



Effect of Glutamine, Glutamic Acid and Nucleotides on the Turnover of Carbon ($\delta^{13}\text{C}$) in Organs of Weaned Piglets

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ABSTRACT: Morphological and physiological alterations occur in the digestive system of weaning piglets, compromising the performance in subsequent phases. This experiment aimed at verifying the influence of glutamine, glutamate and nucleotides on the carbon turnover in the pancreas and liver of piglets weaned at 21 days of age. Four diets were evaluated: glutamine, glutamic acid or nucleotides-free diet (CD); containing 1% glutamine (GD); containing 1% glutamic acid (GAD) and containing 1% nucleotides (ND). One hundred and twenty-three piglets were utilized with three pigs slaughtered at day zero (weaning day) and three at each one of the experimental days (1, 2, 4, 5, 7, 9, 13, 20, 27, and 49 post-weaning), in order to collect organ samples, which were analyzed for the $\delta^{13}\text{C}$ isotopic composition and compared by means of time. No differences were found ($p>0.05$) among treatments for the turnover of the ^{13}C in the pancreas ($T_{50\%} = 13.91, 14.37, 11.07,$ and 9.34 days; $T_{95\%} = 46.22, 47.73, 36.79,$ and 31.04 days for CD, GD, GAD, and ND, respectively). In the liver, the ND presented accelerated values of carbon turnover ($T_{50\%} = 7.36$ and $T_{95\%} = 24.47$ days) in relation to the values obtained for the GD ($T_{50\%} = 10.15$ and $T_{95\%} = 33.74$ days). However, the values obtained for the CD ($T_{50\%} = 9.12$ and $T_{95\%} = 30.31$ days) and GAD ($T_{50\%} = 7.83$ and $T_{95\%} = 26.03$ days) had no differences ($p>0.05$) among other diets. The technique of ^{13}C isotopic dilution demonstrated trophic action of nucleotides in the liver. (**Key Words:** Liver, Pancreas, Pigs, Stable Isotopes)

INTRODUCTION

One of the forms of improving the productivity on farms is the early weaning of piglets. This is a critical step in swine culture, since the piglet's immature digestive system produces low pancreatic and intestinal enzymes for digestion of plant contents in diets (Eggum, 1995; Liu et al.,

2009; Van der Meulen et al., 2010) and also, it presents a low production of hydrochloric acid in stomach (Prohászka and Barón, 1980), which impairs performance in post-weaning period and predisposes animals to digestive problems. Adequate nutrition of weaning piglets is imperative to rapid development of the gastrointestinal tract at post-weaning, which is essential to reduction of problems facing this phase and, consequently to enable the expression of pig's genetic potential.

The inclusion of additives that favors the development of digestive organs becomes important in order to improve animal production results. Various studies have evaluated the utilization of glutamine, glutamate and nucleotides in the diets of weaned piglets (Wu et al., 1996; Wu et al., 2010; Sauer et al., 2012) highlighting improved morphological and physiological responses to weaning, and their use as possible performance enhancers.

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Currently, animal nutrition research seeks new methodologies, among these, the technique of stable isotopes, which can be utilized in animal nutrition and physiology studies, because it allows the measurement of carbon in tissues which are influenced by environmental, nutritional and health factors (Carrijo et al., 2000).

Hobson and Clark (1992) observed that the ^{13}C isotope composition of animal tissues generally resembles those of their diets. Through this method, the stable isotopes of carbon and nitrogen have been used effectively in various studies of different animal species (Carrijo et al., 2006; Denadai et al., 2006; Zuanon et al., 2007; Caldara et al., 2008; Móri et al., 2008) aiming to evaluate the ^{13}C turnover in animal tissues or track the utilization of animal by-products in feed.

