Effects of Biocom as a Replacement of Glutamine on Performance and Blood Biochemical Indexes of Early Weaned Piglets

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ABSTRACT : The objective of this study was to evaluate Biocom (a protein source containing a high level of glutamine and alanyl-glutamine) as a replacement for glutamine (Gln) in nursery pig diets. Forty-two pigs (fourteen pigs per treatment) weaned at 28 d of age were used in a 28-d performance trial using three dietary treatments: control (no Gln), control supplemented with Gln or Biocom. The control diet was composed of corn, soybean meal, whey and fish meal. Individual body weight, pen feed disappearance and diarrhea were monitored. On d 0, 2, 7 and 14 postweaning, respectively, five pigs per treatment were selected and bled from the anterior vena cava to obtain five replicate samples of blood on each dietary treatment for determination of blood biochemical index. Dietary supplementation of Gln and Biocom did not influence performance, plasma Gln and total serum protein concentration ($p>0.05$). However, the addition of Gln and Biocom could prevent serum urea nitrogen and serum cortisol from increasing on d 2 postweaning ($p<0.05$). There were no significant differences ($p>0.05$) in any of the examined parameters between Gln- and Biocom-supplemented diets. In conclusion, dietary Gln did not influence the performance of early-weaned piglets owing to the complex diet containing whey, but could prevent the increase of serum urea and cortisol. Biocom could be used as a replacement for free pure Gln without any negative effect on early-weaned piglets. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 6 : 872-876)***

Key Words : Glutamine, Alanyl-glutamine, Biocom, Performance, Biochemical Indexes, Weanling Pigs

INTRODUCTION

Weaning, the transition from the ingestion of maternal milk to solid foods, often causes abnormalities in intestinal morphology. The alterations include decrease in villus height and increase in crypt depth (Hampson, 1986). As a result, weaned pigs at early age often suffer from diarrhea and reduced growth performance during d 7 to d 14 postweaning. One of the reasons is that weaning stops the supply of glutamine (Gln) from maternal milk (Pluske et al., 1997).

Gln has important and unique metabolic functions, and is considered to be a conditionally essential amino acid in some species (Lacey and Wilmore, 1990; NRC, 1998). Gln is an abundant free amino acid in the plasma of animals (Souba, 1991) and in sow's milk (Wu and Knable, 1994). Gln serves as an essential precursor for the synthesis of proteins, purine and pyrimidine nucleotides, NAD^+ and aminosugars (Krebs, 1980). Gln is also an important fuel source for enterocytes (Windmueller and Spaeth, 1980; Wu et al., 1995).

Biocom (Century Feed Technology Development CO. LTD, Hangzhou, China) is a protein source produced in an enzymatic hydrolysis process. It contains 40% crude protein and has high content of the amino acid Gln and glutamine dipeptide (alanyl-glutamine) (Table 1). Dietary Gln supplement can prevent small intestinal atrophy in 21 d old pigs (Wu et al., 1996; Lee et al., 2003) and in 28 d old pigs (Liu et al., 2002), increase the absorption of sodium in 21 d pigs suffering from rotavirus infection (Rhoads et al., 1990), and maintain muscle Gln level under *E. coli* infection (Yoo et al., 1997). These results indicate Gln is an important nutrient for the early-weaned pigs. However, the effects of dietary Gln on performance of weaned pigs is inconsistent (Wu et al., 1996; Liu et al., 1999; Kitt et al., 2002; Lee et al., 2003; Lai et al., 2004).

The objective of the present study was to investigate the effect of dietary Gln supplementation on performance and blood biochemical indexes of nursery pigs and to evaluate the feasibility of using Biocom to substitute for free Gln in nursery pig diets.

