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Effects of glutamine and glutamate on nursery piglets fed diets with different digestible lysine content

Efeitos da glutamina e do glutamato em leitões alimentados com dietas com diferentes níveis de lisina digestível

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Highlights _

Benefits of adding glutamine and glutamate in the diet on piglet performance. Glutamine/glutamate improved performance independent of the diet's nutritional level. Reducing lysine levels negatively affects the growth performance of piglets

Abstract .

The objective of the study was to evaluate the effects of glutamine and glutamate (Gln/Glu) on the growth performance and immune response of nursery pigs fed different digestible lysine content. Two hundred and sixteen piglets, weaned at 21 days old, were assigned to a randomized block design according to their initial body weight (BW), in a 2 × 2 factorial arrangement with two levels of lysine (control-lys and low-lys) and two levels of Gln/Glu (0 and 12 g kg⁻¹), with nine replicates. At 26 d, piglets consuming the low-lys diet not supplemented with Gln/Glu presented a higher (P < 0.01) incidence of diarrhea than the other treatments. From 21 to 32 d of age, the piglets fed the control-lys diets performed better than those fed low-lys diets (P < 0.01). From 21 to 42 d of age, there was a correlation (P < 0.01) between lysine level and Gln/Glu supplementation for average daily feed intake (ADFI) and feed conversion (FC). Gln/Glu supplementation improved (P < 0.05) the ADFI of pigs fed the low-lys diets, resulting in a higher (P < 0.01) average daily weight gain (ADG) and BW; however, worse (P < 0.05) FC. Piglets consuming control-lys diets had higher (P < 0.05)

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serum urea nitrogen concentration (SUN) and IgG than low-lys piglets. In addition, Gln/Glu supplementation correlated with higher (P < 0.01) SUN. Dietary supplementation of glutamine and glutamate improved the growth performance of weaned piglets from 21 to 42 days of age, regardless of the diets' lysine levels. In addition, reducing lysine levels 10% below the requirement negatively affects the growth performance and the immune response of nursery piglets.

Key words: Glutamate. Glutamine. Lysine. Nutrition. Piglets.

Resumo _

O objetivo do estudo foi avaliar os efeitos da glutamina e do glutamato (Gln/Glu) no desempenho e na resposta imune de leitões alimentados com diferentes níveis de lisina digestível. Duzentos e dezesseis suínos, desmamados aos 21 dias de idade, foram distribuídos em um delineamento de blocos ao acaso de acordo com seu peso corporal (PC) inicial, em um arranjo fatorial 2 × 2 com dois níveis de lisina (baixalis e controle-lis) e dois níveis de Gln/Glu (0 e 12 g kg⁻¹), com nove repetições. Aos 26 dias, os leitões que consumiram o baixa-lis não suplementado com Gln/Glu apresentaram maior (P < 0,01) incidência de diarreia quando comparados aos demais tratamentos. Dentre 21 a 32 dias de idade, os leitões alimentados com dietas controle-lis tiveram melhor desempenho do que aqueles alimentados com dietas baixa-lis (P < 0,01). Dos 21 aos 42 dias de idade, houve correlação (P < 0.01) entre o nível de lis e a suplementação com Gln/ Glu para consumo de ração médio diário (CRM) e conversão alimentar (CA). A suplementação com Gln/ Glu melhorou o CRM dos animais alimentados com dietas de baixo teor de lis, resultando em maior ganho de peso médio diário (GMD) e PC; no entanto, a CA piorou. Os leitões que consumiram dietas controle-lis apresentaram maior concentração sérica de nitrogênio ureico (SUN) (P < 0,05) e IgG (P < 0,05) do que os que consumiram dietas baixa-lis. Além disso, os suplementados com Gln/Glu apresentaram maior (P < 0,01) SUN do que os não suplementados. A suplementação dietética de glutamina e glutamato melhorou o desempenho de crescimento de leitões desmamados de 21 a 42 dias de idade, independentemente dos níveis de lis da dieta. Além disso, a redução dos níveis de lis 10% abaixo do requisito afeta negativamente o desempenho do crescimento e a resposta imunológica dos leitões em creche.

Palavras-chave: Glutamato. Glutamina. Leitões. Lisina. Nutrição.

