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Dietary Bovine Colostrum Increases Villus Height and Decreases Small Intestine Weight in Early-weaned Pigs

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ABSTRACT : This experiment examined the effect of dietary spray-dried bovine colostrum on intestinal histology and organ weights in early-weaned pigs. In a randomised complete block design, twelve 14-day-old weaner pigs were offered a diet containing either 5% spray-dried bovine colostrum or no colostrum (control). Diets were formulated to contain 14.8 MJ/kg DE, 1.26% available lysine and to meet or exceed requirements for other nutrients. Piglets were offered the diets for a period of 14 days. No effect of diet on growth rate or feed intake was observed ($p>0.10$). Small intestine weight was reduced by 12% in piglets consuming dietary bovine colostrum ($p<0.05$). Villous height and crypt depth were increased and decreased, respectively, in the proximal jejunum, mid jejunum and distal ileum of pigs consuming dietary bovine colostrum ($p<0.05$). Mid-jejunal lamina propria $CD4^+$ and $CD8^+$ T lymphocyte density was increased by 28 and 37%, respectively, in piglets consuming dietary bovine colostrum ($p<0.05$). Diet did not affect thickness of tunica muscularis externa or tunica submucosa ($p>0.10$). Collectively, these results suggest a positive effect of dietary bovine colostrum on intestinal morphology and immune status in early-weaned pigs. (**Key Words :** Spray-Dried Bovine Colostrum, Intestinal Histology, Weaner Pigs, Nutrition)

INTRODUCTION

The period immediately after piglets are weaned is characterised by low feed intake, suboptimal growth (or often weight loss) and occasionally diarrhoea (Pluske et al., 1995). Weaning is also associated with dramatic alterations in the morphology and histology of the small intestine. This includes villus atrophy and crypt hyperplasia, along with alterations in the specific activity of brush border enzymes, which collectively could decrease the digestive and absorptive capacity of the intestine (Pluske et al., 1997). Associated with this restructuring of the small intestine are significant alterations in the activity of the gastrointestinal immune system, indicative of an inflammatory state. This, in turn, may damage the mucosa and increase epithelial permeability to luminal antigens, inducing or exacerbating the morphological changes that accompany weaning (King et al., 2003). Combined with the abrupt loss of passive immune protection from sow's milk, this leaves the newly-weaned pig in an immunologically vulnerable situation which is often exacerbated by standard weaning practices, such as mixing, moving, and early weaning of pigs (King et al., 2003).

To improve performance in the post-weaning period, products such as spray-dried plasma, which contain immunoglobulins and other passive immunoprotective factors, have been used in weaning diets. Spray-dried plasma can improve feed intake and growth rate immediately after weaning (van Dijk et al., 2001a), an effect that has been attributed to the high molecular weight immunoglobulin (Ig) fraction (Gatnau et al., 1995; Weaver et al., 1995). The most common hypothesis accounting for the beneficial effects of dietary plasma is that the immunoprotective components exclude antigens from interaction with the intestinal mucosa, and enhance mucosal integrity, reducing indices of intestinal inflammation and leading to an increase in voluntary feed intake (Bosi et al., 2004).

A product similar to spray-dried plasma is spray-dried bovine colostrum (BC), which also contains an array of Ig

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Table 1. Composition and analysis of experimental diets (g/kg air-dry diet)

	Control	Bovine colostrum
Ingredient		
Wheat	428.5	454.5
Delactosed whey powder	200.0	200.0
Fishmeal	100.0	75.0
Meat and bone meal	29.0	0.0
Ring-dried blood meal	25.0	25.0
Skim milk powder	50.0	50.0
Bovine colostrum[1]	0.0	50.0
Soybean meal	80.0	80.0
Sugar	50.0	50.0
Soybean oil	7.6	1.1
L-lysine	1.9	2.0
D, L-methionine	16.2	1.5
L-threonine	3.7	3.1
Salt	3.5	3.5
Tylan[2]	1.5	1.5
Vitamin and mineral premix[3]	3.0	3.0
Calculated analysis		
DE (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] Immulac15 (Specialty Ingredients Division, Fonterra, Hautapu, New Zealand).