MATERIALS AND METHODS

Animals and diets

A basal diet used as the control was formulated based on corn-soybean meal and whey, to contain 3,386 kcal DE/kg, 18.9% crude protein, 1.33% lysine, 0.36% methionine and 0.82% threonine, and to meet or exceed NRC (1998) requirements for all other nutrients (Table 2). The basal diet plus 0.7% free L-Gln (Shanghai Biochemical Research Institution, 031105, chemical pure) or 1% Biocom created the other two dietary treatments. Forty-two pigs (Landrance×Yorkshire, average initial live weight 8.37±0.13 kg) weaned at 28 d of age were assigned, based

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Table 1. Composition of Biocom[1]

Nutrients as analysis (%)	Biocom
Digestable energy (kcal kg^{-1})	3,430
Crude protein	40.00
Lysine	2.22
Methionine	0.56
Methionine+cystine	1.15
Threonine	1.63
Tryptophan	0.62
Glutamine	21.10
Alanyl-glutamine	2.65

[1] Values for glutamine and alanyl-glutamine were measured by reverse phase HPLC, and values for the other nutrients were supplied by the supplier of Biocom (Century Feed Technology Development CO. LTD, Hangzhou, China).

upon weight and sex, to the three dietary treatments with 14 pigs in each treatment and 7 pigs (3 barrows and 4 gilts or 3 gilts and 4 barrows) per pen as a replicate.

Animal husbandry and chemical analysis

All the experimental pigs were housed in the same nursery room with mesh flooring. Each pen has a self-feeder and nipple waterer to allow *ad libitum* consumption of both feed and water. The total experiment was conducted in four phases (Phase 1 = d 0 to 7; Phase 2 = d 7 to 14; Phase 3 = d 14 to 21; Phase 4 = d 21 to 28).

Pigs were individually weighed on d 0, 7, 14, 21 and 28, respectively. Feed intake was recorded daily for each pen. Average daily gain, average daily feed intake and feed:gain ratio were calculated in each phase. Diarrhea was monitored daily throughout the experiment and diarrhea index (DI) was calculated using the following equation: DI (%) = (accumulated number of pigs experiencing diarrhea)/(number of pigs×period)×100.

On d 0, 2, 7 and 14, respectively, five pigs per treatment were selected and bled from the anterior vena cava to obtain five replicates of blood samples in each dietary treatment. Blood samples (3 ml) were centrifuged at 3,000 rpm for 20 min in order to obtain serum. The serum was stored at -20°C and then thawed prior to analysis for blood urea nitrogen, total protein and cortisol. Blood samples (2 ml) were centrifuged at 3,000 rpm for 5 min in order to obtain plasma. Plasma (1 ml) was deproteined with an equal volume of 1.5 M perchloric acid and neutralized with 0.5 ml of 2 M K_2CO_3.

Plasma Gln was analyzed by fluorometric high performance liquid chromatogram method involving precolumn derivatization with o-phthaldialdehyde. Serum urea nitrogen and total protein were measured using kit (Jiancheng Bioengineering Institution, Nanjing, China) and an automatic biochemical analyzer (RT224, BIOTECNICA). Serum cortisol was measured using RIA kit (Navy Radioimmunity Technological Center, Beijing) and radioimmunity analyzer (Rihuan Photoelectric Instrument Co. Ltd., Shanghai, China).

Statistical analysis

Data were statistically analyzed using the one-way Analysis of Variance models procedure of the SAS statistical package (SAS, 1989). The experimental units were the mean value of a pen and a pig for performance and blood parameters, respectively. Duncan's multiple range test was used to separate means when significant ($p<0.05$) effect was detected (Duncan, 1955). Values in the text were mean±SE.

RESULTS

Performance and diarrhea index

Effects of supplemental Gln and Biocom on performance and diarrhea in weaned pigs were presented in