Introduction __

Afterpigletsareweaned,morphological and functional changes occur in their small intestines. The reduction in feed intake associated with weaning has been known to affect intestinal integrity and potentially cause pathology (Gresse et al., 2017). Dietary supplementation with specific nutrients or immune modulators can help improve overall health and performance (Klasing, 2007). Some studies indicate that glutamine and glutamate serve important regulatory functions in nutrient metabolism, protein turnover, and immune function, thereby enhancing growth and feed efficiency in pigs (Hou & Wu, 2018; Ji, Wang, Yang, Hu, & Yin, 2019).

Glutamine has important metabolic functions, and it is considered a conditionally essential amino acid in some species under inflammatory conditions (Newsholme, 2001). It is a major metabolic fuel for rapidly dividing cells, including enterocytes and lymphocytes, and a key regulator of gene expression and cell signaling pathways (Rhoads & Wu, 2009). The gastrointestinal tract requires L-glutamine (Reeds & Burrin, 2001) and its availability from endogenous tissue production may not be sufficient to maintain the structural and functional integrity of the intestinal mucosa (Van der Hulst et al., 1993; James, Lunn, & Elia, 1998). Similar to glutamine, glutamate is a functional amino acid shown to provide energy and participate in nutrient metabolism, immune and oxidative stress responses, regulation of signaling pathways, and synaptic transmission (Burrin & Stoll, 2009; Duan et al., 2016).

Despite the known activities of glutamine and glutamate (Gln/Glu), studies on the effects of supplementation with these amino acids on piglet performance have vielded inconsistent results (Teixeira, Noqueira, Kutschenko, Rostagno, & Lopes, 2014; He et al., 2016; Johnson & Lay, 2017; Leite da Silva et al., 2019), and this variability may be linked to the specific nutritional composition of the diet. Depending on the cost of ingredients and management strategies, among other factors, diets with lysine (lys) levels below those recommended by the nutritional guides (National Research Council [NRC], 2012; Rostagno et al., 2017) are routinely used by the pig feed industry. In these low lys diets, the inclusion of non-essential amino acids is not considered (Ball et al., 2013; Zhang, Xie, Thacker, Htoo, & Qiao, 2013). However, after weaning, some conditionally essential amino acids are required for proper growth performance and immune function (Ruth & Field, 2013). Therefore, glutamine plus glutamate supplementation may enhance performance (Wu et al., 2013), especially in those pigs fed low lys diets.

Although some studies (Cabrera et al., 2013; He et al., 2016) demonstrated that Gln/ Glu affect piglets' performance, the effects of these supplements on piglets fed different nutritional levels of lys have not been studied. Thus, our objective is to evaluate the effects of Gln/Glu on growth performance and immune response of nursery pigs fed different digestible lys content.

Material and Methods ____

All procedures used were approved by the Animal Use Ethics Committee of Ethical Committee on Animal Use of Universidade Federal de Viçosa (protocol 041/2020).

Two hundred and sixteen barrow and female pigs (AGPIC 415 × Camborough), weaned at 21 days old and with initial body weights (BW) of 5.80 ± 0.73 kg, were used in a 21-day trial. Pigs were housed in suspended pens (1.70 × 1.20 m) in a commercialexperimental barn (Oratórios-MG, Brazil). Each pen housed six pigs (0.34 m²/pig) with free access to feed and water. The minimum and maximum temperatures inside the nursery room were 20.8 ± 2.49°C and 30.3 ± 1.79°C, respectively.

Pigs were assigned to a randomized block design according to their initial BW, in a 2 × 2 factorial arrangement with two levels of standardized ileal digestible (SID) lys and two levels of Gln/Glu (AminoGut, Ajinomoto do Brasil, São Paulo, Brazil, min 10% L-glutamine + min 10% L-glutamate), with nine replicates. Pigs were fed in a two-phase feeding, from 21 to 32 and 32 to 42 days. There were four dietary groups: (1) the control-lys diet, which consisted of 1.45% and 1.34% of SID lys



from 21 to 32 and 32 to 42 days, respectively, as recommended by Rostagno et al. (2017); (2) the control-lys + Gln/Glu diet, which was control-lys + 12 g kg⁻¹ of Gln/Glu; (3) the lowlys diet, with 1.30% and 1.21% of SID lys from 21 to 32 and 32 to 42 days, respectively, equivalent to 90% of the SID lys level of the control-lys diets; (4) low-lys + Gln/Glu diet, which was low-lys + 12 g kg⁻¹ of Gln/Glu (Table 1). Glutamine and glutamate were added to the diets in place of the starch. Amoxicillin (0.5 g kg⁻¹ of feed; Amoxan 50, Fatec, Brazil) was added on top of all diets from 21 to 32 d at the manufacturer's recommended rate for pigs and upon prescription.