Due to the problems faced by the piglets at weaning, the objective of the present study was to verify the influence of glutamine, glutamate and nucleotides on turnover of carbon in the pancreas and in the liver of weaned piglets, which are the major annex organs of digestive system. Therefore, a greater development of these organs could help the piglets to improve their growth performance in this critical phase, once these additives have trophic action on them.

MATERIALS AND METHODS

Location and animals

This research project was conducted at São Paulo State University (UNESP) at the Faculty of Veterinary Medicine and Animal Science, Botucatu Campus, with the approval by the Animal Ethics Committee from this institution (protocol number 32/2011-CEUA). All procedures used complied with national regulations concerning experimentation on farm animals.

A total of 123 weaned piglets from commercial lineage (61 females and 62 castrated males), at an average age of 21 days and, 6.27 ± 0.13 kg of average body weight were allocated into randomized blocks, considering 3 piglets per experimental unit divided into categories of weight (light, medium and heavy with an average initial body weight of 5.91 kg, 6.40 kg, and 6.57 kg, respectively) and four treatments. The animals were housed in suspended metal pens of 1.0×1.75 m, which had a partially slatted plastic flooring and were equipped with one feeder, nipple-type drinker and heater.

Diets

The animals were fed *ad libitum* within a nursery phase feeding program to fulfill their nutritional requirements in accordance with Rostagno et al. (2011), as follows: pre-starter I (21- to 35-day-old), pre-starter II (36- to 49-day-old) and starter (50- to 70-day-old). The evaluated treatments were: additive-free diet, control (CD); diet containing 1% glutamine (GD); diet containing 1%

glutamic acid (GAD) and diet containing 1% nucleotides (ND).

The main energy source of these diets was rice grits, raw material coming from the C_3 photosynthetic plant cycle, which showed a ^{13}C isotopic signal distinct from the diets provided to sows (-16.14%). The gestation and lactation diets primarily contained corn as an energy source (a C_4 photosynthetic plant). The percentage composition and isotopic values ($\delta^{13}\text{C}$) of pre-starter I, pre-starter II, starter diets and their calculated nutritional composition are presented in Table 1, 2, and 3.

Slaughters and organs sampling

Slaughters occurred at days 1, 2, 4, 5, 7, 9, 13, 20, 27, and 49 post-weaning, following a manual electrical stunning and exsanguination of 3 piglets per treatment. At day 0 (baseline), three animals were slaughtered to quantify the ^{13}C half-life values ($T_{50\%}$) and the 95% of carbon substitution values ($T_{95\%}$) in organs, which were a function of diets fed sows in the gestation and lactation phases. The remaining 120 animals were blocked by three weight categories (light, medium, and heavy) and, randomly assigned to pens and 4 diets with one piglet slaughtered per weight category per treatment at days 1, 2, 4, 5, 7, 9, 13, 20, 27, and 49 after weaning.

The sampling procedures were concentrated in early days of the experimental period due to the greater speed of ^{13}C isotopic dilution in tissues (Hobson and Clark, 1992) and, samples of the pancreas head and the left lobe of the liver were collected, washed with de-ionized water, identified and immediately frozen at -18°C until isotopic analyses.

Organs processing and isotopic analyses

The previously frozen samples were dried in a forced-circulation air oven (Marconi, MA 035-5, Piracicaba, SP, Brazil) at 56°C for 24-hour for isotopic analyses of the organs. Since the lipid fraction may cause isotopic fractionation at up to 5‰ on ^{13}C values (Piasentier et al., 2003), the samples were degreased with ethyl ether C.P. (*chemically pure*) at 65°C for 4-hour in a Soxhlet apparatus (Tecnal, TE-044, Piracicaba, SP, Brazil). The samples were stored in plastic flasks and milled for 5 minutes at constant rotation (9,700 rpm) in a cryogenic mill (SPEX Sample Prep, Geno/Grinder 2010, Metuchen, NJ, USA) at -190°C , in order to obtain a homogenous material ($<60 \mu\text{m}$). After milling, all samples were weighed (50 to 70 μg) into tin capsules prior to analyses.

