Asian-Aust. J. Anim. Sci.
Vol. 24, No. 5 : 670 - 677
May 2011

www.ajas.info
doi: 10.5713/ajas.2011.90490

Colostrum Protein Isolate Increases Gut and Whole Body Growth and Plasma IGF-I in Neonatal Pigs

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ABSTRACT : The growth rate of the young pig is generally much less than it's potential and may be constrained by endocrine status as well as nutrient intake. Growth factors are present in relatively high quantities in colostrum and play an important part in gut development. It is possible that supplementation of colostrum protein isolate may stimulate gut and whole body growth in the pig. Eight male and 8 female (Large White×Landrace) piglets were weaned at 1 d of age after each pig had obtained colostrum from their dam, and were trained to consume one of two liquid diets. The two diets were based on either a colostrum protein isolate (n = 4 males and 4 females) or whey protein concentrate (n = 4 males and 4 females) and were formulated to contain equal levels of crude protein and amino acids. Pigs were fed their diets *ad libitum* for 28 days after which time 12 pigs were euthanised and various tissues and organs weighed. Pigs were bled for IGF-I analyses at 21 and 28 days of age. Daily gain was higher in pigs consuming the colostrum isolate (171 vs. 216 g/d, p = 0.010), particularly between 2 and 4 weeks of age (212 vs. 298 g/d, p = 0.010). Pigs tended to consume more of the liquid feed containing colostrum isolate (25.5 vs. 29.1 kg, p = 0.074) and gained more live weight per unit of liquid feed (0.203 vs. 0.223 g/g, p = 0.056). There were no effects of sex on growth performance. Pigs consuming the diet supplemented with colostrum isolate had higher (p<0.05) full gut weight (445 vs. 554 g, p = 0.026), empty gut weight (356 vs. 463 g, p = 0.008), stomach weight (42.2 vs. 54.4 g, p = 0.001), small intestine weight (222 vs. 275 g, p = 0.025) and large intestine weight (63.7 vs. 98.0 g, p = 0.005). Plasma IGF-I (99 vs. 150 ng/ml, p<0.001) and IGF-II (265 vs. 406 ng/ml, p<0.001) were higher in pigs fed colostrum isolate. Pigs consuming colostrum protein isolate ate more, grew faster and had higher plasma IGF-I concentrations than pigs consuming a diet with similar macronutrient content but devoid of growth factors. (**Key Words :** Pig, Neonate, Colostrum, IGF-I, Growth)

INTRODUCTION

The growth rate of the young pig is generally less than half it's potential (Boyd et al., 1995) and may be constrained by endocrine status as well as nutrient intake. Growth factors such as insulin-like growth factors, transforming growth factor β and epidermal growth factor are present in relatively high quantities in colostrum (Playford et al., 1999) and play an important part in gut development (Widdowson et al., 1976), especially in compromised states (Pereira-Fantini et al., 2008). Gut development and growth in the piglet is essential for efficient nutrient absorption and protection against bacterial invasion (Dong and Pluske, 2007). The growth factor content of milk decreases with advancing lactation and so an opportunity exists to enhance gut growth and development through supplementation with exogenous growth factors. This is particularly pertinent given that the pig gut can be relatively immature at weaning (Dunshea et al., 2002a; Pluske et al., 2003) and the pig suffers a growth check at or around this time (Dunshea, 2003).

Porcine colostrum stimulates intestinal development in an organ culture system (Ma and Xu, 1997) as well as stimulating milk clotting activity and mucosal mitotic index *in vivo* (Mubiru et al., 1997). Similarly, bovine colostrum also increases small intestinal villus height and crypt depth despite decreasing overall small intestinal weight in early-weaned pigs (King et al., 2008). Piglets which were denied access to colostrum but received milk replacer supplemented with insulin-like growth factor-I (IGF-I) for 4 d had greater small intestinal weight and villus height at jejunum than their control counterparts (Burrin et al., 1996) suggesting a role for IGF-I in gut development. Colostrum supplementation increased IGF binding protein-3 (IGFBP3)

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Received September 21, 2009; Accepted January 13, 2011

in both sham and short bowel resectioned pigs but increased IGF-I in only the pigs undergoing bowel resectioning (Pereira-Fantini et al., 2008). Finally, Dunshea et al. (2002b) found that exogenous IGF-I and particularly long arginine-3 IGF-I (LR3IGF-I) can increase growth rate in artificially-reared neonatal pigs fed *ad libitum* but not in limit-fed neonates. Therefore, the hypothesis tested in the present study was that a colostrum product would increase growth and gut development in the artificially reared neonatal pig.

