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# Effects of housing conditions and glutamine levels on growth performance of post-weaning piglets

## Efeitos da condição de alojamento e do nível de glutamina sobre o desempenho de leitões desmamados

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#### **Abstract**

This study aimed to determine the effects of housing conditions and glutamine (GLN) levels on growth performance and incidence of diarrhea in weaned piglets. Forty-eight piglets were randomly assigned (4x2 factorial design) to one of the eight experimental diets represented by four levels of dietary GLN (0.0, 1.0, 1.5 or 2.0 %) and two housing conditions (AD = adequate and INAD = inadequate). From 22 to 28 days of age all animals were challenged daily with *Escherichia coli* polysaccharides. No effect of GLN level was found on daily gain (ADG), daily feed intake (ADFI) or body weight at 28 days postweaning (BW28). For housing conditions, ADG at 21 days after weaning was greater for animals of treatment AD than INAD, and AD had lower body weight variation than INAD pigs at 21 days after weaning. Considering the whole period, AD had a higher ADG, ADFI and BW28 than INAD. However, an interaction effect was observed for feed:gain rate, in which values decreased linearly (Y = 2.1727 - 0.4017x; R<sup>2</sup>=0.92) just for AD pigs as GLN levels increased. No GLN level effects were observed for diarrhea incidence; however, AD had a higher number of animals without diarrhea and with lower incidence of severe diarrhea than INAD pigs. In conclusion, supplementing GLN doesn't affect ADG, ADFI or BW28 but it improves feed efficiency when housing conditions are adequate.

Key words: Amino acid. Housing condition. Lps. Pigs. Weanling.

#### Resumo

Este estudo visou determinar o efeito das condições de alojamento e dos níveis de glutamina (GLN) sobre o desempenho e a incidência de diarréia em leitões desmamados. Quarenta e oito leitões foram divididos aleatoriamente (modelo fatorial 4x2) e submetidos a oito dietas experimentais representadas por quatro níveis de GLN dietética (0.0, 1.0, 1.5 ou 2.0 %) e duas condições de alojamento (AD = adequada e INAD = inadequada). A partir do 22º dia de idade até os 28 dias de experimentação, todos os leitões foram desafiados com lipopolissacarídeo de *Escherichia coli*. Nenhum efeito do nível de

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GLN foi observado sobre o ganho de peso diário (GDP) consumo diário de ração (CDR), conversão alimentar (CA), e peso dos animais no dia 28 pós-desmame (BW28). Para a condição de alojamento, o valor de GDP no dia 21 pós-desmame foi maior para os animais AD que os INAD e os animais AD apresentaram menor heterogeneidade de peso que INAD. Considerando todo o período experimental, os animais de AD obtiveram maiores GDP, CDR e BW28 que animais INAD. No entanto, houve efeito de interação para CA, com valores decrescendo linearmente (Y = 2,1727 0,4017x; R² = 0.92) para leitões AD conforme aumentou a suplementação de GLN. Nenhum efeito de nível de GLN foi observado para a incidência de diarreia, no entanto, o tratamento AD apresentou maior número de animais sem diarreia e menor número de diarreias severas. Em conclusão, a suplementação com GLN não afeta GDP, CDR e BW28, mas melhora a eficiência alimentar quando as condições de alojamento são favoráveis.

Palavras-chave: Aminoácido. Condição de alojamento. Desmame. Leitões. LPS.

#### Introduction

Currently in Brazil there are no standardized housing systems for weaned pigs, and therefore, a big range of different facilities are observed in pig farms. Although some studies have evaluated individual risk factors related to health status and growth performance (MORES et al., 1998; DALLA COSTA et al., 2000), studies regarding the evaluation of risk factors in housing conditions and their interaction with functional nutritional supplements, such as glutamine (GLN), are even less common or non-existent.

High numbers of risk factors in housing systems impair growth performance, decrease group uniformity, and increase the incidence of diseases, such as diarrhea. Actually, diarrhea is one of the diseases most related to environmental risk factors and management after weaning, and it is generally caused by infections with enterotoxigenic *Escherichia coli* (SILVA et al., 1999).