To determine the isotopic composition of samples, a mass spectrometer (Delta S-Finnigan Mat, Waltham, MA, USA) was used coupled with an elemental analyzer (EA 1108-CHN-Fisons Instruments, Waltham, MA, USA) at the center of Environmental Stable Isotopes - Biosciences Institute of UNESP. The data were expressed in $\delta^{13}\text{C}$

Table 1. Percentage composition and isotopic values of pre-starter I diets¹

Items	Pre-starter I diets			
	CD	GD	GAD	ND
Ingredients (%)				
Rice, grits	57.41	56.41	56.41	56.41
Soybean meal 46%	20.00	20.00	20.00	20.00
Whey protein concentrate	6.80	6.80	6.80	6.80
Maltodextrin	6.66	6.66	6.66	6.66
Corn gluten meal 60%	2.60	2.60	2.60	2.60
Soybean oil	1.48	1.48	1.48	1.48
Glutamine (99%)	-	1.00	-	-
Glutamic acid (98.5%)	-	-	1.00	-
Nucleotides ² (97%)	-	-	-	1.00
Dicalcium phosphate	1.25	1.25	1.25	1.25
Limestone	1.03	1.03	1.03	1.03
Sodium chloride	0.59	0.59	0.59	0.59
L-lysine HCl (78.4%)	0.77	0.77	0.77	0.77
DL-methionine (99%)	0.23	0.23	0.23	0.23
L-threonine (98%)	0.31	0.31	0.31	0.31
L-tryptophan (99%)	0.06	0.06	0.06	0.06
L-valine (96.5%)	0.11	0.11	0.11	0.11
Zinc oxide (77%)	0.34	0.34	0.34	0.34
Choline chloride (60%)	0.07	0.07	0.07	0.07
BHT antioxidant ³	0.02	0.02	0.02	0.02
Mineral premix ⁴	0.10	0.10	0.10	0.10
Vitamin premix ⁵	0.15	0.15	0.15	0.15
Sweetener ⁶	0.02	0.02	0.02	0.02
Isotopic values $\delta^{13}\text{C}$ (‰)	-26.86	-26.44	-26.76	-29.91
Calculated values				
Metabolizable energy (kcal/kg)	3,400	3,400	3,400	3,400
Crude protein (%)	19.00	19.00	19.00	19.00
Digestible lysine (%)	1.45	1.45	1.45	1.45
Digestible methionine (%)	0.52	0.52	0.52	0.52
Digestible valine (%)	1.00	1.00	1.00	1.00
Digestible threonine (%)	0.91	0.91	0.91	0.91
Digestible tryptophan (%)	0.26	0.26	0.26	0.26
Calcium (%)	0.82	0.82	0.82	0.82
Phosphorus (%)	0.45	0.45	0.45	0.45

CD, control diet (no glutamine, glutamic acid or nucleotides); GD, diets containing 1% glutamine; GAD, diets containing 1% glutamic acid; ND, diets containing 1% nucleotides.

¹ The nutritional values of the ingredients were considered as proposed by Rostagno et al. (2011).

² 5'-disodium inosinate and 5'-disodium guanylate.

³ BHT, butylated hydroxy toluene

⁴ Mineral premix supplied per kg of diet: Fe, 40 mg; Cu, 35 mg; Mn, 20 mg; Zn, 40 mg; Co, 0.36 mg; I, 0.84 mg; Se, 0.12 mg.

⁵ Vitamin premix supplied per kg of diet: Vit. A, 25,000 IU; Vit. D₃, 5,000 IU; Biotin, 5 mg; Niacin, 10 mg; Calcium pantothenate, 30 mg; Vit. B₁₂, 70 µg; Vit. B₂, 18 mg; Vit. E, 75 mg; Vit. K₃, 1 mg.