MATERIALS AND METHODS

Colostrum isolate

The colostrum isolate used in the present study was derived from bovine colostrum from which most of the immunoglobulins, lactose and fat had been removed. Briefly, the patented process involved pasteurisation of colostrum, followed by centrifugation to remove fat, biofugation to reduce bioburden, ultrafiltration to remove lactose and salts and finally spray drying. While the concentrations of the various growth factors in the colostrum isolate are incompletely defined, the content of a similar liquid product includes IGF-I (2 mg/kg), IGF-II (2 mg/kg), transforming growth factor β (25 μg/kg) and epidermal growth factor (6 μg/kg) (Playford et al., 1999).

Animals and handling

Sixteen (8 male and 8 female) cross-bred (Large White× Landrace) piglets were weaned from the 2 sows at 24 h of age after each pig had obtained colostrum from their dam. These pigs were injected with 100 mg Fe and had their teeth clipped. After weaning the pigs were transferred to individual wire mesh cages (0.5×0.4×0.5 m high) located in an insulated building in which the ambient temperature was maintained at 30°C with additional heat provided by a 175 watt infra-red lamp. Over the next 48 h pigs were trained to consume one of the two liquid diets (Table 1) through rubber teats attached to a plastic hose inserted into a plastic container. The two diets were based on either a colostrum protein isolate (n = 4 males and 4 females) or whey protein concentrate (n = 4 males and 4 females) and were formulated to contain equal levels of crude protein and amino acids. However, the diets were formulated to be protein deficient (19% of DM) similarly to sow's milk (Auldist et al., 1997) to allow the efficacy of growth factors present in the diets to be expressed. The lysine content was similar to that of sow's milk while the ratios of other essential amino acids to lysine were in excess of the ratios suggested by Chung and Baker (1992). To prepare the diets, the butter, oil and water were heated to 70°C. All dry ingredients were then added to the water and mixed together with anti-foaming agent. The melted butter and oil were then mixed with the water. The diets were homogenised and the vitamins and minerals thoroughly mixed in. Both diets were then frozen until required. Feed was provided *ad libitum* with the reservoirs being emptied, refusals weighed and fresh liquid diet provided twice per day. On day 20, 4 pigs exhibited symptoms of E.coli toxaemia and these pigs were dosed with 0.5 ml of penicillin (Norocillin LA, Norbrook Laboratories Gisborne, Australia) and an oral administration of scourban (BOMAC Laboratories, Auckland, New Zealand). All symptoms disappeared within 1 day. As, a precaution all pigs were dosed with 1.0 ml of Terramycin LA (Pfizer Animal Health, West Ryde, NSW, Australia).

The pigs were offered their diets until they were 4 weeks of age. At this time 3 males and 3 females from each diet group (allocated randomly at the start of the study) were euthanased with an intravenous injection of sodium pentabarbitone. Visceral tissues and organs were removed, the gastrointestinal tract emptied and various tissues and organs weighed. All pigs were bled via venipuncture for

Table 1. Composition of experimental diets (g/kg air-dry basis)

	Whey protein	Colostrum isolate
Ingredient		
Butter oil	60.0	60.0
Soybean oil	10.0	10.0
Skim milk powder	29.6	32.6
Whey protein concentrate	40.0	
Colostrum isolate		43.5
Lactose	40.0	40.0
Glucose	20.4	13.9
L-threonine		0.610
L-valine		0.004
DL-methionine		0.870
L-isoleucine		0.718
L-leucine		0.792
L-phenylalanine	0.305	
Lysine·HCl		1.197
L-histidine	0.073	
L-arginine	0.332	
Vitamins	0.5	0.5
Minerals	0.2	0.7
Water	798.1	794.6
Composition		
Total lysine [a]	17	17
Crude protein [b]	187	185
Gross Energy (GE) (MJ/kg) [a]	24.0	24.0
Lysine (g/MJ GE)	0.71	0.71

[a] Calculated for each diet from analyses of each ingredient.
[b] Determined on diets.

IGF-I and IGF-II analyses at 21 and 28 days of age.

Plasma analyses

Plasma was analysed for IGF-I and IGF-II by radioimmunoassay. IGF-I and -II were dissociated and separated from IGF-binding proteins by size exclusion liquid chromatography of plasma at pH 2.5 before assay (Owens et al., 1990). IGF-I and IGF-II RIAs were calibrated with porcine IGF-I and IGF-II (GroPep, Adelaide), respectively (Francis et al., 1989), and performed as previously described (Carr et al., 1991).

