L-glutamine and L-glutamate in diets with different lactose levels for piglets weaned at 21 days of age

Juliano Pelição Molino[1], Juarez Lopes Donzele[2], Rita Flávia Miranda de Oliveira[2], Alysson Saraiva[3], Douglas Haese[4], Eduardo Ianino Fortes[1], Matheus Faria de Souza[1]

[1] Mestrando em Zootecnia, DZO, UFV.
[2] Departamento de Zootecnia/UFV.
[3] Doutorando em Zootecnia, DZO, UFV.
[4] Departamento de Zootecnia/UVV.

ABSTRACT - This study was developed to evaluate the effects of adding L-glutamine and L-glutamate in diets with no lactose or with 4.0 or 8.0% lactose inclusion for piglets weaned at 21 days old. One hundred and eight piglets with initial weight of 6.12±0.70 kg were allotted in a complete randomized block design, in a 3 × 2 factorial arrangement, with six treatments, six replicates, and three piglets per experimental unit. The experimental diets were supplied from 21 to 35 days. From 36 to 49 days, animals received the same diet with no lactose inclusion, but the animals in the treatments with L-glutamine + L-glutamate in the previous phase continued to receive diets containing these ingredients. There was no interaction between the level of lactose and the inclusion of L-glutamine + L-glutamate on the parameters evaluated. The levels of lactose did not affect the performance of piglets in either of the two periods. Adding L-glutamine and L-glutamate in the diet positively influenced the weight gain of pigs from 21 to 49 days of age and increased the villous height in the duodenum, jejunum and ileum. Inclusion of L-glutamine + L-glutamate in diets for piglets weaned at 21 days of age improves the performance and the intestinal mucosa morphology, regardless of lactose addition.

Key Words: amino acids, crypt depth, glutamic acid, villous height

Introduction

The association of all the stressors related to early weaning of piglets results in reduction in daily feed intake, especially during the first week. The interruption of feed intake in this phase results in intestinal villous atrophy and increased crypt depth (Pierce et al, 2005).

According to Pinheiro & Machado (2007), the performance of piglets in the first week after weaning is strongly correlated with the weight at the end of the nursery phase, and the number of days required to reach slaughter weight.

Some nutritional strategies for piglets at this stage have been taken to mitigate the harmful effects of weaning, including the use of lactose, whose benefit is mainly linked to the increase and maintenance of feed consumption due to the improved palatability of the feed (Bertol et al., 2000). However, diets containing lactose have their cost increased due to the high price of this ingredient, which is also used in human food. Thus, it is necessary to look for ingredients that, like lactose or by different mechanisms, can minimize the detrimental effects of early weaning.

The amino acid glutamine constitutes an ingredient with the potential to improve the performance of piglets through its action in the maintenance of the small intestine mucosal structure (Yi et al., 2005), in addition to its role as precursor of proteins, nucleotides and polyamines (Sources et al., 2007). The action of this amino acid, termed conditionally essential, is related to the multiplication of cells of intense development, such as enterocytes of the intestinal mucosa and immune cells (Abreu & Donzele, 2008; Wu et al., 1996).

Thus, there is a need to evaluate the effect of adding L-glutamine and L-glutamate in diets containing different levels of lactose on the performance and intestinal mucosa structure of piglets weaned at 21 days.

Material and Methods

The experiment was conducted in the Setor de Suinocultura do Departamento de Zootecnia da Universidade Federal de Viçosa. One hundred and eight hybrid piglets weaned at 21±1 day of age and weighing 6.12±0.70 kg were allotted in a completely randomized block design with six treatments, six replicates, and three piglets per experimental unit. Weaning weight and piglets' kinship were considered as criteria in the blocks formation.

Treatments consisted of diets containing 0.0, 4.0, and 8.0% lactose, supplemented with 0.8% L-glutamine + L-glutamate (L-L-Glu + Gln), from 21 to 35 days. From 36 to 49 days, all piglets were fed the same diet free of lactose, and those that were fed 0.80% L-glutamine + L-glutamate from 21 to 35 days received 0.60% L-glutamine + L-glutamate. The experimental diets from 21 to 35 days (Table 1) and from 35 to 49 days (Table 2) were formulated to meet or exceed the requirements of pigs at the pre-starter phase (7 to 15 kg) for all nutrients (Rostagno et al., 2005).

Piglets were housed in suspended metal cages with slatted floors, semi-automatic feeders and nipple drinkers, located in a facility with concrete floor, wood ceiling and covered with ceramic tile. The room temperature was kept at 28 °C, using hoods and/or heaters when needed.

For evaluation of performance, piglets were weighed at the beginning (35 days) and end of the experiment (49 days),

and feed leftovers were collected and weighed daily when the animals reached 35 and 49 days, i.e., at the end of each period, for calculation of average daily feed intake (ADFI), average daily gain (ADG) and feed conversion (FC).