Throughout the trial, feed was weighed before feeding and feed wastage was collected and weighed daily to determine the average daily feed intake (ADFI). Pigs were individually weighed at 21, 32, and 42 d to calculate the average daily gain (ADG). The feed conversion ratio (FC) was obtained by dividing ADFI by ADG. The incidence of diarrhea was visually assessed at 26, 29, 32 and, 35 d and was ranked as 0 = absence or 1 = presence for each pen.

At 42 days of age, blood was obtained from one piglet with body weight closest to the average weight of the experimental unit (pen) by orbital sinus puncture with a hypodermic needle (40 x 1.6 mm) and collected in 10 mL tubes without anticoagulants. Samples were immediately sent to the Viçosa Clinical Laboratory (Viçosa, MG - Brazil) for determination of serum urea nitrogen concentration (SUN; Ureal Cobas C311, Linklab, software PNCQ), immunoglobulin G (IgG Atellica CH IgG_2, CH Analyzer, Siemens Healthineers) and immunoglobulin A (IgA, 9D98-21 Reagent Kit, Architect cSystems).

Table 1

	21 to 32 da	ys of age ²	32 to 42 days of age		
Ingredients ¹	Control-lys	Low-lys	Control-lys	Low-lys	
Corn	352.6	353.2	392.0	392.3	
Soybean meal	176.4	181.0	220.0	224.0	
Dried whey	150.0	150.0	110.0	110.0	
Soybean Micronized	100.0	100.0	85.0	85.0	
Extrude corn	80.0	80.0	80.0	80.0	
Plasma protein	40.0	40.0	25.0	25.0	
Sugar	30.0	30.0	20.0	20.0	
Soybean oil	13.0	13.2	13.6	13.6	
Dicalcium phosphate	12.7	12.7	13.2	13.2	
Limestone	8.7	8.7	8.3	8.3	
Starch	12.0	12.0	12.0	12.0	
Fumaric acid	5.0	5.0	5.0	5.0	
Zinc Oxide	2.5	2.5	2.2	2.2	

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Anti-caking ³	3.0	3.0	2.0	2.0
Choline chloride	1.9	1.9	1.5	1.5
L-lysine	3.8	1.7	3.1	1.3
DL-methionine	2.3	1.4	1.7	0.9
L-threonine	2.5	1.4	1.9	0.8
L-valine	1.0	0.0	0.4	0.0
L-tryptophan	0.5	0.2	0.3	0.1
Salt	0.6	0.6	1.3	1.3
Vit-mineral premix	1.4	1.4	1.4	1.4
BHT	0.1	0.1	0.1	0.1
Calculated nutritional composition				
ME, kcal/kg	3,400	3,400	3,375	3,375
Crude protein, %	21.0	20.8	20.9	20.7
SID lysine⁴, %	1.451	1.305	1.346	1.211
SID threonine, %	0.972	0.874	0.902	0.811
SID met + Cys	0.813	0.731	0.754	0.678
SID tryptophan	0.276	0.248	0.256	0.230
SID valine	1.001	0.919	0.929	0.904
Calcium, %	0.850	0.850	0.825	0.825
Available P, %	0.504	0.504	0.469	0.469
Sodium, %	0.280	0.280	0.230	0.230
Lactose, %	11.25	11.25	8.25	8.25

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¹Experimental diets were obtained by adding 3glutamine and glutamate [AminoGut, Ajinomoto Biolatina, SP, Brazil. L-glutamine (min. 10%) and L-glutamate (min. 10%)] at 12 g kg⁻¹ in exchange to starch.

²Amoxilin (Amoxan 50, Fatec, Brazil) was added on top of all diets from 21 to 32 days of age.

³Tixosil[®] (Solvay, Brazil) prevent the formation of lumps (caking).

⁴SID = standardized ileal digestible.