Table 2. The formulation and composition of diets

Ingredients (%)	Control	Control+ Gln[2]	Control+ Biocom	Nutrients as calculation (%)	Control	Control+ Gln[2]	Control+ Biocom
Corn	60.15	59.45	60.15	DE (kcal/kg)[3]	3,386	3,386	3,386
Soybean meal	23.00	23.00	22.00	Crude protein	18.90	18.90	18.90
Dried whey	10.00	10.00	10.00	Calcium	0.81	0.81	0.81
Fish meal	4.00	4.00	4.00	Total phosphorus	0.66	0.66	0.66
Limestone	0.70	0.70	0.70	Available phosphorus	0.31	0.31	0.31
Dicalcium phosphate	1.00	1.00	1.00	Lysine	1.30	1.30	1.30
Salt	0.30	0.30	0.30	Methionine	0.44	0.44	0.44
L-lysine	0.25	0.25	0.25	Methionine+cystine	0.76	0.76	0.76
Methionine	0.10	0.10	0.10	Threonine	0.76	0.76	0.76
Premix[1]	0.50	0.50	0.50				
Gln[2]	-	0.70	-				
Biocom	-	-	1.00				

[1] Provided per kilogram diet: Fe,150 mg; Cu, 250 mg; Zn, 200 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.5 mg; Vitamin A, 11,250 IU/kg; Vitamin D_3, 2,500 IU/kg; Vitamin E, 20 mg/kg; Vitamin K_3, 2.5 mg/kg; Vitamin B_1, 2.5 mg/kg; Vitamin B_2, 6.0 mg/kg; Vitamin B_6, 3.0 mg/kg; Vitamin B_{12}, 0.08 mg/kg; D-biotin, 0.1 mg/kg; D-pantothenic acid, 12.5 mg/kg; Folic acid, 1.25 mg/kg; Nicotinic acid, 25 mg/kg; ethoxyquin, 0.13 mg/kg.
[2] Gln = free L-Gln (Shanghai Biochemical Research Institution, 031105, chemical pure).
[3] DE = digestible energy.

Table 3. Effects of glutamine and Biocom on performance and diarrhea in weaned pigs[1]

Experimental period	Measured parameters	Control	Control+0.7% Gln	Control+1.0% Biocom
days o to 7	Average daily gain (g)	185.42±28.36	187.50±39.22	135.17±25.14
	Daily feed intake (g)	202.37±9.48	198.1±7.33	193.39±11.51
	Feed:gain ratio	1.36±0.17	1.37±0.48	1.77±0.25
	Diarrhea index (%)	14.50	10.00	11.50
days 7 to 14	Daily gain (g)	398.82±35.93	432.96±29.88	378.02±54.96
	Daily feed intake (g)	516.14±38.00	537.31±13.79	499.22±33.31
	Feed:gain ratio	1.32±0.03	1.26±0.09	1.32±0.02
	Diarrhea index (%)	6.00	4.02	10.01
days 14 to 21	Daily gain (g)	501.20±45.67	519.81±28.68	490.12±33.30
	Daily feed intake (g)	755.15±136.75	813.33±13.28	717.44±12.24
	Feed :gain ratio	1.51±0.27	1.56±0.05	1.46±0.23
	Diarrhea index (%)	13.50	9.50	10.00
days 21 to 28	Daily gain (g)	629.76±51.13	676.91±46.02	567.05±28.54
	Daily feed intake (g)	1108.73±30.11	1104.36±50.22	1020.1±43.26
	Feed:gain ratio	1.75±0.23	1.64±0.21	1.81±0.03
	Diarrhea index (%)	11.50	4.00	12.50
days 0 to 28	Daily gain (g)	420.41±22.70	445.14±19.41	379.04±28.61
	Daily feed intake (g)	590.79±45.45	701.04±7.74	616.19±50.23
	Feed:gain ratio	1.49±0.08	1.46±0.16	1.59±0.01
	Diarrhea index (%)	14.50	8.00	14.50

[1] The number of animals per treatment is 14.

Table 4. Effects of glutamine and Biocom on plasma glutamine concentration of weaned pigs[1]

Item	Control	Control+0.7% Gln	Control+1.0% Biocom
Glutamine concentration (μmol/L)			
day 0	362.82±6.03	362.82±6.03	362.82±6.03
day 2	163.21±14.31	231.22±8.60	189.44±15.61
day 7	241.41±22.04	270.61±9.37	251.20±10.10
day 14	250.81±8.63	311.40±9.53	264.64±2.62

[1] The number of animals per treatment is 5.