At 49 days of age, one piglet from each experimental unit, with body weight closest to the average weight of the cage was slaughtered. Samples of 2 cm in length were taken from the regions corresponding to 4, 50 and 96% of the small intestine, corresponding respectively to the duodenum, jejunum and ileum. The collected material was sent to the Laboratório de Histologia do Departamento de Medicina Veterinária da Universidade Federal de Viçosa. Histological cuts were washed in physiologic solution, fixed in bouin for a 24-h period, dehydrated in ethyl alcohol, diaphanized in xylol, and included in paraffin. Each slide contained two 5 µm thick cuts. Slides were put back in xylol solution for removal of paraffin excess and then rehydrated. The pigments

Table 1 - Composition and nutritional values of experimental diets fed to piglets from 21 to 35 days of age

	Experimental diets[1]					
Ingredient	Lac 8	Lac 8 + L-Gln + L-Glu	Lac 4	Lac 4 + L-Gln + L-Glu	Lac 0	Lac 0 + L-Gln + L-Glu
Corn	46.168	45.363	50.977	50.172	53.126	51.825
Soybean extruded	19.000	19.000	18.000	18.000	23.500	24.000
Soybean meal 45%	17.000	17.000	17.000	17.000	17.000	17.000
Crystalline lactose	4.080	4.080	0.000	0.000	0.000	0.000
Milk powder	7.700	7.700	7.700	7.700	0.000	0.000
L-Gln + L-Glu[2]	0.000	0.800	0.000	0.800	0.000	0.800
Blood plasma	2.000	2.000	2.000	2.000	2.000	2.000
Soybean oil	0.190	0.190	0.450	0.450	0.000	0.000
Dicalcium phosphate	1.760	1.760	1.760	1.760	1.970	1.970
Limestone	0.573	0.573	0.576	0.576	0.640	0.642
Salt	0.336	0.336	0.336	0.336	0.410	0.410
Vitamin premix[3]	0.400	0.400	0.400	0.400	0.400	0.400
Mineral premix[4]	0.240	0.240	0.240	0.240	0.240	0.240
Growth promoter[5]	0.050	0.050	0.050	0.050	0.050	0.050
Antioxidant[6]	0.010	0.010	0.010	0.010	0.000	0.010
L–Lysine HCl	0.258	0.260	0.272	0.274	0.395	0.385
DL–Methionine	0.140	0.143	0.133	0.136	0.164	0.164
L–Threonine	0.080	0.080	0.080	0.080	0.080	0.080
L–Tryptophan	0.015	0.015	0.016	0.016	0.025	0.024
Total	100	100	100	100	100	100
Nutritional calculated composition						
ME (Mcal/kg)	3400	3400	3400	3400	3400	3400
Crude protein (%)	23.93	23.35	22.96	23.38	22.78	23.32
Lactose (%)	8.00	8.00	4.00	4.00	0.00	0.00
Digestible lysine (%)	1.400	1.400	1.400	1.400	1.400	1.400
Digestible met + cys (%)	0.784	0.784	0.784	0.784	0.784	0.784
Digestible threonine (%)	0.882	0.882	0.882	0.882	0.882	0.882
Digestible tryptophan (%)	0.266	0.266	0.266	0.266	0.266	0.266
Calcium (%)	0.850	0.850	0.850	0.850	0.850	0.850
Total phosphorus (%)	0.669	0.669	0.669	0.669	0.669	0.669
Available phosphorus (%)	0.500	0.500	0.500	0.500	0.500	0.500

[1] Lac 8 = 8% lactose, Lac 4 = 4% lactose, Lac 0 = 0% lactose, L-Gln + L-Glu = L-glutamine + L-glutamate.
[2] Commercial product Aminogut®
[3] Provided per kg of the product: vitamin A - 12,000 IU; vitamin D3 - 2,250 IU; vitamin E - 27 mg; vitamin K - 3 mg; thiamin - 2.25 mg; riboflavin - 6 mg; pyridoxine - 2.25 mg; vitamin B12 - 27 mcg; folic acid - 400 mcg; biotin - 150 mcg; pantothenic acid - 22.5 mg; niacin - 45 mg; selenium - 300 mcg.
[4] Provided per kg of the product: calcium - 112 g (minimum); phosphorus - 27.0 g (minimum); sodium - 58.5 g; iron - 1,820 mg; zinc - 2,049 mg; copper - 2,126 mg; manganese - 836 mg; iodine - 29.5 mg; fluorine - 485 mg (maximum); selenium - 8 mg; cobalt - 3.6 mg.
[5] Tylosin.
[6] Butil hydrox toluene.

Table 2 - Composition and nutritional values of experimental diets fed to piglets from 35 to 49 days of age

Ingredient	Experimental diets	
	Basal diet	Basal diet + L-Gln + L-Glu
Corn	57.786	57.180
Soybean extruded	18.000	18.00
Soybean meal 45%	17.000	17.00
L-Gln + L-Glu[1]	0.000	0.600
Blood plasma	2.000	2.000
Soybean oil	0.950	0.950
Dicalcium phosphate	1.990	1.990
Limestone	0.660	0.660
Salt	0.300	0.300
Vitamin premix[2]	0.400	0.400
Mineral premix[3]	0.240	0.240
Growth promoter[4]	0.050	0.050
Antioxidant[5]	0.010	0.010
L–Lysine HCl	0.326	0.328
DL–Methionine	0.166	0.168
L–Threonine	0.103	0.105
L–Tryptophan	0.019	0.019
Total	100.00	100.00
Nutritional calculated composition		
ME (Mcal/kg)	3400	3400
Crude protein (%)	21.34	21.03
Digestible lysine (%)	1.250	1.250
Digestible met + cys (%)	0.750	0.750
Digestible threoine (%)	0.810	0.810
Digestible tryptophan (%)	0.240	0.240
Calcium (%)	0.850	0.850
Total phosphorus (%)	0.690	0.688
Available phosphorus (%)	0.500	0.500