The considered the pen was experimental unit for the analysis of performance data. One piglet from each pen was considered the experimental unit for analysis of serum results. The data were analyzed using the GLM procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC, USA). Means were compared using the Tukey test, and the effects were considered significant at P \leq 0.05. When there was an interaction, Gln/Glu was analyzed within control-lys and low-lys diets. Diarrhea score data were analyzed using

the FREQ procedure of SAS, in which the pen was considered the experimental unit, and the effects were considered significant by the chisquared (X²) test at $P \le 0.05$.

Results and Discussion _____

There was no interaction (P > 0.05) between lys level and Gln/Glu for any piglet performance variable from 21 to 32 days of age (Table 2). During the same phase, piglets



consuming control lys diets had higher (P < 0.01) ADG and 32-d BW and improved (P < 0.01) FC than those fed low-lys diets. However, there was no effect (P > 0.05) of lys

level on ADFI. In addition, there was no effect (P > 0.05) of Gln/Glu supplementation on piglet performance.

Table 2

Effects of glutamine and glutamate on growth performance of nursery pigs fed diets with different lysine content

	Control-lys ¹		Low-lys ²			P-value		
Items	0.0 g kg ⁻¹ Gln/Glu ³	12 g kg⁻¹ Gln/Glu	0.0 g kg⁻¹ Gln/Glu	12 g kg⁻¹ Gln/Glu	SEM	Lys	Gln/Glu	Lys× Gln/Glu
21 to 32 d								
Initial BW, kg	5.8	5.8	5.8	5.8	0.02	-	-	-
ADFI, g day ⁻¹	242	241	238	238	7.66	0.62	0.98	0.96
ADG, g day⁻¹	216	215	186	189	9.03	<0.01	0.89	0.79
FC	1.12	1.13	1.30	1.27	0.03	<0.01	0.78	0.63
32 d BW, kg	8.2	8.2	7.8	7.9	0.10	<0.01	0.91	0.82
21 to 42 d								
ADFI, g day ⁻¹	377	387	323	386	10.06	0.01	<0.01	0.01
ADG, g day⁻¹	287	311	263	287	8.38	<0.01	0.01	0.71
FC	1.32	1.24	1.23	1.38	0.02	0.47	0.36	<0.01
42 d BW, kg	11.8	12.3	11.3	11.7	0.17	<0.01	0.01	0.69

¹Following the recommendation of Rostagno et al. (2017); ²Lysine level of 90% of the recommendation of Rostagno et al. (2017); ³glutamine and glutamate [AminoGut, Ajinomoto Biolatina, SP, Brazil. L-glutamine (min. 10%) and L-glutamate (min. 10%)] at 12 g kg⁻¹ was added to the diets exchanging the starch.

From 21 to 42 day of age, there was an interaction (P < 0.01) between lys level and Gln/ Glu supplementation for ADFI and FC. Piglets provided low-lys diet supplemented with Gln/ Gluhadhigher (P < 0.05) ADFI and worse FC than low-lys piglets receiving no supplementation, whereas Gln/Glu supplementation did not significantly affect (P > 0.05) ADFI and FC of piglets fed control-lys diets.

There was no interaction (P > 0.05) between lysine level and Gln/Glu for ADG and 42 d BW in piglets from 21 to 42 days of age. However, piglets consuming control-lys diets had higher (P < 0.01) ADG and 42 d BW than piglets fed low-lys diets. Moreover, in the same period, the supplementation of Gln/Glu in the diets increased (P = 0.01) the ADG and 42 d BW of piglets, regardless of lysine level.

At 26 days of age, piglets consuming the low-lys diet not supplemented with Gln/Glu experienced a higher (P < 0.01) incidence of diarrhea than piglets fed the other diets (Table 3). However, at 29, 32, and 35 days of age, no diarrhea was observed (P > 0.05) in any pen.