Statistical analyses

The study was designed as a 2×2 factorial with the respective factors being diet (colostrum protein isolate vs. whey concentrate) and sex (male vs. female). Growth performance and carcass data were analysed by ANOVA suitable for a 2×2 factorial with sow as a blocking factor. Plasma IGF-I and IGF-II data for days 21 and 28 were analysed using residual maximal likelihood (REML) analyses suitable for repeated measures as well as subject to multiple regression analyses. The incidence of pigs exhibiting signs of *E. coli* toxaemia on day 20 were analysed using a Chi-squared analyses (using either a yes or no criteria). All ANOVA and regression analyses were performed using Genstat© (8th edition, Lawes Agricultural Trust).

RESULTS

Piglets adapted to consuming liquid feed from the teat system very quickly and all pigs were using the system after 2 days of training. There was no effect of diet on daily gain over the first two weeks of the study (Table 2). However, daily gain was 40% higher in pigs consuming the diet containing colostrum isolate (212 vs. 298 g/d, p = 0.010) over the subsequent 2 week period. As a consequence, daily gain over the entire 4 weeks of the study was 26% higher in pigs consuming the colostrum isolate (171 vs. 216 g/d, p = 0.010). There were no effects of sex on daily gain over any period of the study.

Pigs tended to consume more of the liquid feed containing colostrum isolate (25.5 vs. 29.1 kg, p = 0.074) resulting in a significantly greater intake of air-dry matter (5.13 vs. 5.96 kg air-dry feed, p = 0.043) over the entire 4 week period (Table 2). Pigs consuming the diet containing the colostrum isolate tended to gain more live weight per unit of liquid feed (0.203 vs. 0.223 g/g, p = 0.056), although the body weight gain per unit of air-dry matter consumed was not evident (1.01 vs. 1.09 g/g, p = 0.12).

Pigs consuming the diet supplemented with colostrum isolate had a significantly higher weight full gut (445 vs. 554 g, p = 0.026), empty gut (356 vs. 463 g, p = 0.008), stomach (42.2 vs. 54.4 g, p = 0.001), small intestine (222 vs. 275 g, p = 0.025) and large intestine (63.7 vs. 98.0 g, p = 0.005) when compared to pigs consuming the diet supplemented with whey protein concentrate (Table 3). Female pigs tended to have a heavier large intestine than male pigs (74.0 vs. 92.3 g for males and females, respectively, p = 0.056). The small and large intestinal lengths were greater in pigs consuming the diet containing colostrum isolate. While there was no significant effect of diet on the weight to length ratio of either the small or large intestine, female pigs had a greater weight to length ratio in the large intestine than male pigs (0.43 vs. 0.51 g/cm for males and females, respectively, p = 0.044). As a proportion

Table 2. Effect of colostrum isolate and sex on growth performance of artificially reared pigs

Diet (D)	Whey protein		Colostrum isolate		SED[a]	Significance		
Sex (S)	Male	Female	Male	Female		Diet	Sex	D×S
Liveweight (kg)								
Initial	1.45	1.60	1.63	1.58	0.156	0.50	0.81	0.37
Final	6.42	6.90	7.95	8.20	0.420	0.016	0.42	0.80
Daily gain (g/d)								
0-2 weeks	137	130	137	149	7.6	0.36	0.59	0.36
2-4 weeks	196	229	295	302	21.4	0.010	0.36	0.61
0-4 weeks	165	176	210	221	10.6	0.010	0.28	0.97
Total feed Intake (kg)								
Liquid	24.6	26.5	29.0	29.1	1.75	0.074	0.59	0.63
Air dry	4.95	5.32	5.95	5.97	0.352	0.043	0.59	0.63
Feed efficiency (g/g)								
Liquid	0.20	0.20	0.22	0.23	0.009	0.056	0.70	0.47
Air dry	1.02	1.00	1.06	1.11	0.042	0.12	0.70	0.47

[a] Standard error of the difference for main effects of diet or sex. For standard error of the difference for the interaction between diet and sex multiply by 1.414. n = 4 for each diet×Sex cell.