[1] Commercial product Aminogut®.
[2] Provided per kg of the product: vitamin A - 12,000 IU; vitamin D3 - 2,250 IU; vitamin E - 27 mg; vitamin K - 3 mg; thiamin - 2.25 mg; riboflavin - 6 mg; pyridoxine - 2.25 mg; vitamin B12 - 27 mcg; folic acid - 400 mcg; biotin - 150 mcg; pantothenic acid - 22.5 mg; niacin - 45 mg; selenium - 300 mcg.
[3] Provided per kg of the product: calcium - 112 g (minimum); phosphorus - 27.0 g (minimum); sodium - 58.5 g; iron - 1,820 mg; zinc - 2,049 mg; copper - 2,126 mg; manganese - 836 mg; iodine - 29.5 mg; fluorine - 485 mg (maximum); selenium - 8 mg; cobalt - 3.6 mg.
[4] Tylosin.
[5] Butil hidrox toluene.
L-Gln + L-Glu = L-glutamine + L-glutamate; Me = metabolizable energy; met + cys = methionine + cystine.

used were hematoxylin and eosin. After staining, the slides were dehydrated again. For the morphological readings a OLYMPUS BX50 optical microscope (40 × magnification) coupled to the image analyzer "Image-Pro Plus 1.3.2" (1994) was used. Thirty villi and their respective 30 crypts were selected, well targeted, longitudinally sectioned, and had their heights measured. The villous:crypt ratio was also computed.

On the day of slaughter, samples of intestinal content of pigs in the colon proximal were collected in sterile containers and frozen immediately. Samples were subsequently sent to the Laboratório de Microbiologia Industrial do Departamento de Microbiologia da Universidade Federal de Viçosa. The total DNA extraction of the digesta was done by following the protocol described by Lu et al. (2003) with some modifications made in the Laboratório de Microbiologia Industrial do Departamento de Microbiologia da Universidade Federal de Viçosa. To amplify the rDNA 16S gene, corresponding to total Eubacteria, universal primers of Eubacterias with the following sequences of nucleotides were used: primer 2: 5'ATTACCGCGGCTGCTGG3' and p r i m e r : 5 ' C G C C C G C C G C G C G C G G C G G G CGGGGCGGGGGCACGGGGGGGCCTACGGGAGGCAGCAG3' (Muyzer et al., 1993). To amplify the rDNA 16S region corresponding to Lactobacillus spp. specific primers with the sequences were used as follows: CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCGCCC C T T T A Y G C G A A C A Y Y T R G G K G T CCAAACATCACVCCRACTT. The intestinal microbiota genetic profile was assessed by the electrophoresis technique in gel with gradient denaturant – DGGE (Muyzer et al., 1993). The data obtained by using the program Bionumerics, version 5.10, were used to calculate the richness, measured in operational taxonomic units (OUT), the Shannon-Weaver diversity (H') and equitably (E') indexes, and with similarity corresponding to the diets containing different lactose levels.

The variable R was generated using a binary matrix in which the absence of DNA band corresponding to each OUT was coded as zero (0) and the presence as one (1). For each level of lactose, the frequency of occurrence of each OUT was obtained by counting the number of presences in the treatments. The index H 'was calculated based on the abundance, expressed as optical density of the band in the lane of the gel, using the formula:

$$H' = \frac{2,3}{N}\left(N \log N - \sum ni \log ni\right),$$

where N = optical density in the lane, obtained by the sum of the bands optical densities in the lane; ni = optical density of each band in the lane.

E' was calculated as:

$$E = \frac{H'}{\log R}$$

The performance and morphological intestinal mucosa data obtained, corresponding to the pigs fed diets containing lactose supplemented or not with L-glutamine + L-glutamate, were subjected to analysis of variance and F test at 5% probability, using the program SAEG (Statistical Analysis System and Genetics, version 8.1), developed at the Universidade Federal de Viçosa. In the absence of interaction between the factors studied, the study proceeded to the analysis of the factors alone.

The evaluation of the composition of the intestinal microbiota of piglets was carried out using the program Bionumerics, version 5.10.

Table 3 -   Effect of L-glutamine + L-glutamate (L-Gln + L-Glu) on performance of piglets weaned at 21 days of age, assessed from 21 to 35 and from 21 to 49 days of age

	No L-Gln + L-Glu	Plus L-Gln + L-Glu	CV (%)
21 − 35 days			
Weight at 21 days	6.11	6.21	1.41
Weight at 35 days	9.15	9.57	6.84
Weight gain (g/day)	215	239	20.38
Feed intake (g/day)	278	300	16.18
Feed conversion	1.30	1.26	8.72
21 − 49 days			
Weight at 21 days	6.11	6.21	1.41
Weight at 35 days	15.65	16.76	7.34
Weight gain (g/day)	341	376	11.97
Feed intake (g/day)	480	519	11.06
Feed conversion	1.41	1.38	5.99

[1] Significant at 5% probability.
CV = coefficient of variation.

Results and Discussion

There was no interaction (P>0.05) between the factors studied on any parameters evaluated.