Table 3

Effects of glutamine and glutamate on number of pens with diarrhea (total of nine) of nursery pigs fed diets with different lysine content

	Control-lys ¹		Low		
Days of age	0.0 g kg ⁻¹ Gln/Glu ³	12 g kg⁻¹ Gln/Glu	0.0 g kg⁻¹ Gln/Glu	12 g kg⁻¹ Gln/Glu	P-value
26	2/9	0/9	8/9	2/9	<0.01
29	1/9	2/9	1/9	2/9	0.84
32	1/9	2/9	1/9	1/9	0.49
35	0/9	1/9	1/9	0/9	0.54

¹Following the recommendation of Rostagno et al. (2017); ²Lysine level of 90% of the recommendation of Rostagno et al. (2017); ³glutamine and glutamate [AminoGut, Ajinomoto Biolatina, SP, Brazil. L-glutamine (min. 10%) and L-glutamate (min. 10%)] at 12 g kg⁻¹ was added to the diets exchanging the starch.

There was no interaction (P > 0.05) between lys level and Gln/Glu in blood analysis results (Table 4). Piglets consuming controllys diets had higher (P < 0.05) SUN than low-lys piglets. Similarly, those supplemented with Gln/ Glu had higher (P < 0.01) SUN than those not supplemented. Piglets consuming control-lys diets had higher (P < 0.05) IgG concentrations than those fed low-lys diets and Gln/Glu did not affect (P > 0.05) IgG. In addition, IgA was not affected (P > 0.05) by any diet.

Table 4

Effects of glutamine and glutamate on serum parameters of nursery pigs at 42 days of age fed diets with different lysine content

	Control-lys ¹		Low-lys ²			P-value		
Items	0.0 g kg ⁻¹ Gln/Glu ³	12 g kg⁻¹ Gln/Glu	0.0 g kg⁻¹ Gln/Glu	12 g kg⁻¹ Gln/Glu	SEM	Lys	Gln/Glu	Lys× Gln/Glu
Urea, mg dL ⁻¹	18.2	23.3	16.0	19.1	1.37	0.04	<0.01	0.65
lgG, mg dL ⁻¹	229.0	202.0	179.6	170.1	20.29	0.05	0.37	0.66
lgA, mg dL ⁻¹	6.9	7.3	6.7	7.1	0.56	0.71	0.46	0.98

¹Following the recommendation of Rostagno et al. (2017); ²Lysine level of 90% of the recommendation of Rostagno et al. (2017); ³glutamine and glutamate [AminoGut, Ajinomoto Biolatina, SP, Brazil. L-glutamine (min. 10%) and L-glutamate (min. 10%)] at 12 g kg⁻¹ was added to the diets exchanging the starch.

Glutamine and glutamate are involved in crucial metabolic functions and may be considered essential amino acids for weaned piglets. However, study results have been inconsistent regarding the effects of supplementation of these amino acids on piglet performance. We hypothesized that Gln/ Glu performance improvement would be more



evident in piglets fed a diet providing poorer nutrition. Thus we supplemented Gln/Glu (0.0 or 12 g kg⁻¹) in diets formulated according to the ideal amino acid profile recommended by the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017) and in diets formulated with only 90% of the recommended lysine, also following the ideal amino acid profile.

At 26 days of age, piglets consuming the low-lys diet experienced significantly more diarrhea than pigs consuming the low-lys diet supplemented with Gln/Glu. Besides lys, the low-lys diet was deficient in other essential amino acids, which may have caused intestinal disorders (Wang, Qiao, & Li, 2009; Mou, Yang, Yin, & Huang, 2019). Otherwise, the results would indicate that Gln/Glu helps prevent gut disorder. This observation is consistent with other studies that associated reduced post-weaning diarrhea with glutamine and glutamate supplementation (Cabrera et al., 2013; Teixeira et al., 2014). These two amino acids are major metabolic fuels for enterocytes and are key regulators of gene expression that maintain the intestinal mucosa's structural and functional integrity (Rhoads & Wu, 2009; Duan et al., 2016).

However, the observed diarrhea did not alter the piglets' performance from 21 to 32 days of age once there were no Gln/Glu supplementation effects on performance, independent of the lys level. According to Liu et al. (2018), the efficiency of additives on weaned piglet diets may be influenced by multiple factors, such as the health status of the animals and the use of other feed additives. Concerning these additives, in the present study, the absence of Gln/Glu's effect on performance from 21 to 32 days of age may be linked to the use of amoxicillin, fumaric acid, and zinc oxide. These additives are known to improve performance and gut health (Vandael et al., 2020; Bonetti, Tugnoli, Piva, & Grilli, 2021). However, because our study was conducted on a commercial-experimental farm, we had to use practical diets that followed the standards adopted by the pig farm.

