

# Dietary supplementation with glutamine or glutamic acid for weanling piglets

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**Abstract** – The objective of this work was to evaluate the effects of dietary glutamine and glutamic acid supplementation on carbon turnover ( $\delta^{13}\text{C}$ ) in the muscles of piglets, on animal performance, and on feed economic feasibility. The diets consisted of: control (C), 1% glutamine (G), and 1% glutamic acid (GA). In experiment I, 111 piglets were used to assess carbon turnover in the *Masseter*, *Psoas major*, and *Abdominal internal oblique* muscles in terms of their isotopic composition and  $^{13}\text{C}$  substitution over time. In experiment II, 72 piglets were assigned randomly to blocks (eight replicates per treatment and three animals per experimental unit), in order to evaluate animal performance and feed economic feasibility. The GA diet promoted the best  $^{13}\text{C}$  acceleration in the studied muscles. No diet effects were observed on performance variables. The G and GA diets presented higher costs than the C diet. Although supplementation with 1% glutamine or glutamic acid accelerates carbon turnover in the studied muscles, which suggests a faster recovery during post-weaning and proves the anabolic effect of these additives, its use is not economically viable for weanling piglets.

**Index terms:** *Sus scrofa*, additives, anabolic effect, carbon turnover, economic feasibility, stable isotopes.

## Suplementação dietética de glutamina ou ácido glutâmico para leitões recém-desmamados

**Resumo** – O objetivo deste trabalho foi avaliar os efeitos da suplementação dietética de glutamina e ácido glutâmico no turnover do carbono ( $\delta^{13}\text{C}$ ) em músculos de leitões, no desempenho dos animais e na viabilidade econômica das rações. As dietas consistiram em: controle (C), 1% de glutamina (G) e 1% de ácido glutâmico (AG). No experimento I, 111 leitões foram usados para avaliar o turnover do carbono nos músculos *Masseter*, *Psoas major* e *Abdominal internal oblique*, quanto à composição isotópica e à substituição do  $^{13}\text{C}$  no tempo. No experimento II, 72 leitões foram alocados em blocos ao acaso (oito repetições por tratamento e três animais por unidade experimental), para avaliar o desempenho dos animais e a viabilidade econômica das rações. A dieta AG promoveu a melhor aceleração de  $^{13}\text{C}$  nos músculos estudados. Não se constatou efeito das dietas nas variáveis de desempenho. As dietas G e AG apresentaram maiores custos que a dieta C. Embora a suplementação com 1% de glutamina ou ácido glutâmico acelere o turnover do carbono nos músculos estudados, o que sugere rápida recuperação no pós-desmame e comprova o efeito anabólico desses aditivos, seu uso não é economicamente viável para leitões recém-desmamados.

**Termos para indexação:** *Sus scrofa*, aditivos, efeito anabólico, turnover de carbono, viabilidade econômica, isótopos estáveis.

### Introduction

The technification of swine production and the reduction of the lactation period have increased the number of piglets produced per sow per year. Currently, piglets are early-weaned around three weeks of age in Brazil, a fact that requires attention due to their nutritional requirements and digestive system immaturity. The composition of piglet diets is

altered at weaning, once milk is replaced by a solid diet, predisposing animals to nutritional stress which may result in feed intake reduction, poor digestibility, and diarrhea (Sulabo et al., 2010). The reduced secretion of hydrochloric acid by the piglet's stomach and the limited production and activity of pancreatic and intestinal enzymes are also an issue of concern (Heo et al., 2013).



During the first post-weaning days, piglets do not consume enough feed to meet their nutritional requirements and, as a consequence, their body weight does not increase. This may affect economically pig production and has a detrimental effect on future growth performance (McLamb et al., 2013).

Previous studies have assessed a potential role for dietary glutamine and glutamic acid as performance-enhancing additives for weaned pigs. Glutamine, among other functions, acts as metabolic fuel for the fast-turnover cells, and glutamic acid replaces glutamine in several of its roles, including those of energy generation and amino acid synthesis (Watford, 2015), ultimately promoting better performance of weaned pigs (Bünzen et al., 2008).