Table 3. Effect of colostrum isolate and sex on gastrointestinal and visceral organs of artificially-reared pigs

Diet (D)	Whey protein		Colostrum isolate		SED[a]	Significance		
Sex (S)	Male	Female	Male	Female		Diet	Sex	D×S
Full gut (g)	417	508	583	573	37.0	0.026	0.33	0.23
Empty gut (g)	322	390	458	468	25.0	0.008	0.18	0.30
Stomach (g)	39.9	44.5	55.7	53.2	1.85	0.001	0.59	0.11
Caecum (g)	17.8	18.4	22.4	17.4	1.5	0.20	0.29	0.12
Liver (g)	248	278	348	363	18.7	0.004	0.28	0.74
Spleen (g)	27.1	25.9	24.4	22.0	4.2	0.47	0.68	0.90
Small intestine								
Weight (g)	210	250	287	289	16.4	0.017	0.25	0.30
Length (cm)	914	966	1,122	1,038	53.0	0.045	0.77	0.26
Weight:length (g/cm)	0.23	0.26	0.26	0.28	0.014	0.12	0.11	0.69
Weight:empty gut (g/g)	0.65	0.64	0.63	0.62	0.017	0.52	0.54	0.72
Large intestine								
Weight (g)	54.6	76.6	93.4	108.9	7.55	0.005	0.056	0.69
Length (cm)	132	161	214	213	13.8	0.005	0.37	0.32
Weight:length (g/cm)	0.41	0.49	0.45	0.53	0.031	0.22	0.044	0.96
Weight:empty gut (g/g)	0.17	0.20	0.21	0.24	0.008	0.006	0.016	0.93

[a] Standard error of the difference for main effects of diet or sex. For standard error of the difference for the interaction between diet and sex multiply by 1.414. n = 3 for each diet×sex cell.

of empty gut weight pigs fed the colostrum diet (0.18 vs. 0.22, p = 0.006) and females (0.19 vs. 0.22, p = 0.016) had heavier large intestines than their counterparts. Pigs fed the diet containing the colostrum isolate had heavier livers than pigs fed the diet containing whey protein concentrate (263 vs. 356 g, p = 0.004).

Plasma IGF-I (99 vs. 150 ng/ml, p<0.001) and IGF-II (265 vs. 406 ng/ml, p<0.001) concentrations were higher in pigs fed colostrum isolate. Plasma IGF-I increased between days 21 and 28 of treatment (115 vs. 134 ng/ml, p = 0.008). There was no effect of sex on either plasma IGF-I or IGF-II. A simple linear regression between plasma IGF-I and growth rate over the previous week suggests a significant positive correlation (R^2 = 0.25, p = 0.008). However, inclusion of dietary treatment and sow in the model suggests that much of the difference in plasma IGF-I is explained by the different dietary treatments. Thus, while the effect of previous growth rate on plasma IGF-I was still apparent (p = 0.066), there was an additional increase in IGF-I of 34 ng/ml due to the colostrum isolate (Figure 1).

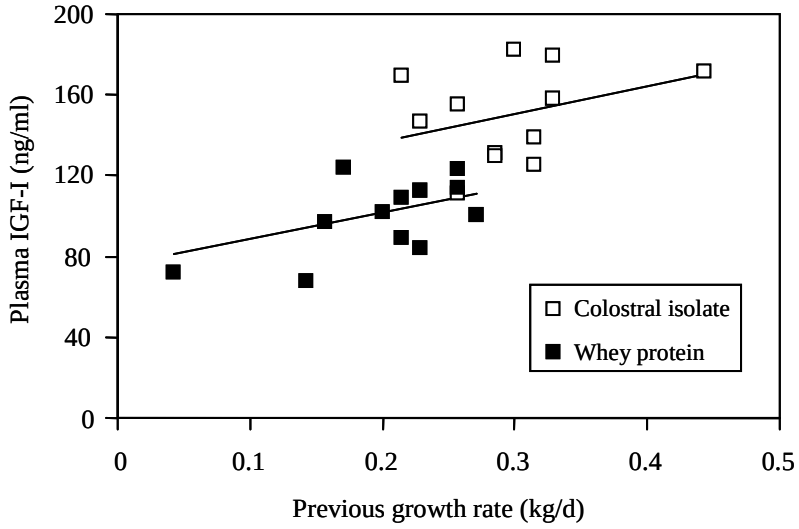

Figure 1. Relationship between plasma IGF-I and average growth rate over the week prior to blood sampling for pigs consuming liquid diets containing either colostral isolate or whey protein (n = 24). Regression equation is: IGF-I = 129.2 (SE = 21.5)+145.9 (75.1) Growth +36.3 (11.0) colostral isolate, R^2 = 0.67. Data have been adjusted for effect of sow.