Under adverse immunologic conditions, such as infections, the systemic demand for GLN is higher than its absorption/synthesis rate, and consequently, it becomes a conditionally essential substance for the animals (WU et al., 2011). Considering the important and unique metabolic functions performed by this amino acid, its supplementation for weaned pigs appears to be a promising alternative to counteract or alleviate intestinal disorders and improve performance. In fact, diets supplemented with 2% GLN had beneficial effects in alleviating growth depression

in *E. coli*-challenged early-weaned pigs (YI et al., 2005).

Given that the effects of GLN in the first days after weaning are well known, this study aimed to evaluate the effects of different dietary GLN levels on growth performance and incidence of diarrhea later in the nursery phase of pigs subjected to two different housing conditions.

#### **Material and Methods**

Animals and treatments

Experimental procedures were approved by the Committee on Ethics in Animal Use (CEUA), protocol number 15.185.2011.74 of the Universidade Estadual de Londrina.

Forty-eight Agroceres-PenArlan piglets, 24 castrated males and 24 females, were selected at weaning with  $6.16 \pm 0.78$  kg body weight (BW) and  $21 \pm 2$  days of age. Immediately after weaning, piglets were randomly housed by pairs (one castrated male and one female; according to their BW) in nursery facilities with one of two different housing conditions: adequate, AD) slatted plastic flooring pens (0.7 m<sup>2</sup> per pig), equipped with linear feeders (0.15 cm per animal) and adjustable nipple drinkers with a flow rate of 0.95 L min<sup>-1</sup>; and inadequate, INAD) solid concrete flooring pens (1.4 m<sup>2</sup> per pig) half covered with wood shavings, equipped with wooden linear feeders (0.15 cm per animal) during the first 14 days of the experiment and, thereafter, semi-automatic metal feeders (0.30 cm per animal) and non-adjustable 90° angle nipple drinkers placed at 37.5 cm high with a flow rate of 1.8 L min<sup>-1</sup>.

For both housing conditions, curtains were used to control room temperature and infrared lamps provided additional heating. Additionally, all piglets were randomly assigned (according to their BW) to one of the four experimental diets: GLN-0) basal prestarter and starter diets without L-GLN (Ajinomoto Brazil) (Table 1); GLN-1) basal pre-starter and starter

diets supplemented with 1.0% of L-GLN; GLN-1.5) basal pre-starter and starter diets supplemented with 1.5% of L-GLN; and GLN-2) basal pre-starter and starter diets supplemented with 2.0% of L-GLN. Diets were initially formulated without GLN and after preparation, the different levels of GLN were added. The basal iso-nutrient experimental diets (Table 1) exceeded the recommended levels for all other nutrients (ROSTAGNO et al., 2011).

**Table 1.** Composition of the basal diet (as feed basis).

Ingredient, %	Pre-starter 1 (1-14 d post-weaning)	Starter 2 (15-28 d post-weaning)		
Corn	53.25	67.77		
Soybean meal 45%	23.73	20.88		
Dried skim milk	10.00	04.00		
Soybean oil	03.79	00.23		
Sugar	03.00	02.00		
Dried whey	02.00	02.00		
Dicalcium phosphate	01.66	01.36		
Limestone	00.64	00.70		
Salt	00.51	00.36		
L-lysine HCL	00.63	0.34		
DL-methionine	00.24	00.05		
L-threonine	00.30	0.10		
L-tryptophan	00.05	00.01		
Mineral and vitamin premix <sup>3</sup>	00.20	00.20		
Total	100	100		

<sup>&</sup>lt;sup>1</sup>The calculated compositions for metabolizable energy, crude protein, lysine, arginine, Ca, and P of the basal pre-starter diet were 3,400 kcal kg<sup>-1</sup>, 20.0%, 1.5%, 0.97%, 0.85% and 0.5%, respectively.

Regardless of the experimental housing conditions or GLN level, from 22 to 28 days of age post-weaning, all piglets were challenged daily with *E. coli* polysaccharides (LPS; serotype 0111: B4 L2630, Sigma Aldrich), at 0.3 µg per animal as described by Parra et al. (2013). During the 28-day experimental period (weaning = day 0), all animals were fed *ad libitum*.