Adding L-glutamine + L-glutamate in the diets did not affect (P>0.05) the performance of pigs from 21 to 35 days of age (Table 3). However, in absolute values, the inclusion of L-glutamine + L-glutamate resulted in an increase of 4.6, 11.1, and 7.9% in the body weight at 35 days, average daily gain (ADG), and average daily feed intake (ADFI) of piglets, respectively, followed by a reduction of 3.2% in feed conversion (FC), also in absolute values.

From 21 to 49 days of age (Table 3), there was effect (P>0.05) of L-glutamine + L-glutamate on body weight at 49 days and on the body weight gain of piglets which increased 7.1 and 10.2%, respectively, compared with those fed diets without L-glutamine + L-glutamate. The ADFI and FC in this phase were not influenced by adding of L-glutamine + L-glutamate in the diets.

Beneficial effect of adding L-glutamine + L-glutamate in the diet on piglet performance was observed by Wu et al. (1996), Kitt et al. (2003) and Zou et al. (2006). Assessing four levels of glutamine supplementation (0, 0.2, 0.6, and 1.0%) on the performance of weaned piglets analyzed at weaning and in the first and second subsequent weeks, Wu et al. (1996) found a significant improvement in the order of 25% in the feed efficiency of piglets fed glutamine compared with those fed the control diet from 7 to 14 days after weaning.

In diets for weaned piglets challenged with LPS (*E. coli* lipopolysaccharide), Kitt et al. (2003) found that the inclusion of 5% glutamine maintained satisfactory the performance of the animals, which was not seen in those challenged and non-supplemented. In studies with piglets weaned at 21 days, Zou et al. (2006) found improvement in the FC within 10 days after weaning and for ADG within 20 days after weaning in response to the inclusion of 1% glutamine in the diet, followed by reduced rate of diarrhea and increased serum levels of growth hormone.

The inclusion of L-glutamine + L-glutamate resulted in an increase (P>0.05) in the villi height of the three segments of the small intestine evaluated, which were 7.7, 10.4 and 6.6% higher, respectively, in the duodenum, jejunum and ileum than those presented by piglets that were not supplemented with L-glutamine + L-glutamate (Table 4). However, the inclusion of L-glutamine + L-glutamate did not affect (P>0.05) crypt depth in any of the intestinal segments, and the values found in both groups of animals were very close to each other (Table 4).

The villous:crypt ratio, which is the result of the division between the measurement of villus height and the depth of its respective crypt, in the ileum was positively affected (P>0.05) by inclusion of L-Glu + L-Gln in the diet, evidently by the increasing in villi height (Table 4). Piglets fed diets containing L-glutamine + L-glutamate in their composition showed yet, in absolute values, villous:crypt ratio in the duodenum and jejunum 2.1 and 7.3% higher compared with those whose diets did not contain L-glutamine + L-glutamate. These results confirmed those found by Wu et al. (1996) and Yi et al. (2005).

Studying the effect of glutamine on intestinal villus height of piglets after weaning, Wu et al. (1996) reported that in addition to substantial improvement in performance, the inclusion of 1.0% glutamine in the diets of the piglets was able to prevent villus height atrophy of the jejunum in both first and the second week after weaning.

Table 4 -   Effect of L-glutamine + L-glutamate (L-Gln + L-Glu) on the intestinal mucosa structure of piglets weaned at 21 days of age, assessed at 49 days of age

	No L-Gln + L-Glu	Plus L-Gln + L-Glu	CV (%)
Duodenum			
Villous height[1]	482	519	9.66
Crypt depth	217	219	12.03
Villous:crypt ratio	2.30	2.35	9.21
Jejunum			
Villous height[1]	442	488	12.75
Crypt depth	190	198	13.62
Villous:crypt ratio	2.32	2.49	11.81
Ileum			
Villous height[1]	393	419	8.49
Crypt depth	171	172	14.47
Villous:crypt ratio[1]	2.31	2.50	11.47

[1] Significant at 5% probability.
CV = coefficient of variation.

Comparing the effect of adding glutamine or plasma in diets for piglets weaned at 17 days of age and challenged with *Escherichia coli*, Yi et al. (2005) found that the inclusion of 2% glutamine or 7% plasma significantly increased villus height and crypt depth of piglets with regard to the non-supplemented challenged treatment, although the inclusion of glutamine provided better performance. The authors attributed the beneficial effect of glutamine, mainly to the maintenance of the intestinal mucosa structure, electrolyte absorption, and intestinal homeostasis preventing diarrhea.

Comparing the effect of adding glutamine or plasma in diets for piglets weaned at 17 days of age and challenged with *Escherichia coli*, Yi et al. (2005) found that the inclusion of 2% glutamine or 7% plasma significantly increased villus height and crypt depth of piglets concerning the non-supplemented challenged treatment, although the inclusion of glutamine provided better performance. The authors attributed the beneficial effect of glutamine mainly to the maintenance of the intestinal mucosa structure, electrolyte absorption, and intestinal homeostasis preventing diarrhea.

The nutrition of piglets is closely linked to the immune system. It is known that early weaning is related to immunosuppression, reduced feed intake, and susceptibility to intestinal disorders. According to Newsholme (2001), the increased demand for glutamine in conditions of stress increases the release of glutamine by the muscle, resulting in decreased concentration of this amino acid in the muscle, and elevated rates of protein degradation, which is not desirable in growing pigs.