The use of stable isotope ratio analysis as a tool for the characterization of an animal's diet by tracing the feeding system has drawn attention in swine meat production. Commonly,  $C_3$  and  $C_4$  plants are distinguished based on Kranz and non-Kranz anatomy. The  $C_4$  plants result in  $^{13}C$ -enrichment (i.e., elevated carbon isotopes ratio), compared with  $C_3$  plants, and, by examining the  $^{13}C/^{12}C$  ratio, it is possible to determine whether animals predominantly ate  $C_3$  or  $C_4$  plants. Moreover, the isotope dilution technique provides accurate estimates of an element replacement over time in different tissues. The intake of diets and subsequent proportion of  $\delta^{13}C$  in the metabolism, different from that observed in animal tissue, result in the isotopic dilution of carbon in this tissue and ultimately leads to a new steady-state ratio of carbon isotopes (O'Brien et al., 2015). In addition to the aforementioned, the search for new additives that improve piglets' performance should also be considered.

The objective of this work was to evaluate the effects of dietary glutamine and glutamic acid supplementation on carbon turnover ( $\delta^{13}C$ ) in the muscles of piglets, on animal performance, and on feed economic feasibility.

## Materials and Methods

The research was carried out at Universidade Estadual Paulista Júlio de Mesquita Filho (Unesp), with approval of the Animal Ethics Committee of this institution (protocol No. 159/2013) and in alignment with the directive 2010/63/EU.

A total of 183 weanling piglets, females and castrated males of a crossbred commercial lineage (Landrace x

Large White), were housed in a nursery facility with a ceiling height of 3.5 m, side curtains, and suspended metal pens of 1.0x1.75 m, equipped with one feeder, one nipple-type drinker, and one heater. The pens had partially slatted plastic floors, and the heater was placed on a compact concrete floor. The internal temperature of the nursery facility was controlled by adjusting the side curtains and managing the heaters. Throughout the trial, the maximum and minimum average temperature recorded were  $25.7\pm 2.0$  and  $18.0\pm 1.2^\circ C$ , respectively, and the average relative humidity was 65%.

Piglets were fed ad libitum within a phase feeding program to attend their nutritional requirements, according to Rostagno et al. (2011), as follows: pre-starter (21 to 35 days of age), starter I (36 to 50 days of age), and starter II (51 to 80 days of age) diets. The evaluated treatments were: additive-free diet – control (C), diet containing 1% glutamine (G), and diet containing 1% glutamic acid (GA), as shown in Tables 1 and 2 (experiment I).

The main energy source of these diets was rice grits, a raw ingredient from the  $C_3$ -photosynthetic pathway plant with a  $^{13}C$ -isotopic signal distinct from those of diets provided for sows, because gestation and lactation diets primarily contained corn as an energy source (a  $C_4$ -photosynthetic pathway plant).

A total of 111 weaned piglets with an average age of 21 days and  $6.27\pm 0.13$  kg of initial body weight were allocated randomly into blocks with three treatments. At 1, 2, 4, 5, 7, 9, 13, 20, 27, 35, 48, and 59 days after weaning, three piglets per treatment were slaughtered after electronarcosis. At the baseline (day 0), three piglets were also slaughtered, in order to express the isotopic composition of their tissue, which was a result of the diets provided to sows during the gestation and lactation periods. The sampling procedures were concentrated in the first days of the experimental period due to the higher speed of  $^{13}C$  isotopic incorporation into the muscles.

After slaughter, 4-cm<sup>2</sup> segments were subsampled, washed with deionized water and packed into plastic bags, labeled, and immediately frozen ( $-18^\circ C$ ) for further isotopic analyses. In order to perform isotopic analyses, *Masseter*, *Psoas major*, and *Abdominal internal oblique* muscle samples were previously defrosted and dried in a MA-035/5 circulating-air oven (Marconi Equipamentos para Laboratório Ltda., Piracicaba, SP, Brazil) at  $56^\circ C$  for 24-hour.

As the lipid fraction might cause isotopic fractionation up to 5‰ of the  $^{13}\text{C}$ -values (Piasentier et al., 2003), the samples were degreased with ethyl ether (chemically pure) at 65°C for 4 hours and then subjected to cryogenic milling (SPEX SamplePrep, 2010 Geno/Grinder, Metuchen, NJ, USA) for 8 min at high speed (9,700 rpm) under -190°C in order to obtain homogeneous material (particle size <60 µm).

Then, all samples were weighed (50 to 70 µg) into tin capsules for the quantification of carbon isotopes

( $^{13}\text{C}/^{12}\text{C}$ ) by isotope ratio mass spectrometry in the Finnigan Mat Delta-S equipment coupled to the EA-1108 CNHS elemental analyzer (Thermo Scientific Inc., Waltham, MA, USA) at Centro de Isótopos Estáveis of Unesp.