Table 3. Compared to the control, the supplementation of Gln or Biocom didn't result in a significant differences ($p>0.05$) in average daily gain, average daily feed intake and feed:gain ratio. However, pigs fed Gln-supplemented diet had numerically higher ($p>0.05$) feed intake and growth rate than those on the control or Biocom-supplemented diet during the total period.

Pigs on diet with Gln addition had the lowest diarrhea among the three dietary treatments during each phase though no significant differences ($p>0.05$) were observed.

Blood biochemical indexes

Plasma glutamine : Effects of supplemental Gln and Biocom on plasma Gln concentration of weaned pigs were presented in Table 4. The plasma Gln concentration of pigs in the three dietary treatments was all decreased on d 2 postweaning, but pigs fed Gln-supplemented diet had the lowest reduction in the plasma Gln concentration. Pigs fed Biocom-supplemented diet also had a slightly higher concentration of plasma Gln ($p>0.05$) than those in the control on d 2, 7 and 14 postweaning.

Table 5. Effects of glutamine and Biocom on serum biochemical index of weaned pigs

Item	Control	Control+0.7% Gln	Control+1.0% Biocom
Total protein (g/L)			
day 0	60.59±0.25	60.59±0.25	60.59±0.25
day 2	59.14±0.61	59.53±0.43	59.49±0.08
day 7	59.87±0.18	59.94±0.29	59.80±0.46
day 14	59.94±0.32	60.28±0.26	60.06±0.27
Urea nitrogen (mmol/L)			
day 0	3.77±0.65^{a}	3.77±0.65^{a}	3.77±0.65^{a}
day 2	7.13±0.51^{b}	4.95±0.92^{a}	5.13±0.89^{ab}
day 7	6.81±0.59^{b}	4.25±0.36^{a}	4.86±0.51^{ab}
day 14	5.23±0.75^{ab}	3.19±0.50^{a}	3.54±0.33^{a}
Cortisol (μg/L)			
day 0	57.83±5.72^{a}	57.83±5.72^{a}	57.83±5.72^{a}
day 2	94.81±13.3^{b}	56.70±10.50^{a}	59.70±9.86^{ab}
day 7	67.26±2.58^{ab}	50.66±4.88^{a}	59.79±4.64^{a}
day 14	46.92±2.17^{a,b}	42.48±6.86^{a}	46.78±4.68^{a}

$^{a, b}$ Means within a criterion with no common superscripts in same column (row) differ significantly ($p<0.05$).

Serum biochemical indexes : Effects of supplemental Gln and Biocom on the content of total serum protein, urea nitrogen and cortisol were presented in Table 5. No significant differences ($p>0.05$) were observed in the content of total serum protein among the three treatments on any tested day.

Serum urea nitrogen concentration significantly increased ($p<0.05$) in the control on d 2 and 7 postweaning compared to that tested on d 0, but little change took place in pigs fed Gln or Biocom inclusion diet. Subsequently, pigs fed Gln-supplemented diet had a significantly lower ($p<0.05$) concentration of serum urea nitrogen than those in the control on d 2 and 7 postweaning. However, there was no difference ($p>0.05$) in the concentration of serum urea nitrogen between Gln and Biocom.

Serum cortisol concentration in pigs fed the control diet increased by 64% on d 2 postweaning compared to that on d 0. Little change took place in pigs fed Gln- or Biocom-supplemental diet and the difference in the concentration of serum cortisol approached statistical significance ($p<0.05$) between the control and the Gln inclusion diet on d 2 postweaning. On d 7 and 14 postweaning, no significant ($p>0.05$) differences were observed in the serum cortisol concentration among the three treatments.