In a study with piglets infected or not by *Escherichia coli*, Yoo et al. (1997) found that supplementation with glutamine was able to maintain the concentration of glutamine in the muscle, and the concentrations of lymphocytes and leukocytes. However, the authors reported no effect of supplementation with glutamine on the non-challenged piglets, suggesting that supplementation with glutamine is important, among other factors, for the maintenance of its extracellular concentration, preventing the mobilization of muscle glutamine to meet the needs of the immune system.

According to Karinch et al. (2001), moderate or severe infections change the flow of glutamine in the body. The complexity of glutamine metabolism in different sanitary conditions of piglet production may explain why, in this research, even no effect (P> 0.05) of lactose was detected on the performance (Table 5) nor on the intestinal mucosa structure (Table 6); the inclusion of glutamine in the diet provided significant improvement in the ADG from 21 to 49

days of age and in the villi height. In general, the animals have good health, indicating low level of health challenge. Under these conditions, the small intestine is the organ of highest uptake of glutamine in the body, and it is provided primarily by the muscle (Karinch et al., 2001), which would explain the increase in villus height and, consequently, the increase in the ADG.

Under conditions of systemic inflammatory response, such as induction of immune challenge observed in the study of Kitt et al. (2003), Yi et al. (2005), and Yoo et al. (1997) the liver becomes the most important organ of glutamine uptake and exportation of this amino acid for the synthesis of immune cells due to the increase in the production of these specific cells (Karinch et al., 2001).

The effect of L-glutamine + L-glutamate in this study was self-evident although no inflammatory response was induced in the piglets. In addition to the low health challenge reflected by the low number of piglets suffering from diarrhea during the experiment and the overall health of the piglets, the environmental condition of sanitary challenge typically found on the first weeks after early weaning was attenuated by the good sanitary conditions of the rooms and by adding blood plasma and antibiotics in the experimental diets. The results obtained by Abreu et al. (2010), in a study evaluating the combination of glutamine, nucleotides and plasma in diets for weanling pigs showed that the response of piglets to plasma, the association of plasma + nucleotides or plasma + glutamine supplanted the response in performance afforded by glutamine alone, suggesting, furthermore, that the inclusion of plasma may attenuate the inflammatory response of piglets at weaning.

The levels of lactose did not influence (P>0.05) performance (Table 5) or intestinal mucosa structure of piglets (Table 6).

These results do not confirm the findings of Hauptili et al. (2005), who observed improved performance of piglets weaned at 21 days of age during the nursery phase by increasing the level of inclusion of whey powder in the diets, which was attributed to the lactose present in the food.

The lactose level in diets for piglets weaned at 21 days recommended by Bertol (1999) is 12 to 14% on the first week. In addition, Ludke et al. (1998) point out that in feeding programs for young piglets the need of milk powder inclusion in the diet rises as early as weaning is carried out, which is confirmed by Mahan et al. (2004), who worked with piglets weaned at different ages.

However, the effect of the addition of lactose in the diet of piglets may vary depending on the environment in which they are housed, as we can see in the study of Cromwell et al. (2008), who found, in three different experimental stations,

different responses in piglet performance when the level of dietary lactose increased.

The inclusion of crystalline lactose, milk powder or whey powder in diets for early weaned piglets has been adopted mainly due to its effect on feed intake, especially in the first week after weaning. The maintenance of feed intake prevents villi atrophy of the intestinal mucosa (Wu et al., 1997), which is necessary for digestion and absorption of nutrients, and therefore, performance.

However, in this study, there was no effect of adding lactose in the piglets' diet on the intestinal mucosa structure,

These results corroborate those achieved by Pierce et al. (2006), who, although did not find any significant effect of lactose on villous height, crypt depth or villous:crypt ratio of piglets, observed increase in ration intake in the same period.

In a study to evaluate the replacement of dietary lactose by maltodextrin in diets for piglets weaned at 21 days of age, emphasizing its role as an energy source for piglets, Hauptili (2009) verified the viability of full replacement of lactose by maltodextrin without compromising performance.
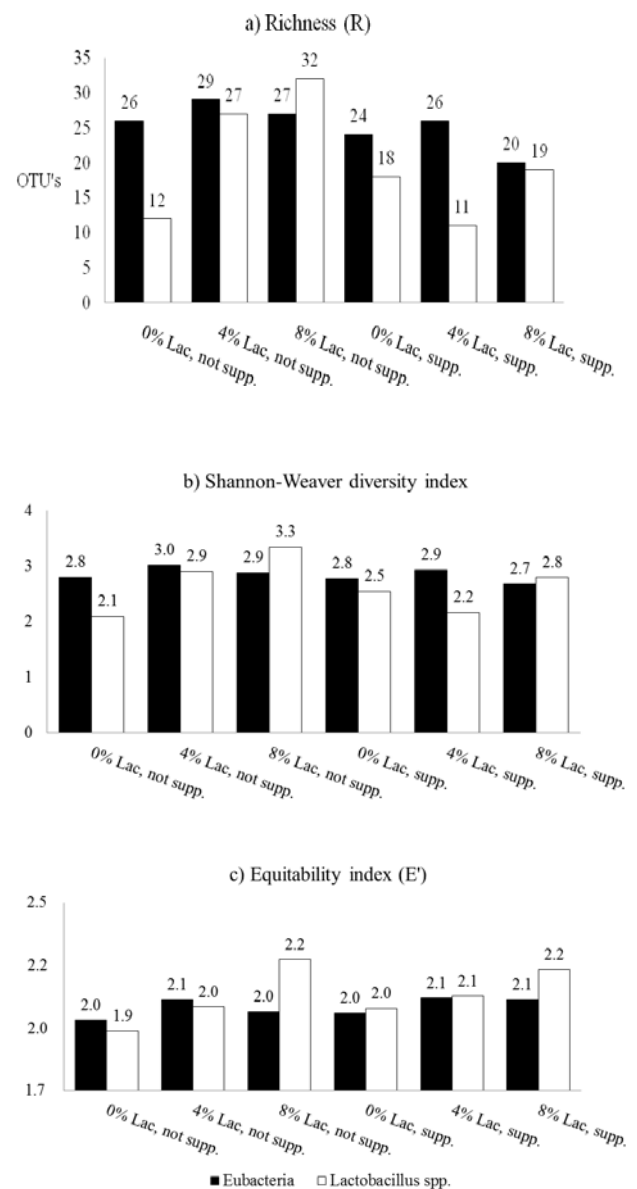
Table 5 - Performance of piglets fed diets with lactose, assessed from 21 to 35 and from 21 to 49 days of age[1]