[2] Tylan (Elanco Animal Health, Auckland, New Zealand).

[3] Vitastart (Vitec Nutrition Ltd, Auckland, New Zealand). Supplied per kg 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, 2,000 IU; Vitamin E, 70 mg; 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.

and antimicrobial factors. Inclusion of BC in weaning diets has similarly been shown to improve piglet feed intake and growth rate immediately after weaning (Pluske et al., 1999a, King et al., 2001), but the mechanisms of action have not been defined. When BC has been used in supplementary feeds for piglets pre-weaning, intestinal villus height has increased (King et al., 1999) and intestinal T lymphocyte proliferation during weaning has reduced (Pluske et al., 1999b). The hypothesis tested in this experiment was that inclusion of BC in a diet for early-weaned pigs would improve intestinal morphology and immune status in the two weeks following weaning.

MATERIALS AND METHODS

Animals and feeding

Experimental procedures were approved by the Massey University Animal Ethics Committee.

Twelve, 14-day-old mixed-sex piglets (Large White× Landrace; 3.6±0.1 kg) from 5 litters were obtained from a commercial piggery. These were blocked by litter of birth and live weight and randomly assigned to receive either control diet (CON) or a diet containing 50 g/kg 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 ambient temperature was maintained at 30°C with a 12 h light/dark cycle. Diets were offered *ad libitum* for the duration of the 14 d experiment. Piglet live weight was measured at d 0, 7 and 14, and feed intake was recorded at d 7 and 14.

The CON and BC diets were both based on wheat and delactosed whey (Table 1), and were formulated to contain 14.8 MJ/kg digestible energy (DE), 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. The BC diet contained 50 g/kg BC, providing a calculated concentration of 7.5 g/kg IgG. Diets were fed in meal form. The BC product (Immulac; Fonterra, Specialty Ingredients Division, Hautapu, New Zealand) contained 810 g/kg crude protein, 45 g/kg lysine, 25 g/kg methionine+cysteine, 35 g/kg threonine and 20.3 MJ/kg gross energy (GE) (air dry product).

Post-mortem procedure

On d 14 of the experiment (piglet age = 28 d) all piglets were euthanised and post-mortem measurements taken. Piglets were sedated with 0.2 ml/kg Stresnil (SmithKline Beecham Animal Health, Auckland, New Zealand) 20 min prior to slaughter, and euthanised by intravenous injection of 125 mg/kg sodium pentobarbitone (Chemstock Animal Health, Christchurch, New Zealand). The abdomen was opened, the entire gastrointestinal tract removed, and scissors were used to disconnect the small intestine at the gastric pylorus and the ileo-caecal valve. The small intestine was weighed, laid out on a stainless-steel tray and its length measured. A section at proportionally 25, 50 and 75% along the intestine was clamped with haemostats, excised, flushed with chilled isotonic saline, weighed, and placed immediately into Bouin's fluid fixative (24% formalin, 5% glacial acetic acid, 71% picric acid) for 24 hours, followed by 70% ethanol. The thymus, spleen, liver and pancreas were removed and weighed. The stomach, caecum, and small and large intestine were weighed, then emptied of digesta, washed with water, blotted dry, and re-weighed. Processing time, from euthanasia to obtaining gut samples, was approximately 10 min. Regions at 0.25, 0.5 and 0.75% along the small intestine are referred to by their respective approximate positions in the small intestine, i.e. proximal jejunum, mid-jejunum and distal ileum.

Histology and immunocytochemistry

After fixation, 4 ring-shaped lengths of small intestine from each of the 3 regions were excised, dehydrated and embedded in paraffin wax. From each of these, a transverse

section (6 μm) was cut, stained with haematoxylin and eosin, and examined under a light microscope. Measurements of villus height and crypt depth were taken from areas where the plane of section ran vertically from the tip of a villus to the base of the adjacent crypt. For each section, image analysis software (Sigma Scan; Jandel Scientific, San Rafael, CA) and a light microscope were used to measure 10 of the tallest villi from villus tip to crypt mouth, and 10 associated crypts from crypt mouth to base (after Hampson, 1986). Measurement of epithelial cell height on each of the 10 villi was performed by taking 6 measurements at even distances along the villus. Tunica submucosa and tunica muscularis externa thicknesses were measured at a minimum of 48 points at even distances around the intestinal sections.

