two species to consumption of porcine plasma.

8.3.3 Mechanisms of action

Currently, the hypothesised mechanism of action of dietary spray-dried plasma is based on the provision of passive intestinal immune protection by components of plasma such as immunoglobulins (Chapter 2). Fractionation of spray-dried plasma on a molecular weight

basis has demonstrated that the most active fraction is that of high molecular weight, which is composed principally of immunoglobulins (Chapter 2). The similar immunoglobulin compositions of spray-dried plasma and colostrum suggest that they may share a common mechanism of action, via the provision of passive immune protection in the intestine. As described in Chapter 2, there are components other than immunoglobulins present in plasma and colostrum that are capable of performing antibacterial actions, such as glycoproteins and glycoconjugates, which should also be considered as potentially active components of whole plasma and colostrum.

Provision of passive immune protection in the intestinal lumen is likely to decrease the interaction of luminal bacteria and antigens with the intestinal epithelium (Chapter 1). This effect may be especially significant in the immediate post-weaning period, when the passive protection afforded by maternal immunoglobulins in milk has been abruptly removed, and intestinal permeability is increased, potentially allowing luminal antigens access to the intestinal lamina propria (Chapter 1). In this situation, the provision of passive immune protection within the gut lumen may decrease the incidence of intestinal inflammation after weaning. Observation of indices of intestinal inflammation was therefore emphasised in Chapters 3, 4 and 5, in an attempt to elucidate the mechanism of action of colostrum and plasma products.

In Chapter 3, the consumption of bovine colostrum increased intestinal villus height and reduced crypt depth, suggestive of a reduction in both epithelial cell extrusion from villus tips and epithelial cell mitosis in intestinal crypts, and indicating a state of reduced inflammation in the intestine. However, this was accompanied by an increase in the density of both $CD4^+$ and $CD8^+$ T lymphocytes in the lamina propria of the small intestine, which indicates an increase in the activity of these components of the gastrointestinal immune system. As discussed in Chapter 3, an explanation for these observations is that pigs consuming the bovine colostrum diet developed oral tolerance to the novel proteins contained therein, and the development of oral tolerance induced expansion of intestinal T cells. This may also have stimulated a concurrent secretory immune response (Challacombe and Tomasi, 1980) which could bolster the immune status of the intestine, along with the passive immunological protection afforded by antibacterial components of colostrum. The results of this experiment are therefore not incompatible with the "passive protection" hypothesis, but also suggest another hypothesis – that dietary bovine colostrum may improve immunity by

stimulating the oral tolerance mechanisms of the intestinal immune system. It should be noted that these hypotheses are not mutually exclusive, so both could potentially contribute to the beneficial effects of dietary colostrum and plasma.

Both hypotheses are supported by the histological results of Chapter 4, which suggested that consumption of diets containing bovine plasma and colostrum provided passive immune protection in the small intestine of weaner pigs, resulting in improved villus height in more distal regions of the small intestine. However, an effect that was common among all diets containing the test proteins was an increase in crypt goblet cell density in the small intestine compared to pigs consuming the control diet, which suggests the induction of secretory immunological activity in the intestine, supporting the "oral tolerance" hypothesis. No consistent effect of the test proteins on lamina propria $CD4^+$ and $CD8^+$ T lymphocyte subsets was observed in Experiment 4, although both $CD4^+$ and $CD8^+$ T cell numbers of pigs consuming the bovine plasma diet were not significantly increased compared to "baseline" piglets who were killed immediately after weaning, whereas all other groups killed one week after weaning displayed expansion of $CD4^+$ and $CD8^+$ T lymphocyte subsets. This may have been due to the numerically higher average daily feed intake of pigs offered the bovine plasma diet, which may reduce intestinal inflammation (Chapter 1), or may be further support for the "passive protection" hypothesis, indicating reduced inflammation in the intestine as a result of reduced antigenic stimulation.

The results of Chapter 5 provide what may be interpreted as further support for the "passive protection" hypothesis, with pigs consuming diets containing bovine plasma and colostrum displaying a significantly greater negative response to *E. coli* challenge compared to challenged pigs offered the control diet, as indicated by observations of intestinal histology. As discussed in Chapter 5, this may indicate a more immunologically "naïve" response on the part of pigs consuming dietary bovine colostrum and plasma, which implies that immune stimulation was reduced during the period preceding administration of the challenge due to the passive protection provided by bovine plasma and colostrum (Touchette et al. 2002; Carroll et al., 2002). However, these results also imply that both bovine plasma and colostrum were ineffective at protecting the weaner piglet during times of significant immune challenge.

8.3.4 Future research

Based on the findings of this study, some suggested areas of future research are:

- Serial slaughter of pigs consuming diets containing bovine colostrum or plasma in the weeks after weaning to observe indices of intestinal health.
- Examine the effect of dietary plasma and colostrum on other indices of intestinal immunology, including quantification of other leukocyte populations, observation of indices of leukocyte activation and expression of inflammatory mediators, particularly during pathogenic challenge.
- Determine the optimal inclusion rate of dietary bovine colostrum in weaning diets used in production situations, addressing both performance and economic benefits.
- Fractionation of bovine colostrum and evaluation of the benefits of the different fractions on feed intake and growth rate of weaner pigs.
- Explore the potential for plasma and colostrum products to be used in the broiler or wider poultry industry, including determining the effect of environmental and animal health status on response to dietary plasma and colostrum, and determining the optimum inclusion rate of these products in poultry diets.
- Investigate the possible application of plasma and colostrum components to human infant disorders, e.g. using piglet models of total parenteral nutrition.

8.4 CONCLUSION

This study reviewed the current literature on the immune status of pigs over the weaning period. It confirms observations of increased immunological activity in the intestine after weaning, and suggests that these are not necessarily related to a hyperimmune response to dietary soy proteins. The current literature on the use and benefits of spray-dried plasma and colostrum, and their possible mechanisms of action were also reviewed. This study demonstrates the potential for plasma and colostrum products to improve the local immune status of the intestine in weaner pigs. It provides data that supports the current hypothesis of the mechanism of action of dietary plasma, and suggests that this hypothesis may be extended to account for the beneficial effect of dietary bovine colostrum. However, it also suggests that other mechanisms may be involved in mediating the effects of dietary plasma and colostrum, possibly involving antigenic stimulation of the secretory immune response in

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