#### Data collection

To define housing conditions (AD *vs* INAD), the protocols described by Mores et al. (1998) and Dalla Costa et al. (2000) were applied considering feeder space per pig (FSP), drinker height (DH), drinker maximum flow rate (MFR) and floor type to determine the quality of the facilities. The evaluation of diarrhea was performed according to Madec and

<sup>&</sup>lt;sup>2</sup>The calculated compositions for metabolizable energy, crude protein, lysine, arginine, Ca, and P of the basal starter diet were 3,230 kcal kg<sup>-1</sup>, 17.4%, 1.0 %, 0.96 %, 0.72 % and 0.4 %, respectively.

<sup>&</sup>lt;sup>3</sup>Provided per kilogram of diet: vitamin A, 18,000 UI; vitamin D<sub>3</sub>, 3,600 UI; vitamin E, 40 UI; vitamin K, 3.6 mg; vitamin B<sub>1</sub>, 2.8 mg; vitamin B<sub>6</sub>, 3 mg; vitamin B<sub>12</sub>, 36  $\mu$ g; niacin, 60 mg; pantothenic acid, 32 mg; biotin, 200  $\mu$ g; folic acid, 8 mg; Fe as ferrous sulfate, 200 mg; Cu as copper sulfate, 50 mg; Mn as manganese oxide, 11 mg; Zn as zinc oxide, 180 mg; I as calcium iodate, 2.0 mg.

Josse (1983). Average daily feed intake (ADFI), average daily gain (ADG), and body weight were recorded at the beginning and end of the experimental period and feed:gain rate (FCR) was calculated. Facility temperatures were recorded daily.

#### Statistical analysis

Data were analyzed using the ExpDes package of R statistical program (FERREIRA et al., 2011) according to a randomized arrangement of treatments in blocks with the eight treatments as the main independent variables. The pen with two pigs was considered the experimental unit. The residual error term was used to test the treatment effects. To assess the incidence and intensity of diarrhea, the chi-square test was applied. Differences were considered significant at  $P \le 0.05$  and tendencies at  $0.05 < P \le 0.10$ . All results are expressed as adjusted means  $\pm$  SEM.

#### **Results and Discussion**

Initial BW was not different among treatments  $(6.17 \pm 0.78 \text{ kg})$  and temperature variations were similar for both housing conditions (between  $22.6 \pm 0.8$  °C and  $30.7 \pm 1.3$  °C).

No effect of GLN level (P > 0.05; Table 2) was found on ADG, ADFI or body weight at day 28 post-weaning (BW28). These results are in line with another study (YI et al., 2005), in which there was no effect of treatment throughout the overall experimental period. In contrast to our findings, some studies demonstrated that dietary supplementation with GLN or glycyl-GLN dipeptide improved growth performance in the first week after weaning in piglets challenged with E. coli K88+ or LPS (YI et al., 2005; JIANG et al., 2009). These results suggest that at an older age (28 days post-weaning) these effects might be overshadowed by normal physiologic growth. However, it is noteworthy to mention that the level of 0.3 µg of LPS used in the present experiment is much lower than the level used by other authors e.g. 200 μg kg<sup>-1</sup> body weight of LPS in Jiang et al (2009). In a previous study, Lozano et al. (2014) showed that challenging weaned pigs with 0.3 µg of LPS per pig per day did not promote any moderate or transient cortisol alterations. According to García-Herrera et al. (2008), increased cortisol values are expected in LPS challenged animals due to the effects of endotoxemia. Therefore, it is reasonable to consider that the present level of LPS was not enough to promote physiologic stress and alter GLN metabolism.

Table 2. Growth performance of piglets at 28 days after weaning according to dietary glutamine level and housing condition.