Lamina propria $CD4^+$ and $CD8^+$ T lymphocytes were identified by immunocytochemistry. Tissue from the mid-jejunal region of each piglet was transversely sectioned (6 μm) onto glass slides. Sections were de-paraffinised in 2, 7 min changes of xylene, 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 min. Sections were then washed in 0.01 M phosphate buffered saline (PBS; pH 7.2), and antigen retrieval achieved by incubating sections in 0.1 M phosphate-citrate buffer (pH 6.0) for 60 min at 60°C. Sections were then washed in 3 changes of PBS and non-specific binding sites blocked by incubation with 1% bovine serum albumin for 5 min 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 for 1 h at room temperature, washed in 3 changes of PBS and incubated with a 1:200 dilution of biotinylated anti-mouse IgG (Amersham Biosciences UK Ltd., Buckinghamshire, UK) for 30 min at room temperature. Sections were again washed in 3 changes of PBS and incubated with a 1:200 dilution of biotin-streptavidin-peroxidase preformed complex (Amersham Biosciences UK Ltd., Buckinghamshire, UK) for 15 min at room temperature. Sections were then washed in 3 changes of PBS, 3-3 diaminobenzidine solution applied, and sections allowed to react for 3 min. 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 (after 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 1×10^4 μm^2 of crypt lamina propria.

Statistical analysis

Data were subjected to analysis of variance 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 individual empty body weight, as was small intestine length (m). To analyse growth rate, feed intake, organ weight, small intestine length and immunocytochemistry a linear model was fitted which included diet as a fixed effect and litter of birth as a random effect. Villus height, crypt depth, epithelial cell height, ratio of villus height to crypt depth, tunica muscularis externa and tunica submucosa were analysed using a linear model including dietary treatment, region of the small intestine, and the interaction between dietary treatment and region as fixed effects, and piglet nested within diet as a random effect.

Where a significant treatment effect was observed, Fisher's least significant difference test was performed to determine differences between least-square means. Significance was determined at $p<0.05$ and trends identified at $0.05<p<0.10$. Pearson correlation analysis was used where appropriate. Data are presented as least-square means with associated pooled standard error of the mean (SEM).

RESULTS

Histology

Villus height, crypt depth, epithelial cell height, ratio of villus height to crypt depth, tunica muscularis externa and tunica submucosa were all significantly affected by pig nested within diet ($p<0.001$), which accounted for 12-35% of the variation in these variables.

Villus height (Table 2) was affected by region of the small intestine and showed an interaction between diet and region of the small intestine ($p<0.05$). 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 ($p<0.05$). 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 ($p<0.05$).

Crypt depth (Table 2) was affected by diet ($p<0.0001$) and region of the small intestine ($p<0.05$), but no region-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, producing an overall reduction in crypt depth of 13% in the BC treatment ($p<0.05$). Pooled across treatments,

Table 2. Morphological measurements in the different section of the small intestine of weaner piglets offered a control diet or a diet containing 5% bovine colostrum

	Dietary treatment		SEM
	Control	Bovine colostrum	
Villus height (μm)			
Proximal jejunum	444[1a]	478[1b]	7
Mid jejunum	397[2a]	465[1b]	
Distal ileum	323[3a]	386[2b]	
Mean	388	443	25
Crypt depth, μm			
Proximal jejunum	234[1a]	208[1b]	4
Mid jejunum	227[1a]	197[12b]	
Distal ileum	232[1a]	194[2b]	
Mean	231[a]	200[b]	7
Villus height:crypt depth			
Proximal jejunum	1.93[1a]	2.35[1b]	0.05
Mid jejunum	1.80[2a]	2.39[1b]	
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	0.4
Mid jejunum	27.2	28.2	
Distal ileum	23.6	23.4	
Mean	25.9	26.3	0.6

* Least-square means 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 ($p<0.05$).

crypt depth was highest in the proximal jejunum ($p<0.05$), with more distal regions showing similar values (221, 212 and 213 μm, respectively, SEM 2.5 μm).