The data expressed the  $\delta^{13}\text{C}$  notation in relation to Pee Dee Belemnite (PDB), an international standard, with analysis deviation at the order of 0.2‰;  $\delta^{13}\text{C}$  were calculated by the equation:  $\delta^{13}\text{C}_{(\text{sample, standard})} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$ , in which  $\delta^{13}\text{C}$  is the enrichment of

**Table 1.** Centesimal composition of the pre-starter, starter I, and starter II diets for piglets.

| Ingredients                | Pre-starter diets |       |       | Starter I diets |       |       | Starter II diets |       |       |
|----------------------------|-------------------|-------|-------|-----------------|-------|-------|------------------|-------|-------|
|                            | C                 | G     | GA    | C               | G     | GA    | C                | G     | GA    |
| Rice grits                 | 57.41             | 56.41 | 56.41 | 60.51           | 59.51 | 59.51 | 64.25            | 63.25 | 63.25 |
| Soybean meal 46%           | 20.00             | 20.00 | 20.00 | 25.00           | 25.00 | 25.00 | 30.00            | 30.00 | 30.00 |
| Whey concentrate protein   | 6.80              | 6.80  | 6.80  | 3.70            | 3.70  | 3.70  | -                | -     | -     |
| Maltodextrin               | 6.66              | 6.66  | 6.66  | 3.17            | 3.17  | 3.17  | -                | -     | -     |
| Corn gluten meal 60%       | 2.60              | 2.60  | 2.60  | 1.69            | 1.69  | 1.69  | 1.30             | 1.30  | 1.30  |
| Soybean-oil                | 1.48              | 1.48  | 1.48  | 1.53            | 1.53  | 1.53  | 1.50             | 1.50  | 1.50  |
| Glutamine 99%              | -                 | 1.00  | -     | -               | 1.00  | -     | -                | 1.00  | -     |
| Glutamic acid 98.5%        | -                 | -     | 1.00  | -               | -     | 1.00  | -                | -     | 1.00  |
| Dicalcium phosphate        | 1.25              | 1.25  | 1.25  | 1.50            | 1.50  | 1.50  | 1.23             | 1.23  | 1.23  |
| Limestone                  | 1.03              | 1.03  | 1.03  | 0.90            | 0.90  | 0.90  | 0.83             | 0.83  | 0.83  |
| Salt                       | 0.59              | 0.59  | 0.59  | 0.62            | 0.62  | 0.62  | 0.46             | 0.46  | 0.46  |
| L-lysine HCl 78.4%         | 0.77              | 0.77  | 0.77  | 0.55            | 0.55  | 0.55  | 0.09             | 0.09  | 0.09  |
| DL-methionine 99%          | 0.23              | 0.23  | 0.23  | 0.21            | 0.21  | 0.21  | -                | -     | -     |
| L-theonine 98%             | 0.31              | 0.31  | 0.31  | 0.22            | 0.22  | 0.22  | -                | -     | -     |
| L-tryptophan 99%           | 0.06              | 0.06  | 0.06  | 0.02            | 0.02  | 0.02  | -                | -     | -     |
| L-valine 96.5%             | 0.11              | 0.11  | 0.11  | 0.03            | 0.03  | 0.03  | -                | -     | -     |
| ZnO 77%                    | 0.34              | 0.34  | 0.34  | -               | -     | -     | -                | -     | -     |
| Choline chloride 60%       | 0.07              | 0.07  | 0.07  | 0.07            | 0.07  | 0.07  | 0.07             | 0.07  | 0.07  |
| Antioxidant <sup>(1)</sup> | 0.02              | 0.02  | 0.02  | 0.02            | 0.02  | 0.02  | 0.02             | 0.02  | 0.02  |
| Premixes <sup>(2)</sup>    | 0.25              | 0.25  | 0.25  | 0.25            | 0.25  | 0.25  | 0.25             | 0.25  | 0.25  |
| Sweetener <sup>(3)</sup>   | 0.02              | 0.02  | 0.02  | 0.02            | 0.02  | 0.02  | 0.02             | 0.02  | 0.02  |