	Lactose level from 21 to 35 days (%)			CV (%)
	0	4	8	
Initial weight (kg)	6.16	6.18	6.13	
Final weight (kg)	9.11	9.65	9.30	
ADG	207	248	226	18.32
DFI	264	311	291	20.07
FC	1.27	1.26	1.29	12.41
	Lactose level from 21 to 49 days (%)			CV (%)
	0-0[2]	4-0[3]	8-0[4]	
Final weight (kg)	16.05	16.68	15.87	
ADG	353	375	347	12.70
DFI	491	522	485	14.50
FC	1.38	1.39	1.40	7.50

[1] Not significant at 5% probability.
[2] 0% lactose from 21 to 35, and 0% lactose from 36 to 49 days of age.
[3] 4% lactose from 21 to 35, and 0% lactose from 36 to 49 days of age.
[4] 8% lactose from 21 to 35, and 0% lactose from 36 to 49 days of age.
CV = coefficient of variation; ADG = average daily gain; DFI = daily feed intake; FC = feed conversion.

Table 6 - Intestinal mucosa structure of piglets fed diets with lactose, assessed at 49 days of age[1]

	Lactose levels			CV (%)
	0-0[2]	4-0[3]	8-0[4]	
Duodenum				
Villous height	503	480	519	12.92
Crypt depth	222	220	213	16.14
Villous:crypt ratio	2.29	2.31	2.30	10.69
Jejunum				
Villous height	464	451	480	14.02
Crypt depth	207	208	168	16.26
Villous:crypt ratio	2.42	2.17	2.37	12.85
Ileum				
Villous height	390	426	403	12.17
Crypt depth	186	161	168	16.52
Villous:crypt ratio	2.15	2.36	2.42	13.58

[1] Not significant at 5% probability.
[2] 0% lactose from 21 to 35, and 0% lactose from 36 to 49 days of age.
[3] 4% lactose from 21 to 35, and 0% lactose from 36 to 49 days of age.
[4] 8% lactose from 21 to 35, and 0% lactose from 36 to 49 days of age.
CV = coefficient of variation.

Figure 1 - a) Richness (R), b) Shannon-Weaver Diversity Index (H') and c) Equitability (E) of Eubactéria comunities and *Lactobacillus* spp. of intestinal digesta of piglets fed diets containing 0.4 or 8% lactose supplemented or not with L-Glu + L-Gln.

However, part of dietary lactose may escape enzymatic digestion in the stomach and serve as a substrate for intestinal microbial fermentation. Delcenserie et al. (2008) accurately described the variety of physiological effects brought about by some genera of microorganisms, particularly regarding the production of inflammatory cytokines. Therefore, the existence of a relationship between the level of lactose inclusion in the diet of piglets and the intensity of sanitary challenge are feasible to be verified, given the importance of the study in the intestinal microbial diversity.

Operational Taxonomic Units (OTUs) quantitatively represent the microbial population of a given environment (Figure 1).

It was observed that the level of 4% inclusion of lactose in the diet provided the highest counts of UTOs, both in non-supplemented (29 UTOs) and supplemented diets (27 UTOs) with L-glutamine + L-glutamate. Regarding the community of *Lactobacillus* spp. of piglets fed diets with no addition of L-glutamine + L-glutamate the increase in the lactose level from 0 to 8% propitiated a crescent increase in the UTOs count.
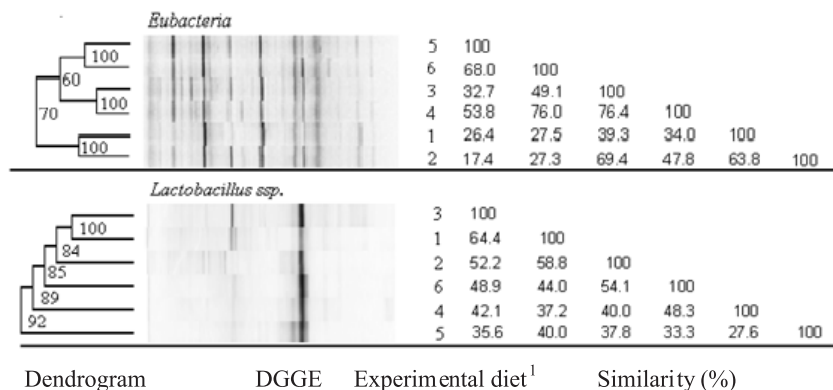
However, the pattern of response to the inclusion of these amino acids in the diet for the amount of *Lactobacillus spp.* was not maintained as the diet with 4% lactose and L-glutamine + L-glutamate resulted in the lowest value of UTO's (11).