Ratio of villus height to crypt depth (Table 2) was affected by region of the small intestine ($p<0.0001$) and diet ($p<0.05$), and a trend for a region-by-diet interaction was observed ($p = 0.09$). Ratio of villus height to crypt depth was consistently higher in piglets offered the BC diet ($p<0.05$), resulting in an overall increase in crypt depth of 31% in this treatment. 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 decreased only in the distal ileum ($p<0.05$). Pooled across both treatments, 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).

Epithelial cell height (Table 2) was affected by region of the small intestine but not diet or region-by-diet interaction ($p>0.10$). Pooled across both treatments, epithelial cell height 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).

Table 3. Influence of dietary spray-dried bovine colostrum on $CD4^+$ and $CD8^+$ T lymphocyte populations[1,2] in mid-jejunal crypt lamina propria of weaner piglets piglets offered a control diet or a diet containing 5% bovine colostrum

	Dietary treatment		SEM
	Control	Bovine colostrum	
$CD4^+$	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] Least-square mean values with pooled standard error (SEM).
[2] Number of positive T lymphocytes per $1x10^4$ μm^2 crypt lamina propria.
[a, b] Least-square means with different superscripts are significantly different ($p<0.05$).

Tunica muscularis externa and tunica submuscularis thicknesses were affected by region only ($p<0.05$), with no effect of diet ($p>0.10$; data not shown).

The density of mid-jejunal lamina propria $CD4^+$ T lymphocytes (Table 3) 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 ($p< 0.05$). Litter of birth accounted for 25% of the total variation in $CD4^+$ T lymphocyte density. The density of $CD8^+$ T lymphocytes (Table 3) 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 ($p<0.05$). 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 or litter of birth ($p>0.10$).

Average villus height showed a trend to correlate with $CD8^+$ T lymphocyte density ($r = 0.57$, $p = 0.06$) but no other variable ($p>0.10$). Overall average crypt depth was positively correlated with small intestine weight ($r = 0.74$, $p = 0.006$).

Feed intake, weight gain, empty body weight and organ weights

Daily weight gain and feed intake were not affected by litter of birth or dietary treatment during either the first or second week of the experiment, or both weeks combined ($p>0.10$; data not shown).

Empty body weights were unaffected by diet ($p>0.10$; data not shown), however a significant effect of litter of birth was observed ($p<0.05$). The weight of organs was unaffected by diet ($p>0.10$; data not shown), with the exception of small intestine weight, which was 12% lower in pigs offered the BC diet compared to those offered the CON diet (4.67 and 5.32% of empty body weight, respectively, SEM 0.17%; $p<0.05$). Litter of birth also affected small intestine weight ($p<0.05$), and showed a trend for an effect on thymus weight ($p = 0.08$) and small intestine length ($p = 0.09$). Small intestine length did not

differ between treatment groups ($p>0.10$; data not shown).

DISCUSSION

This experiment demonstrated that the addition of 50 g/kg BC to a diet offered for two weeks after early weaning can alter piglet intestinal morphology and T lymphocyte density, and reduce small intestine weight compared to piglets offered a diet containing no BC. The increase in villus height observed in the pigs fed BC was similar to that observed in weaner pigs fed diets containing spray-dried plasma (Gatnau et al., 1995; Touchette et al., 1999), although other experiments have shown no effect of dietary plasma on villus height (Jiang et al., 2000; van Dijk et al., 2001b). The effect of other dietary compounds on villus height after weaning has been investigated. An increase in villus height was observed when lactitol and tributyrin were added to weaner diets (Hou et al., 2006) but no changes in intestinal morphology were reported when different types of soy protein were fed to piglets after weaning (Kim et al., 2007; Yang et al., 2007).