<sup>(1)</sup>B.H.T., Butylated hydroxytoluene. <sup>(2)</sup>Mineral and vitamin premixes, supplied per kg of diet: 100 mg Fe, 10 mg Cu, 40 mg Mn, 100 mg Zn, 1 mg Co, 1.5 mg I, 0.78 mg Se, 10,000 IU vitamin A, 2,000 IU vitamin D3, 0.2 mg biotin, 30 mg niacin, 100 mg calcium pantothenate, 30 µg vitamin B12, 6 mg vitamin B2, 50,000,000 IU vitamin E, and 2 mg vitamin K3. <sup>(3)</sup>Composed of sodium saccharin, neohesperidin, and silicon dioxide. C, control diet; G, diet containing 1% glutamine; and GA, diet containing 1% glutamic acid. Nutritional values of ingredients proposed by Rostagno et al. (2011). Phase feeding program of piglets from experiment I (pre-starter, 21 to 35 days old; starter I, 36 to 50 days old; starter II, 51 to 80 days old) and experiment II (pre-starter, 21 to 35 days old; starter I, 36 to 50 days old; and starter II, 51 to 55 days old).

the isotopic ratio  $^{13}\text{C}/^{12}\text{C}$  of the sample to the standard; and R represents the ratio of the heavier ( $^{13}\text{C}$ ) to the lighter ( $^{12}\text{C}$ ) stable isotopes (dimensionless).

In order to evaluate the  $^{13}\text{C}$ -substitution speed of the samples, the following exponential function over time was employed (Ducatti et al., 2014):  $\delta^{13}\text{C}_{(t)} = \delta^{13}\text{C}_{(f)} + [\delta^{13}\text{C}_{(i)} - \delta^{13}\text{C}_{(f)}] e^{-kt}$ , in which  $\delta^{13}\text{C}_{(t)}$  is the isotopic enrichment of the tissue at any time (t);  $\delta^{13}\text{C}_{(f)}$  is the isotopic enrichment of the tissue at the equilibrium or final condition;  $\delta^{13}\text{C}_{(i)}$  is the isotopic enrichment of the tissue at the beginning; k is the turnover constant, in units of  $\text{time}^{-1}$ ; and t is the time ( $\text{days}^{-1}$ ) since diet substitution.

The half-life of carbon ( $T_{50\%}$ ) in muscles at  $t = T$  necessary for initial atom substitution by the final atoms was determined by the equation:  $t = (-1 / k) \ln(1 - F)$ , in which t is the time of the initial atom substitution until the final substitution (days); ln is the Naperian logarithm (natural); F is the value of atom substitution, which can vary among 0.0 and 0.99; and k is the turnover constant ( $\text{day}^{-1}$ ) (Ducatti et al., 2014).

The calibration of the mass spectrometer was performed with  $\text{CO}_2$  6.0 (99.999%) from a cylinder, which was previously calibrated with the official reference material IVA-33802174 (IVA Analysentechnik e. K., Meerbusch, Germany) with standard deviation for measurements of 0.2‰. The  $^{13}\text{C}/^{12}\text{C}$  standard isotope ratio was expressed as the delta ( $\delta$ ) per mil (‰) value relative to the V-PDB primary standard. At the beginning of each run, two pulses of  $\text{CO}_2$  reference gas were admitted into the inlet system for about 20 s. The constant flow rate during this period has given peaks a flat-top appearance. The level of  $\text{CO}_2$  corresponding to 5 V at m/z 44 was used to calibrate the system.

In addition, an internal working standard (coal) was used with an analytical uncertainty of measurement of  $-28 \pm 0.2\%$  for carbon isotopes. It was calibrated versus IVA-33802174 with a certified value of  $\delta^{13}\text{C}_{\text{V-PDB}} = -39.73 \pm 0.16\%$ . The samples were analyzed in duplicate, and the standards were analyzed in four replicates with values averaged. The precision of the method was set at 12‰, which refers to the amplitude of isotopic

**Table 2.** Calculated dietary composition of the pre-starter, starter I, and starter II diets of piglets<sup>(1)</sup>.