Table 4. Effect of colostrum isolate and sex on plasma IGF-I and IGF-II concentrations at 4 weeks of age

Diet (D)	Whey protein		Colostrum isolate		SED[b]	Significance[a]		
Sex (S)	Male	Female	Male	Female		Diet	Sex	Day
Plasma IGF-I (ng/ml)								
Day 21	93	96	123	150	11.4	<0.001	0.22	0.008
Day 28	99	111	156	171				
Plasma IGF-II (mg/ml)								
Day 21	268	231	423	405	24.1	<0.001	0.17	0.95
Day 28	296	266	378	417				

[a] There were no significant interactions ($p<0.10$).
[b] Standard error of the difference for main effects of diet or sex. For standard error of the difference for effect of day divide by 2.285.

The incidence of pigs exhibiting signs of *E. coli* toxaemia on day 20 was significantly greater in pigs consuming the diet containing whey protein isolate (4/8 and 0/8 for pigs consuming the whey protein concentrate and the colostrum isolate diets, respectively; $x^2 = 5.33$, $p<0.05$). There was no effect of sex on the incidence of *E. coli* toxaemia.

DISCUSSION

Supplementation of a sow milk replacer with colostrum isolate increased growth rate over that of a commonly used protein, whey protein concentrate. As a result, pigs consuming the colostrum isolate weighed 1.4 kg more at 4 weeks of age than the pigs consuming the diet containing the whey protein concentrate. While this may not appear to be a large weight advantage, a 1.4 kg increase in live weight at around 3-4 weeks of age can be the equivalent of up to 1 weeks growth at slaughter at 5 months of age (Mahan et al., 1998). In studies where daily gain has been increased around weaning through liquid feeding, these increases in live weight have also resulted in increased live weight for age (Dunshea et al., 1999a,b; 2000; Kim et al., 2001). The increase in growth rate in pigs consuming a colostrum protein isolate is consistent with previous findings in pigs consuming bovine colostrum. For example, Dunshea et al. (2002c) found that pigs fed colostrum- and porcine plasma-based mash diets grew, 11% faster and ate 12% more food than pigs fed diets containing more conventional ingredients**.** In a New Zealand study conducted on a commercial piggery, Pluske et al. (1999) reported that 28-day-old weaned pigs fed a spray-dried bovine colostrum preparation for 10 days after weaning grew faster, an effect caused by increased voluntary feed intake. Pigs fed the colostrum powder were heavier at 14 days after weaning, an advantage maintained through to slaughter at 85 kg. In a subsequent study conducted on the same farm, King et al. (2001) found that pigs weaned at 28 days of age and fed diets containing either spray-dried bovine plasma or colostrum powder ate more and grew faster than pigs offered a diet without these products. On the other hand, King et al. (2008) found that there was no difference in the growth rate of weaned pigs supplemented with 5% colostrum powder in a solid diet. However, intestinal morphology and immune status were improved.

Supplementation with colostrum isolate also resulted in an increase in gut growth in the present study. Although, the increased gut growth was largely commensurate with the increase in live weight, the enhanced growth of the large intestine in pigs fed the colostrum isolate may confer an ability to adapt to solid diets with a higher fibre content. This in turn could reduce the post-weaning check that occurs when piglets are weaned from liquid to solid diets.

The increase in gut and whole body growth and plasma IGF-I may be due to growth factors in the colostrum isolate. Although not measured in the present study it is recognised that growth factors are present in relatively high quantities in colostrum and play an important part in gut development (Widdowson et al., 1976). The various growth factors that have been found to be abundant in colostrum include IGF-I, EGF, betacellulin, insulin and relaxin (Hammon et al., 2000; Bastian et al., 2001; Frankshun et al., 2009). The removal of immunoglobulins from the colostrum isolate used in the present study resulted in a further enrichment of the growth factor content. There is evidence that some component of colostrum, distinct from macronutrient composition, promotes gut growth and plasma IGF-I (Burrin et al., 1996; Wester et al., 1998). In the present study, plasma IGF-I was positively correlated with previous daily gain as has been demonstrated in sucking neonatal pigs (Dunshea et al., 1999b) or pigs consuming cows milk (Dunshea et al., 2002b). However, in pigs fed colostrum isolate there was an additional increase in plasma IGF-I (+36 ng/ml) at any liveweight. Wester et al. (1998) also found much higher plasma IGF-I concentrations in piglets fed a diet containing colostrum compared to piglets consuming milk replacer. Also, piglets that were not allowed access to colostrum but received milk replacer supplemented with IGF-I for 4 d had greater small intestinal weight and jejunum villus height than their control