The reduction in crypt depth observed in piglets offered the BC diet suggests that the greater villus height observed in these animals is attributable to decreased villus atrophy rather than increased epithelial mitosis (Al-Mukhtar et al., 1982). Crypt depth displayed a strong positive correlation with small intestine weight, which is consistent with reports of mucosal volume and weight increasing with epithelial mitosis (See et al., 1990). Given that other variables which can affect small intestine weight (small intestine length, thickness of tunica muscularis externa and tunica submucosa) were not affected by diet, a relative increase in epithelial mitosis could explain the simultaneous increase in crypt depth and small intestine weight in control pigs in the present experiment.

A moderate expansion of CD4+ and CD8+ T lymphocyte subsets was observed in piglets consuming the BC diet. Expansion of lamina propria T lymphocyte subsets in the intestine is characteristic of local immune system activation is observed at weaning, where it is usually accompanied by morphological restructuring of the intestinal mucosa (Vega-López et al., 1995; McCracken et al., 1999). Spreeuwenberg et al. (2001) reported negative correlations between mucosal T lymphocyte density and villus height in weaner piglets during a sampling period of up to four days after weaning. Activated T lymphocytes can produce an array of proinflammatory cytokines which bolster the inflammatory response, inducing crypt hyperplasia, villus atrophy and expression of matrix metalloproteinases that degrade extracellular matrix proteins (MacDonald et al., 1999). However, there is evidence that activated porcine mucosal T lymphocytes are biased towards the induction of immunological tolerance and secretory immune responses, rather than inflammatory and cellular immune responses (Stokes et al., 2001). The mild expansion of lamina propria T lymphocyte subsets in piglets consuming BC in the present experiment may therefore be associated with induction of immunological tolerance to the numerous novel proteins present in bovine colostrum (Smith et al., 2002). This is compatible with the observation that $CD4^+$ T lymphocyte density showed no relationship with morphological indices in the small intestine, and $CD8^+$ T lymphocyte density showed a trend for a positive correlation with average villus height.

The tolerogenic activation and expansion of T lymphocytes is often accompanied by the development of a secretory immune response in the small intestine (Challacombe and Tomasi, 1980). The promotion of a secretory immune response in the small intestine may bolster local intestinal immunity, offering another mechanism by which lymphocyte density may affect villous height in the present experiment.

Immunosuppressive activation of T lymphocytes in the lamina propria may influence the many immune and non-immune cell populations (such as other lymphocytes, plasma cells, macrophages, dendritic cells, granulocytes, mast cells, eosinophils, neutrophils, fibroblasts, fibrocytes) residing in the lamina propria. A reduction in overall cellularity of the lamina propria has been observed to accompany a reduction in gut weight in piglets offered plasma-containing diets (Jiang et al., 2000). This, combined with the reduction in crypt depth, may account for the observed reduction in small intestine weight in BC-fed piglets.

The physiological mechanism(s) through which BC acts in the intestine and systemically remain to be fully elucidated. Given the presence of immunoglobulins in colostrum and their resistance to complete proteolysis in the small intestine of the young pig (Morel et al., 1995) as in the adult human (Roos et al., 1995), they likely afford some passive protection to 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 Ig composition comparable to that of BC, and the results of the present experiment accommodate this as a possible mechanism of action. However BC also contains numerous non-specific anti-microbial and anti-viral factors such as lactoferrin, lactoperoxidase, oligosaccharides and glycoconjugates (Gopal and Gill, 2000; Van Hooijdonk et al., 2000), as well as hormones that are capable of reducing mucosal injury (Playford et al., 1999), and stimulating repair (Zijlstra et al., 1994), which may also be involved. The presence of these components and the effects observed in this experiment suggest that BC should be considered as one of the growing range of immunologically active ingredients for use in pig nutrition

(Gatnau et al., 1995; Touchette et al., 1999; Kong et al. 2007), and monogastric nutrition more generally, particularly during times of immune stress.

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