| Diet component   | Pre-starter diet |        |        | Starter I diet |        |        | Starter II diet |        |        |
|--|------------------|--------|--------|----------------|--------|--------|-----------------|--------|--------|
|  | C                | G      | GA     | C              | G      | GA     | C               | G      | GA     |
| Metabolizable energy <sup>(2)</sup> (MJ kg <sup>-1</sup> ) | 14.235           | 14.235 | 14.235 | 14.164         | 14.164 | 14.164 | 14.109          | 14.109 | 14.109 |
| Crude protein (%)  | 19.00            | 19.00  | 19.00  | 19.55          | 19.55  | 19.55  | 19.90           | 19.90  | 19.90  |
| Digestible lysine (%)                                      | 1.45             | 1.45   | 1.45   | 1.33           | 1.33   | 1.33   | 1.01            | 1.01   | 1.01   |
| Digestible methionine (%)                                  | 0.52             | 0.52   | 0.52   | 0.50           | 0.50   | 0.50   | 0.31            | 0.31   | 0.31   |
| Digestible valine (%)                                      | 1.00             | 1.00   | 1.00   | 0.92           | 0.92   | 0.92   | 0.20            | 0.20   | 0.20   |
| Digestible theonine (%)                                    | 0.91             | 0.91   | 0.91   | 0.84           | 0.84   | 0.84   | 0.64            | 0.64   | 0.64   |
| Digestible tryptophan (%)                                  | 0.26             | 0.26   | 0.26   | 0.24           | 0.24   | 0.24   | 0.23            | 0.23   | 0.23   |
| Lactose-equivalent (%)                                     | 10.00            | 10.00  | 10.00  | 5.01           | 5.01   | 5.01   | -               | -      | -      |
| Ca (%)   | 0.82             | 0.82   | 0.82   | 0.83           | 0.83   | 0.83   | 0.72            | 0.72   | 0.72   |
| Digestible P (%)   | 0.45             | 0.45   | 0.45   | 0.45           | 0.45   | 0.45   | 0.34            | 0.34   | 0.34   |

<sup>(1)</sup>C, control diet; G, diet containing 1% glutamine; and GA, diet containing 1% glutamic acid. Nutritional values of ingredients proposed by Rostagno et al. (2011). Phase feeding program of piglets from experiment I (pre-starter, 21 to 35 days old; starter I, 36 to 50 days old; starter II, 51 to 80 days old) and experiment II (pre-starter, 21 to 35 days old; starter I, 36 to 50 days old; and starter II, 51 to 55 days old). <sup>(2)</sup>3,400, 3,383, and 3,370 kcal kg<sup>-1</sup> for the pre-starter, starter I, and starter II diets, respectively.

signals between C<sub>3</sub>- and C<sub>4</sub>-plants. The certainty of the method was set as 0.2‰ standard deviation.

The data of the carbon isotopic enrichment ( $\delta^{13}\text{C}$ , ‰) over time were adjusted to the first-order equation by a nonlinear regression analysis plotted by the OriginPro software, version 8.0 (OriginLab Corporation, Northampton, MA, USA).

For the evaluation of growth performance and feed economic feasibility (experiment II), 72 weaned piglets with an average age of 21 days and  $7.24 \pm 1.37$  kg of initial body weight were randomly assigned to blocks with eight replicates per treatment and three animals per experimental unit. The animals were subjected to the same management practices of experiment I and to a phase feeding program according to Rostagno (2011), as follows: pre-starter (21 to 35 days of age), starter I (36 to 50 days of age), and starter II (51 to 55 days of age) diets (Tables 1 and 2).

The variables evaluated were: daily feed intake, daily weight gain, and the feed:gain ratio. The growth performance results were evaluated in four periods: when the piglets were 21 to 28, 21 to 35, 21 to 48, and 21 to 55 days old. Data were subjected to the analysis of variance using the mixed procedure of the SAS software, v.9.2 (SAS Institute Inc., Cary, NC, USA).

Evaluation of feed economic feasibility considered the cost of a kilogram of feed, the feed costs per kilogram of weight gain, the economic efficiency index (EEI) and the cost index (CI) for each treatment, following the methodology described by Gomes et al. (2012):  $\text{EEI} = (\text{FCe} \times 100) / \text{CTei}$ ;  $\text{CI} = (\text{CTei} \times 100) / \text{FCe}$ , in which FCe is the lowest feed cost per kilogram of weight gain observed among treatments, and CTei is the cost of the considered treatment.

The feed costs and prices of the ingredients used in feed formulation were those practiced in the municipality of Botucatu, in the state of São Paulo, Brazil, in May, 2017, which were converted to US\$ using the monthly average dollar exchange rate.

## Results and Discussion

The  $\delta^{13}\text{C}$  mean values of the *Masseter*, *Psoas major*, and *Abdominal internal oblique* muscles of piglets at weaning day (-16.93, -15.54, and -15.70‰, respectively) analyzed before the beginning of experiment I were similar to those of the feed provided to sows at the

lactation period (-16.14‰), reflecting the isotopic signal of corn (C<sub>4</sub> plant).