DISCUSSION

Wu et al. (1996) reported that dietary Gln supplementation prevented jejunal atrophy during the first week postweaning and increased the gain:feed ratio by 25% during the second week postweaning in pigs weaned at 21 d of age. Liu et al. (1999) reported that dietary Gln supplementation prevented jejunal atrophy and increased the weight gain during the first week postweaning in pigs weaned at 28 d of age. Lackeyram et al. (2001) also reported that 0.8% Gln supplementation in corn-soybean meal based diets was effective in enhancing body weight gain, the small intestine and other visceral organ growth of piglets weaned at 10-d old during a 12 d study. However, No improvement in performance was observed in this study. The result is similar to those reported by Lee et al. (2003). In their study dietary Gln supplementation did not influence performance of pigs weaned at 21 d of age. This may be due to the diet containing 10% whey. Kitt et al. (2002) also reported that supplementation of Gln to a simple diet resulted in improved feed efficiency in pigs, compared to Gln supplementation to a complex diet containing whey, plasma and fish meal. There was also no significant difference ($p>0.05$) in performance between the pigs (28 d-weaned) fed Gln-supplemented diet and those fed Biocom-supplemented diet, These results indicated dietary Gln and Biocom had similar effect on pigs weaned at 28 d of age.

Dietary Gln supplement did not influence the concentrations of plasma Gln on d 14 or d 28 postweaning (Lee et al., 2003). Yoo et al. (1997) reported that the plasma Gln concentration was unchanged by the addition of 4% Gln for pigs weaned at 21 d of age. In the present study, the plasma Gln concentration was also not significantly ($p>0.05$) affected by the addition of Gln or Biocom. Because dietary Gln is a major fuel for the small intestinal mucosa and essential precursors for intestinal synthesis of glutathione, nitric oxide, polyamines, purine and pyrimidine nucleotides, and amino acids (such as alanine, citrulline and proline), and is obligatory for maintaining the intestinal integrity and intestinal mucosal mass (Wu, 1998). The demands for Gln arise during the transition to weaning, and in response to intestinal injury (Lobley et al., 2001). So the plasma Gln concentration of pigs decreased in the first week postweaning, and then increased.

No significant differences ($p>0.05$) were observed in the concentration of total serum protein among the three dietary treatments in this study. House et al. (1994) found that in 3-d-old piglets supplemented with 10 g of Gln per 100 g of parenteral amino acids, body weight tended to increase but total protein, fat and ash were unaltered. It is reported that 4.5% Gln supplementation in total parenteral nutrition (TPN) increased villus height and villus absorptive area in the jejunum but did not alter protein or DNA mass of 4-d-old neonatal piglets (Burrin et al., 2000). In current experiment, the content of serum urea nitrogen increased greatly in control pigs on d 2 postweaning. Flynn and Wu (1997) reported that plasma concentrations of urea were 127% greater in postweaning pigs than in sucking pigs. This indicated an increase in the conversion of ammonia into urea in the body. Serum urea concentration in pigs fed Gln- or Biocom-supplemented diet was lower than in control pigs, which suggested that dietary Gln might inhibit the conversion of ammonia into urea.

Weaning in pigs is associated with increased plasma concentrations of cortisol (hydrocortisone), the major circulating glucocorticoid in pigs (Worsae and Schmidt, 1980). This steroid hormone is a potent inducer of hepatic urea cycle enzymes including arginase (Morris, 1992). In this study serum cortisol concentration increased to a peak on d 2 postweaning and then decreased to the preweaning value, and this was consistent with the report by Wu et al. (2000), who reported that plasma cortisol concentrations increased to a peak value on d 2 postweaning in pigs weaned at 21 d of age, and then declined to the preweaning value on d 7 postweaning. The concentration of cortisol of pigs in the control was higher ($p<0.05$) than that of pigs in the other two treatments on d 2 postweaning, so it could be concluded that Gln supplementation could prevent cortisol increasing.

In conclusion, dietary Gln could prevent the increase of serum urea nitrogen and cortisol in early-weaned pigs, but did not influence the plasma Gln concentration and total serum protein content. The dietary addition of Gln did not improve the performance of early-weaned piglets due to the complex diet containing 10% whey. Biocom could be used as a replacement of free pure Gln to inclusion in weanling piglet diet without resulting in any negative effect.

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