counterparts (Burrin et al., 1996). Using lower doses of IGF-I, Xu et al. (1994) found no effect on piglet gut growth but a significant increase in the size of the pancreas of pigs receiving infant formula supplemented with IGF-I for 24 h. While IGF-I is relatively stable in the gastrointestinal lumen of sucking pigs (Shen and Xu, 2000), little IGF-I is absorbed intact from the intestine (Burrin et al., 1996; Donovan et al., 1996; Xu and Wang, 1996). Therefore, it is unlikely that absorption of IGF-I from the gut is the source of the increased circulating IGF-I observed in pigs fed the diet containing colostrum isolate. Therefore, the question remains as to whether exogenous IGF-I or other growth factors found in colostrum increase endogenous production of IGF-I. Colostrum does not appear to have any effect on ileal tissue IGF-I or IGF receptor concentrations or the expression of any of the binding proteins (Pereira-Fantini et al., 2008).

Certainly, IGF-I is implicated as either causative factor of, or a reporter of, the increased whole-body and gut growth observed in pigs fed colostrum or colostrum isolate. Systemic infusion of IGF-I and analogues increased whole body and gut growth in piglets consuming cows milk or nursing the sow (Shocknecht et al., 1997; Dunshea et al., 2002b). Also, skeletal muscle myofibrillar protein synthesis was increased by 28% in pigs fed colostrum as compared to pigs fed a formula matched for macronutrient content but devoid of growth factors (Fioretto et al., 2000). Dunshea et al. (1999b) provided evidence of a causal relationship between growth rate and plasma IGF-I and IGFBP-3, since both plasma IGF-I and IGFBP-3 were highly correlated with previous growth rate. Owens et al. (1994) showed that plasma IGF-I concentrations are more highly correlated with current than with future growth rate and suggested plasma IGF-I may be a reporter of, rather than a mediator of, growth performance in the pig. Indeed, these authors have further analysed a very large data set (n>3,500) and have found that plasma IGF-I concentrations at 5 weeks of age are negatively correlated with growth rate over the period from 19-24 weeks of age and positively related to backfat thickness at 24 weeks of age (Owens et al., 1997). Furthermore, Owens et al. (1999) reported that are positive relationships between circulating levels of insulin-like growth factor (IGF)-I and growth rate, circulating levels of IGF-I and gain:feed, and circulating levels of IGF-II and backfat depth. This implies that IGF-II plays a role in regulation of growth in postnatal pigs and that this role is different from that of IGF-I. Thus, the increase in plasma IGF-I and IGF-II in pigs consuming colostrum isolate may be a reflection of increased growth or metabolic activity in these pigs.

Gut development and growth in the piglet is essential for efficient nutrient absorption and protection against bacterial invasion. For example, the small intestine is the route of absorption of many enteric bacteria and is also the site of inflammation caused by these bacteria and the weight of the small intestine was increased by the colostrum protein isolate in the present study. Therefore, it is interesting to note that there was no incidence of *E. coli* toxaemia in pigs fed the diet containing the colostrum isolate whereas half the pigs consuming the diet containing whey protein concentrate exhibited symptoms of *E. coli* toxaemia.

Another interesting observation from this study was the greater large intestinal mass and thickness in female pigs when compared to male pigs. Although this increased large intestinal development was not associated in significantly greater growth rate in the female pigs, we have consistently shown that female pigs eat more and grow more quickly than male pigs during the first week or so after weaning (Dunshea et al., 1999a;2003; Dunshea, 2001). Also, female pigs appear to have a more developed gastrointestinal system and pancreatic enzymic capacity than male pigs when weaned at either 14 or 28 days of age (Pluske et al., 2003). Others have found that neonatal male rats were more susceptible than female rats to early dietary deficiencies, retardation and handling stresses (Crutchfield and Dratman, 1980). Therefore, it may be possible that the protein deficient diets used in the present study to allow the expression of any growth-promoting activity of the colostrum isolate were better handled by female pigs.

IMPLICATIONS

The growth factor content of sow's milk decreases with advancing lactation and so an opportunity exists to enhance gut growth and development through supplementation with exogenous growth factors. An efficacious method of incorporating growth factors into the diet of neonatal pigs is by dietary supplementation with colostrum isolate. Pigs consuming colostrum isolate ate more, grew faster and had higher plasma IGF-I concentrations than pigs consuming a diet with similar macronutrient content but devoid of growth factors.

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