The trial duration (59 days) was not enough for  $^{13}\text{C}$  turnover of muscles to reach the equilibrium plateau in control diets; however, diets containing additives proved their trophic action, accelerating  $^{13}\text{C}$  incorporation in muscles, resulting in the observed lower half-life values (Table 3).

Halley & Soffe (2016) reported that the growth rate of different body tissues varies according to the animal's growth and physiological maturity. A mean difference of about 45 days on the *Abdominal internal oblique* muscle  $^{13}\text{C}$ -turnover in the control diet was observed in comparison with the other diets (G and AG); however, between these diets, there were only minor differences ( $T_{50\%} = 47.74$  and 46.13 days, respectively).

Muscle tissues have different mechanical and metabolic functions, acting as a source of amino acids to other tissues, and all muscles have a high content of glutamine. The predominant oxidative metabolism of muscle fibers obtain energy through various reserves as free fatty acids, amino acids, creatine phosphate, and glycogen (Listrat et al., 2016); however, the muscle of the predominant oxidative metabolism contains nearly twice the amount of free glutamine in relation to muscles of the predominant glycolytic metabolism (Glancy & Balaban, 2011).

The *Abdominal internal oblique* muscle is characterized as a predominant oxidative muscle, and the supplementation with glutamine and glutamic acid increased amino acid availability, which may have

**Table 3.** Isotopic enrichments ( $\delta^{13}\text{C}$ ) over time and half-life values ( $T_{50\%}$ ) of the muscles of piglets by diets.

| Diet                              | Equations  | R <sup>2</sup> | T <sub>50%</sub><br>(days) |
|-----------------------------------|--|----------------|----------------------------|
| <i>Abdominal internal oblique</i> |  |                |                            |
| Control                           | $\delta^{13}\text{C} = -42.90 + 27.33e^{-0.00752t}$  | 0.97           | 92.18                      |
| 1% glutamine                      | $\delta^{13}\text{C} = -32.77 + 17.45e^{-0.014519t}$ | 0.94           | 47.74                      |
| 1% glutamic acid                  | $\delta^{13}\text{C} = -32.55 + 17.07e^{-0.015014t}$ | 0.92           | 46.13                      |
| <i>Psoas major</i>                |  |                |                            |
| Control                           | $\delta^{13}\text{C} = -68.00 + 52.46e^{-0.003425t}$ | 0.94           | 202.37                     |
| 1% glutamine                      | $\delta^{13}\text{C} = -36.28 + 20.89e^{-0.010651t}$ | 0.96           | 65.07                      |
| 1% glutamic acid                  | $\delta^{13}\text{C} = -74.28 + 17.53e^{-0.013462t}$ | 0.93           | 51.49                      |
| <i>Masseter</i>                   |  |                |                            |
| Control                           | $\delta^{13}\text{C} = -64.13 + 48.73e^{-0.003625t}$ | 0.98           | 191.19                     |
| 1% glutamine                      | $\delta^{13}\text{C} = -36.05 + 20.88e^{-0.010521t}$ | 0.92           | 65.88                      |
| 1% glutamic acid                  | $\delta^{13}\text{C} = -31.82 + 16.73e^{-0.014459t}$ | 0.94           | 47.94                      |

occurred due to a better energy supply, accelerating the  $^{13}\text{C}$  turnover in this muscle (Table 3); the same was observed for *Psoas major* and *Masseter* muscles in this study. The  $^{13}\text{C}$  half-life values of the C diet were lower than those of the other diets, on average, about 144 days for the *Psoas major* muscle and 134 days for the *Masseter* muscle.

Glutamine has carbon, oxygen, nitrogen, and hydrogen in its chemical formula, and it represents the most abundant free amino acid in extracellular fluid, serving as fuel for the metabolism of fast-turnover cells (Cruzat & Newsholme, 2017). Caldara et al. (2008) reported that glutamine supplementation in the diets of growing and finishing pigs has accelerated the  $^{13}\text{C}$  turnover of the *Psoas major* and *Masseter* muscles; therefore, glutamine has exerted anabolic stimulus on these tissues.

The diet containing 1% glutamic acid has promoted greater  $^{13}\text{C}$  incorporation in the *Psoas major* and *Masseter* muscles (Table 3) due to their lower  $^{13}\text{C}$  half-life values ( $T_{50\%} = 51.49$  and 47.94 days, respectively).