Similarly, the Shannon-Weaver Diversity Index (H') (Figure 1) followed the results of Richness (R). Considering only the community of Eubacteria, the level of 4% lactose resulted in higher values of UTOs being verified by H' equal to 3.01 and 2.92, respectively, for piglets fed both supplemented and non-supplemented diets with L-glutamine + L-glutamate.

The Equitability (E') is indicative of uniformity in the distribution of groups of microorganisms found in an environment, demonstrating a possible existence of dominance (Figure 1). The higher the E', the higher the balance between populations. The results concerning Eubacteria consistently indicated that 0% lactose inclusion resulted in the lowest values of E' in both non-supplemented (1.98) and supplemented (2.01) diets with L-glutamine + L-glutamate, which allows to infer about a likely dominance exerted by a particular microorganism in relation to others. Regarding the groups of *Lactobacillus spp.*, there was an increase in E' when the level of lactose was increased in the piglets fed both non-supplemented and supplemented diets, which was 2.22 and 2.18, respectively.

With regard to the Richness and intestinal microbiota diversity of piglets, although the association of L-glutamine + L-glutamate with 4 and 8% lactose did not follow the same pattern of response for *Lactobacillus* spp. of piglets fed non-supplemented diets, it is not possible to say that the inclusion of L-glutamine + L-glutamate influenced the composition of the microflora.

Industrial amino acids, in general, have high digestibility, being fully used until the ileum. Stool et al. (1998) postulate that glutamine is degraded along the entire small intestine, which would not allow its fermentation by bacteria in the large intestine, where samples of digesta were collected. Collectively, the results suggest that the inclusion of lactose had a beneficial effect on the microbiota and the supplementation with L-Glu + L-Gln had no influence on the microbiota. The electrophoresis in denaturing gradient gel (DGGE) shows the profiles of similarity among treatments contributing to the consolidation of the results of H', E' and R (Figure 2).

Figure 2 - Dendrogram with Cophenetic Coefficient, DGGE electrophoretic profiles and Similarity (%) of rDNA gene sequences of the colon proximal digesta of piglets fed diets containing 0, 4, and 8% lactose for Eubactéria and *Lactobacillus spp.*

[1] Experimental diets: 1 = diet with 8% lactose with no L-glutamine + L-glutamate (L-Gln + L-Glu), 2 = diet with 8% lactose with L-Gln + L-Glu, 3 = diet with 4% lactose with no L-Gln + L-Glu, 4 = diet with 4% lactose with L-Gln + L-Glu, 5 = diet with 0% lactose with no L-Gln + L-Glu, 6 = diet with 0% lactose with L-Gln + L-Glu.

The occurrence of a high similarity in the intestinal microbiota of piglets that were fed diets containing the same levels of lactose (T1 and T2 = 8%, T3 and T4 and T5 = 4%, and T6 = 0% lactose) was verified for the community of Eubacteria, regardless of supplementation with L-glutamine + L-glutamate.

The similarity observed between treatments with the same level of lactose was 68.0, 76.4, and 63.8, respectively, for the levels 0.4 and 8% lactose. However, it was observed that the treatments with 0 and 4% lactose, with or without L-glutamine + L-glutamate inclusion presented average similarity, lower than that seen between treatments with the same level of lactose. The similarity is indicative of the resemblance between microbial communities in micro-ecosystems, in which case the digesta of animals is strongly influenced by the diet (Williams et al. 2001).

The analysis of intestinal microbiota consistently indicated that the level of lactose exerted influence on the total bacterial community in the colon of piglets, regardless of the presence of L-glutamine + L-glutamate in the diets.

Conclusions

The inclusion of L-glutamine + L-glutamate in diets for piglets weaned at 21 days of age improves performance and morphology of the intestinal mucosa, regardless of the addition of lactose. The level of 8% lactose improves the composition of the intestinal microbiota of piglets.

References

ABREU, M.L.T.; DONZELE, J.L. Glutamina na nutrição de leitões. In: SIMPÓSIO SOBRE MANEJO E NUTRIÇÃO DE AVES E SUÍNOS – CBNA, 5., 2008, Cascavel. **Anais...** Cascavel, 2008. p.75-80.

ABREU, M.L.T.; DONZELE, J.L.; SARAIVA, A. et al. Glutamina, nucleotídeos e plasma suíno em rações para leitões desmamados. **Revista Brasileira de Zootecnia,** v.39, n.3, p.520-525, 2010.

BERTOL, T.M. **Alimentação dos leitões na creche de acordo com a idade de desmame**. Instrução Técnica para o produtor: Área de comunicação empresarial. Concórdia: Embrapa Suínos e Aves, 1999. 2p.