	Housing condition	Glutamine level (%)				Arramaga	CEM
	Housing condition	0	1.0	1.5	2.0	Average	SEM
ADG, kg	AD	0.20	0.26	0.27	0.23	0.23 a	0.12
	INAD	0.17	0.15	0.18	0.16	$0.16^{b}$	0.14
ADFI, kg	AD	0.38	0.41	0.31	0.37	0.37 a	0.11
	INAD	0.32	0.33	0.29	0.31	0.31 b	0.12
F:G	$\mathrm{AD^1}$	2.24	1.61	1.59	1.42	1.72	0.52
	INAD	1.89	2.25	1.88	2.01	2.01	0.53
BW28,kg	AD	11.39	13.41	13.46	12.07	12.58 a	2.25
	INAD	11.12	10.54	11.52	10.23	10.85 b	2.65

Housing condition adequate = AD; housing condition inadequate = INAD; ADG = average daily gain; ADFI = average daily feed intake; F:G= feed:gain rate; BW28= body weight at day 28 post-weaning; AD = adequate housing condition; INAD = inadequate housing condition

a.b Different superscript letters within a column indicate a significantly different effect (P < 0.05)

 $<sup>^{1}</sup>$  Y= 2.1727 - 0.4017x;  $R^{2}$  = 0.92).

According to Wu et al. (2011), a large dosage of GLN (e.g. 2% GLN in the corn and soybean meal-based diet for weanling piglets) may impair growth performance. The present study showed that GLN supplementation at 2.0% of the diet for pigs during the 28 days post-weaning did not promote any negative effects on growth performance.

For housing condition, before challenging the pigs with LPS, the ADG at 21 days after weaning (ADG21) was greater for AD than INAD animals (228 g vs 160 g, respectively; P<0.05). Additionally, AD animals had lower BW variation at 21 days after weaning (BW21-CV) than INAD pigs (13.4% vs 18.6%, respectively; P < 0.05). According to Mores et al. (1998), the ADG21 and BW21-CV are useful information to evaluate the effects of risk factors affecting the growth performance of weaned pigs. Therefore, the present results indicate that the set of factors comprising the AD facilities were adequate and beneficial to support growth of piglets until 28 days post-weaning. Although differences were found for BW21-CV between both housing conditions, according to Mores et al. (1998), this variation remained within the normal range for this category.

Considering the whole experimental period, AD had higher (P < 0.05) ADG, ADFI and BW28 than INAD animals (Table 2). The area of 0.16 m<sup>2</sup> per piglet is adequate until 12.2 kg BW (STOJANAC et al., 2014); therefore, it is unlikely that the area per piglet (0.7 and 1.4 m<sup>2</sup> for AD and INAD, respectively) disturbed growth performance in the present study. Additionally, for both housing conditions, the feeder space per pig of 0.15 cm was adequate to maximize feed intake (MORES et al., 1998). However, the substitution of the feeder after 14 days for INAD facilities may have influenced growth performance because those animals had to readapt to the new feeder, leading to low feed intake during the first day. Another important factor to be addressed is drinker height. For AD facilities, drinkers were adjustable and able to be positioned at 5 cm above the animal's back. According Dybkjaer et al. (2006), this positioning reduces the time for the piglets to establish adequate water intake. In contrast, for INAD facilities, drinkers were fixed at a 90° angle and 37.5 cm in height above the floor, impairing water intake (DALLA COSTA et al., 2000). According to Dybkjaer et al. (2006), feed intake after weaning is related to water intake, and thus, the adequate drinker position for the AD facility may have facilitated water intake and consequently may have impacted growth performance. Also, Dalla Costa et al. (2000) showed that a maximum flow rate of 1.0 L min<sup>-1</sup> improves water intake after weaning. In this regard, the maximum flow rate from INAD facilities was 80% higher than the recommendation and might have impaired water intake and consequently growth performance.

An effect of the interaction (P<0.05) between GLN levels and housing condition (Table 2) was observed for feed:gain rate (F:G), in which values linearly decreased (Y= 2.1727 - 0.4017x; R<sup>2</sup> = 0.92) for AD pigs as GLN supplementation levels increased, but not for INAD animals. Indeed, compared to AD-unsupplemented pigs (GLN-0) and AD supplemented with 2.0% GLN (GLN-2) there was improvement of the F:G by 36.6%. The animals of nursery I, that received feed supplemented with 1.0% GLN, showed better feed conversion when compared to nursery II (1.61 vs. 2.25, respectively) (Table 2). The animals of nursery I that received feed with 2% GLN presented a difference in F:G when compared to animals of nursery II (1.42 vs 2.01). The increase in supplementation from 1% to 2% GLN resulted in improvement by 11.7% for F:G (i.e. 190 g fewer feed per 1 kg of live weight gain).