Glutamate is responsible for 40% of glutamine synthesis (Yelamanchi et al., 2016) when in the diet,

and can easily replace glutamine in several metabolic pathways (Watford, 2015). Besides that, Zai et al. (2012) found that 95% of glutamate is metabolized by intestinal mucosa, being a highly oxidative substrate that serves as a precursor to other biologically active molecules such as ATP or can be converted into other amino acids, as glutamine, ornithine, and aspartate. This fact may explain glutamic acid action on  $^{13}\text{C}$  incorporation in the studied muscles.

The *Abdominal internal oblique* muscle had faster  $^{13}\text{C}$  replacement compared with the other studied muscles. Listrat et al. (2016) explained that intermuscular variation is usually related to metabolic and contractile properties determined by the type of fiber distribution in the muscle. Almeida et al. (2012) stated that the skeletal muscle is a heterogeneous tissue, composed mainly of connective, vascular, and nerve fibers, but that, depending on its location and function, may have differ contents.

Different  $^{13}\text{C}$  half-life values in muscles were obtained for other species. Cruz et al. (2005) reported  $^{13}\text{C}$  half-life values of 5 to 8 days for the *Pectoralis major* muscle in broilers aged 1 to 30 days. Similar

**Table 4.** Performance variables of piglets fed experimental diets.

| Variable                      | Diet    |           |               | Coefficient of variation (%) | p-value <sup>(1)</sup> |
|-------------------------------|---------|-----------|---------------|------------------------------|------------------------|
|                               | Control | Glutamine | Glutamic acid |                              |                        |
| Mean initial body weight (kg) | 7.23    | 7.24      | 7.23          |                              |                        |
| Mean final body weight (kg)   | 22.65   | 21.76     | 22.09         |                              |                        |
| Period 1 (21 to 28 days old)  |         |           |               |                              |                        |
| DFI (g per day)               | 0.289   | 0.278     | 0.271         | 13.41                        | 0.6351                 |
| DWG (g per day)               | 0.201   | 0.205     | 0.202         | 13.34                        | 0.9501                 |
| F:G                           | 1.38    | 1.35      | 1.33          | 7.04                         | 0.5965                 |
| Period 2 (21 to 35 days old)  |         |           |               |                              |                        |
| DFI (g per day)               | 0.443   | 0.434     | 0.419         | 9.70                         | 0.5166                 |
| DWG (g per day)               | 0.305   | 0.293     | 0.282         | 9.25                         | 0.2886                 |
| F:G                           | 1.45    | 1.48      | 1.48          | 7.57                         | 0.8135                 |
| Period 3 (21 to 48 days old)  |         |           |               |                              |                        |
| DFI (g per day)               | 0.651   | 0.615     | 0.619         | 7.40                         | 0.2725                 |
| DWG (g per day)               | 0.403   | 0.373     | 0.377         | 6.92                         | 0.0903                 |
| F:G                           | 1.62    | 1.65      | 1.64          | 4.77                         | 0.7637                 |
| Period 4 (21 to 55 days old)  |         |           |               |                              |                        |
| DFI (g per day)               | 0.765   | 0.727     | 0.739         | 5.94                         | 0.2429                 |
| DWG (g per day)               | 0.453   | 0.427     | 0.437         | 5.73                         | 0.1530                 |
| F:G                           | 1.67    | 1.70      | 1.69          | 3.04                         | 0.8863                 |

<sup>(1)</sup>Tukey's test, at 5% probability. DFI, daily feed intake; DWG, daily weight gain; and F:G, feed:gain ratio.

results average  $^{13}\text{C}$  half-life value of 3.82 days were found by Ducatti et al. (2014) for the *Pectoralis major* muscle of broilers at different periods of growth. Bahar et al. (2009), studying two bovine muscles (*Longissimus dorsi* and *Psoas major*) reported  $^{13}\text{C}$  half-life values of 133 and 157 days, respectively.

There were no effects ( $p>0.05$ ) of diets on daily feed intake, daily weight gain, and the feed:gain ratios in the performance periods covered by experiment II (Table 4).

Glutamine is one of the most important amino acids (quantitatively) within organs. At catabolic state and with bowel injuries, such as those occurring during piglet weaning, it may become an important dietary component for maintaining the structure and function of the intestinal metabolism (Maiorka et al., 2016). It is the most abundant intracellular amino acid found in organisms (Yelamanchi et al., 2016) and is considered a nonessential amino acid capable of generating energy or synthesizing other amino acids (Watford, 2015).