BERTOL, T.M.; SANTOS FILHO, J.I.; LUDKE, J.V. Níveis de suplementação com lactose na dieta de leitões desmamados. **Revista Brasileira de Zootecnia**, v.29, p.1387-1393, 2000.

CROMWELL, G.L.; ALEE, G.L.; MAHAN, D.C. Assesment of lactose levels in the mid- to late-nursery phase on performance of weanling pigs. **Journal of Animal Science**, v.86, n.1, p.127-133, 2008.

DELCENSERIE, V.; MARTEL, D.; LAMOUREUX, M. et al. Immunomodulatory effects of probiotics in the intestinal tract. **Current Issues Molecular Biology**, v.10, n,1-2, p.37-54. 2008.

HAUPTLI, L.; LOVATTO P.A.; SILVA, J.H.S. et al. Níveis de soro de leite integral na dieta de leitões na creche. **Ciencia Rural**, v.35, n.5, p.1161-1165, 2005.

HAUPTLI, L **Maltodextrina e óleos como fontes de energia para leitões**. 2009. 57f. Tese (Doutorado em Zootecnia) - UNESP, Botucatu.

KARINCH, A.M.; PAN, M.; LIN, C.M. et al. Glutamine metabolism in sepsis and infection. **Journal of Animal Science**, v.131, p.2535S-2538S, 2001.

KITT, S.J.; MILLER, P.S.; FISCHER, R.L. Effects of glutamine on growth performance and intestinal development of immune challenged wealing pigs fed chemically defined diets. **Nebraska Swine Report**, p.34-38, 2003.

LU, J.; IDRIS, U.; HARMON, B. et al. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. **Applied Enviroment Microbiology**, v.69, p.6816-6824, 2003.

LUDKE, J.V.; BERTOL, T.M.; SHEUERMANN, G.N. Manejo da alimentação. In: SOBESTIANSKY, Y.; WENTZ, I.; SILVEIRA, P.R.S. (Eds.) **Suinocultura intensiva**: produção, manejo e saúde do rebanho. Brasília: Serviço de Produção de Informação; Embrapa Suínos e Aves, 1998. p.74-80.

MAHAN, D.C.; FASTINGER, N.D.; PETERS, J.C. Effects of diet complexity and dietary lactose levels during three starter phases on post weaning pig performance. **Journal of Animal Science**, v.82, n.9, p.2790-2797, 2004.

MUYZER, G.; DE WALL, E.C.; UITTERLINDEN, A.G. Profiling of complex microbial populations bydenaturing gradient gel electrophoresis analysis of polymerase chain reaction – amplified genes coding for 16s rRNA. **Applied and Environmental Microbiology**, v.59, p.695-700, 1993.

NEWSHOLME, P. Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? **Journal of Nutrition**, v.131, p.2515S-2522S, 2001.

PIERCE, K. M.; SWEENEY, T.; BROPHY, P.O. et al. The effect of lactose and inulin on intestinal morphology, selected microbial populations and volatile fatty acid concentrations in the gastro-intestinal tract of the weanling pig. **Animal Science**, v.82, n.3, p.311-318, 2006.

PIERCE, K.M.; CALLAN, J.J.; McCARTHY, P. et al. Performance of weanling pigs offered low or high lactose diets suplplemented with avilamycin or inulin. **British Society of Animal Science**, v.80, p.313-318, 2005.

PINHEIRO; R.W; MACHADO, G.S. Desempenho do leitão a primeira semana pós desmama: como atingir e porquê gerenciar este parâmetro. In: SIMPÓSIO DE SUINOCULTURA, 2., 2007, Lavras. **Anais...** Lavras, 2007. p.125-145.

ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L. et al. **Tabelas brasileiras para aves e suínos**: composição de alimentos e exigências nutricionais. Viçosa, MG: Universidade Federal de Viçosa, 2005. 141p.

STOOL, B.; HENRY, J.; REEDS, P.J. et al. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. **Journal of Nutrition**, v.128, p.606-614, 1998.

WILLIAMS, B.A.; VERSTEGEN, M.W.A.; TAMMINGA, S. Fermentation in the large intestine and its relationship to animal health. **Nutrition Research Review**, v.14, p.207-227, 2001.

WU, G.; MEIER, S.A.; KNABE, D.A. Dietary glutamine supplementation prevents jejunal atrophy in weaned pigs. **Journal of Nutrition**, v.126, p.2578-2584, 1996.

YI, G.F.; CARROLL, J.A.; ALLEE, G.L. et al. Effect of glutamine and spray-dried plasma on growth performance, small intestinal morphology, and immune responses of *Escherichia coli* K88+-challenged weaned pigs. **Journal of Animal Science**, v.83, p.634-643, 2005.

YOO, S.S.; FIELD, C.J.; MCBURNEY, M.I. Glutamine supplementation maintains intramuscular glutamine concentration and normalize lymphocyte function in infected early weaned pigs. **Journal of Nutrition**, v.127, p.2253-2259, 1997.

ZOU, X.T.; ZHENG G.H.; FANG, X.J.; JIANG, J.F. Effects of glutamine on growth performance of weanling piglets. **Czech Journal of Animal Science**, v.51, p.444-448, 2006.