This result is in line with a previous study (LOZANO et al., 2014) in which dietary supplementation with 2% GLN improved FCR of weaned pigs challenged with LPS. The positive effect of GLN in alleviating mucosal villi atrophy in animals challenged with LPS and increasing absorption of dietary nutrients (CABRERA et al., 2013) might have been potentiated by the adequate housing conditions of AD facilities.

For the final weight, nursery II presented the best gain indexes for weight and feed intake (Table 2), confirming its improved environmental quality and its effect on performance parameters.

However, for all these parameters, it is possible that the higher incidence of severe diarrhea in INAD animals may have impaired their performance. No GLN level effect was observed for incidence of diarrhea (P > 0.05). It is well known that LPS-challenged piglets provide a suitable animal model to study the effects of dietary supplementation on the incidence of diarrhea. Although metabolic changes could be involved with the development of post-weaning diarrhea after a 0.3 µg LPS challenge, as reported by Parra et al. (2013), Lozano et al. (2014) did not observe any major physiologic alteration in weaned pigs challenged with 0.3 µg of LPS. The present result for GLN effect is in line with other experiments using nutritional additives aimed to reduce postweaning diarrhea (SILVA et al., 2007; DALTO et al., 2011). The diarrhea episode is individual and leads to reduction of intestinal villi, and decreased enzymatic activity, digestibility and absorption of nutrients (RIOPEREZ et al., 1991; CERA et al., 1988; VENTE SPREEUWENBERG et al., 2003), accompanied by temporary immunosuppression

which helps explain these results.

For housing conditions, however, AD pigs had a greater number of animals without diarrhea (Table 3) compared to INAD pigs (12 vs 4, respectively; P < 0.05). Considering slight and severe diarrhea, AD pigs had a higher incidence of slight (9 vs 8, respectively) and lower incidence of severe (3 vs 12, respectively; P < 0.05) diarrhea compared to INAD pigs. Silva et al. (1999) studied the eco-pathology of post-weaning diarrhea and reported that a small area of space and feeder space per pig are among the factors most related with post-weaning diarrhea. In the present study, both housing conditions met the recommendation for these variables, however, drinker height, maximum flow rate, and floor type may have contributed to the higher incidence of diarrhea in INAD animals. The lower drinking efficiency, non-adjustable 90° angle drinkers placed at a height of 37.5 cm off the floor increased the waste of water. Silva et al. (1999) reported that low water intake efficiency was related to the incidence of diarrhea. In addition, the high waste of water increased the humidity of the solid concrete flooring pens which were half covered with wood shavings, and according to Madec and Josse (1983), high humidity in the pens is related to multifactorial diseases such as diarrhea.

**Table 3.** Number of piglets that had diarrhea in two different housing conditions.

	Housing		
Degree of presentation	Nursery I	Nursery II	Total
Without diarrhea (0 days)	12a	4b	16
Little diarrhea (1-3 days)	9b	8 <sup>a</sup>	17
Significant diarrhea ( > 4 days)	3b	12a	15
Total	24	24	48

Means followed by different letters in the line differ by chi-square test 5%. Nursery I: slatted plastic flooring pens; Nursery II: solid concrete flooring pens.

#### Conclusion

Dietary supplementation with GLN didn't enhance growth performance during the 28 days

post-weaning in pigs; however, when housing conditions were adequate, increased levels of GLN improved the efficiency of feed utilization.

Adequate housing conditions (AD facilities) presented better results than INAD facilities, indicating that simple management and environment factors may produce remarkable results in terms of growth performance and incidence of diarrhea after weaning.

The standardization of nursery facilities using information already available in the literature may aid in reducing infection pressure, improving growth performance, and possibly potentiating the effects of nutritional additives as shown in the present study.

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