In contrast, during the same growth phase, the piglets fed the control-lys diets performed better than those fed low-lys diets. As lys is the first limiting amino acid in pig diets, the ideal protein is set in ratio to lys and pig diets are usually formulated to contain a specific level of lys to ensure an adequate supply of other amino acids (Boisen, 1997; Ball et al., 2013). Thus, these results were expected once we utilized 90% of the lys recommendation found in the Brazilian Tables (Rostagno et al., 2017). Other studies evaluating lys levels on piglet performance during the first nursery phase also reported that levels below 14.0 g kg-1 of SID lys led to poorer ADG, ADFI, and FC of the animals (Oresanya, Beaulieu, Beltranena, & Patience, 2006; Taylor, Toplis, Wellock, & Miller, 2012; Zhou et al., 2018).

From 21 to 42 d old, Gln/Glu supplementation improved the ADFI of pigs fed the low-lys diets, resulting in a higher ADG and BW; however, worse FC. Altogether, these results indicate that Gln/Glu is beneficial once a higher ADFI and ADG are targeted in the initial phases. Gln/Glu supplementation also improved ADG and BW at 42 d in the controllys group.

Previous studies also observed several benefits of adding dietary glutamine and glutamate on piglet performance (Teixeira et al., 2014; He et al., 2016; Johnson & Lay, 2017). Assessing four levels of Gln/Glu supplementation (0, 5, 10, and 15 g kg⁻¹) on



the performance of weaned piglets, Teixeira et al. (2014) reported that 10 g kg⁻¹ dietary supplementation improved the ADG, ADFI, and feed efficiency of piglets from 21 to 42 days of age. He et al. (2016) observed improvements in the performance of weaned piglets fed 10 g kg⁻¹ glutamine for 28 days during the nursery phase. In addition, Johnson and Lay (2017) reported that piglets fed diets with 2 g kg⁻¹ of L-glutamine presented greater body weight and feed intake than those fed diets with or without an antibiotic as a growth promoter.

Regarding the lys level, from 21 to 42 days of age, pigs fed the control-lys had higher ADG and 42 d BW than those fed the low-lys diet. The SID lys level used in the control-lys was 14.5 and 13.5 g kg⁻¹ (21 to 32 and 32 to 42 days of age, respectively) as recommended in the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017). This level is similar to that recommended in the NRC (2012): 15.0 and 13.5 g kg⁻¹ in similar phases. Thus, it may be assumed that the reduction in 10% of dietary lys affected the supply of amino acids, which was reflected in poorer performance, and that the low-lys diets (13.5 and 12.1 g kg⁻¹ from 21 to 32 and 32 to 42 d old, respectively) were nutritionally inadequate for pigs in that phase.

In the present study, the SUN concentrations at 42 days of age were consistent with the higher CP levels in the diets, indicating greater catabolism of amino acids as piglets are subjected to a greater nitrogen supply (Fang et al., 2019).

There was no effect of Gln/Glu on IgG and IgA concentrations. However, pigs consuming control-lys diets had higher IgG than those fed low-lys (P < 0.05; marginal means 215 vs. 175 mg dL⁻¹). The humoral immune response results from B-cell activation,

which results in the production of specific immunoglobulins (Halas & Nochta, 2012). IgA and IgG immunoglobulins are directly involved in the immune response against infections and pathogens. Thus, the lower serum IgG concentration in piglets fed a low-lys diet may indicate that control-lys diets provided an adequate amount of amino acids to enhance the humoral immunity of weanling piglets (Liao, Wang, & Regmi, 2015). The reason for this response is worthy of future investigation.

Contrary to our initial hypothesis, glutamine and glutamate improved performance independent of the diet's nutritional level, contributing to a nutritional strategy that can help to reduce the challenges associated with piglet weaning. Additionally, the current study results indicate that substantial consideration must be given to the dietary lys levels in weaned diets and reinforce the importance of nutritional manuals as guides in piglet nutrition.

Conclusions _

Dietary supplementation of glutamine and glutamate (Gln/Glu) improved the growth performance of weaned piglets from 21 to 42 days of age, regardless of the diets' lys levels. In addition, reducing lys levels 10% below the requirement negatively affects the growth performance and the immune response of nursery piglets.

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