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

A thesis presented in partial fulfilment of the requirements for the degree of

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at Massey University, Palmerston North,

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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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Date: 25/03/04

"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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