Although these additives exert trophic effects on mucosa (Amorim et al., 2017) and serve as precursors for energy generation, they were not able to show improvements on the performance of piglets. Several studies have evidenced improvements on piglets' growth performance by adding glutamine (Kitt et al., 2002; Abreu et al., 2010) and glutamate (Molino et al., 2012) in diets; however, this could not be proved in the present study, since the growth performance of piglets fed these additives was similar to those fed control diets.

Yi et al. (2005) and Tucci et al. (2011) added glutamine (2 and 1%, respectively) in weanling piglet diets and found no differences on performance. This could be attributed to the fact that no immune challenge was imposed during the trial period, which may have inhibited the action of the additives, although piglets had been stressed during weaning. Similar data were found in this study, due to the low immune challenge applied to animals raised under a sanitary segregation condition and fed complex diets containing highly digestible ingredients, such as dairy products and zinc oxide.

Cabrera et al. (2013) observed improvements on the feed:gain ratio at the first three weeks post-weaning of piglets fed Aminogut (supplementation with glutamine or glutamate associated with glutamine); however, for other performance variables, no differences were found.

Concerning experimental diets prices, diets containing additives showed higher costs than the C diet (Table 5). The cost per kilogram of live weight gain was greater for the G diet, which represented an increase of about 5.93, 17.09, 23.46, and 41.56% in each diet cost compared with the C diet during the four periods evaluated 1, 2, 3 and 4, respectively. The C-diet has also achieved better economic efficiency ratios and average cost indexes in those periods.

Schimanko et al. (2013) and Engelage et al. (2017) pointed out that lower costs of feed ingredients are the key to the success of swine production. Therefore, reductions in the diet costs are extremely interesting for pig farmers, because they increase the chance of economic gains. The comparison between the G and GA diets indicated that the use of the GA diet resulted in lower cost per kilogram of live weight gain, better economic efficiency ratio, and better average cost index in the studied periods.

**Table 5.** Feed cost per kilogram of weight gain (US\$ kg<sup>-1</sup>)<sup>(1)</sup>, economic efficiency index, and mean cost index of piglets fed control, 1% glutamine, and 1% glutamic acid at periods 1, 2, 3, and 4.

| Variable                     | Diet    |           |               |
|------------------------------|---------|-----------|---------------|
|                              | Control | Glutamine | Glutamic acid |
| Period 1 (21 to 28 days old) |         |           |               |
| Feed cost                    | 1.09    | 1.16      | 1.03          |
| Economic efficiency index    | 100.00  | 88.98     | 99.64         |
| Mean cost index              | 100.00  | 112.38    | 100.36        |
| Period 2 (21 to 35 days old) |         |           |               |
| Feed cost                    | 1.08    | 1.27      | 1.15          |
| Economic efficiency index    | 100.00  | 85.29     | 94.09         |
| Mean cost index              | 100.00  | 117.25    | 106.29        |
| Period 3 (21 to 48 days old) |         |           |               |
| Feed cost                    | 0.85    | 1.05      | 0.92          |
| Economic efficiency index    | 100.00  | 81.11     | 93.32         |
| Mean cost index              | 100.00  | 123.29    | 107.15        |
| Period 4 (21 to 55 days old) |         |           |               |
| Feed cost                    | 0.47    | 0.67      | 0.90          |
| Economic efficiency index    | 100.00  | 70.61     | 85.77         |
| Mean cost index              | 100.00  | 141.63    | 116.60        |

<sup>(1)</sup>Feed price calculated based on dollar value on May, 2017, with US\$ 1.00 = R\$ 3,2437 and exchange rate of R\$ 1,00 = US\$ 0.3083, according to Banco Central do Brasil (2017).

## Conclusions

1. Although supplementation with 1% glutamine or glutamic acid (GA) accelerates the carbon turnover in the studied muscles – which suggests a faster recovery during post-weaning and proves the anabolic effect of these additives – its use is not economically viable for weanling piglets

2. The use of the GA diet results in lower cost per kilogram of live weight gain, better economic efficiency ratio, and better average cost index, but the high costs of this additive restrict its use and preclude its inclusion in the diets of weaned piglets.

## Acknowledgments

To Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp, process No. 2011/17844-1) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process No. 501190/2013-9), for financial support.

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Received on February 16, 2017 and accepted on June 8, 2017