⁶ Composed by sodium saccharin, neohesperidin and silicon dioxide.

notation, in relation to the Pee Dee Belemnite (PDB) an international standard, with analyses deviation at the order of 0.2‰ and calculated by the equation:

Table 2. Percentage composition and isotopic values of pre-starter II diets¹

Items	Pre-starter II diets			
	CD	GD	GAD	ND
Ingredients (%)				
Rice, grits	60.51	59.51	59.51	59.51
Soybean meal 46%	25.00	25.00	25.00	25.00
Whey protein concentrate	3.70	3.70	3.70	3.70
Maltodextrin	3.17	3.17	3.17	3.17
Corn gluten meal 60%	1.69	1.69	1.69	1.69
Soybean oil	1.53	1.53	1.53	1.53
Glutamine (99%)	-	1.00	-	-
Glutamic acid (98.5%)	-	-	1.00	-
Nucleotides ² (97%)	-	-	-	1.00
Dicalcium phosphate	1.50	1.50	1.50	1.50
Limestone	0.90	0.90	0.90	0.90
Sodium chloride	0.62	0.62	0.62	0.62
L-lysine HCl (78.4%)	0.55	0.55	0.55	0.55
DL-methionine (99%)	0.21	0.21	0.21	0.21
L-threonine (98%)	0.22	0.22	0.22	0.22
L-tryptophan (99%)	0.02	0.02	0.02	0.02
L-valine (96.5%)	0.03	0.03	0.03	0.03
Zinc oxide (77%)	-	-	-	-
Choline chloride (60%)	0.07	0.07	0.07	0.07
BHT antioxidant ³	0.02	0.02	0.02	0.02
Mineral premix ⁴	0.10	0.10	0.10	0.10
Vitamin premix ⁵	0.15	0.15	0.15	0.15
Sweetener ⁶	0.02	0.02	0.02	0.02
Isotopic values $\delta^{13}\text{C}$ (‰)	-27.11	-27.76	-26.14	-27.30
Calculated values				
Metabolizable energy (kcal/kg)	3,383	3,383	3,383	3,383
Crude protein (%)	19.55	19.55	19.55	19.55
Digestible lysine (%)	1.33	1.33	1.33	1.33
Digestible methionine (%)	0.50	0.50	0.50	0.50
Digestible valine (%)	0.92	0.92	0.92	0.92
Digestible threonine (%)	0.84	0.84	0.84	0.84
Digestible tryptophan (%)	0.24	0.24	0.24	0.24
Calcium (%)	0.83	0.83	0.83	0.83
Phosphorus (%)	0.45	0.45	0.45	0.45

CD, control diet (no glutamine, glutamic acid or nucleotides); GD, diets containing 1% glutamine; GAD, diets containing 1% glutamic acid; ND, diets containing 1% nucleotides.

¹ The nutritional values of the ingredients were considered as proposed by Rostagno et al. (2011).

² 5'-disodium inosinate and 5'-disodium guanylate.

³ BHT, butylated hydroxy toluene.

⁴ Mineral premix supplied per kg of diet: Fe, 40 mg; Cu, 35 mg; Mn, 20 mg; Zn, 40 mg; Co, 0.36 mg; I, 0.84 mg; Se, 0.12 mg.

⁵ Vitamin premix supplied per kg of diet: Vit. A, 25,000 IU; Vit. D₃, 5,000 IU; Biotin, 5 mg; Niacin, 10 mg; Calcium pantothenate, 30 mg; Vit. B₁₂, 70 µg; Vit. B₂, 18 mg; Vit. E, 75 mg; Vit. K₃, 1 mg.

⁶ Composed by sodium saccharin, neohesperidin and silicon dioxide.

$$\delta^{13}\text{C} (\text{sample, standard}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where:

$\delta^{13}\text{C}$ = the enrichment of the isotopic ratio $^{13}\text{C}/^{12}\text{C}$ of the

Table 3. Percentage composition and isotopic values of starter diets¹

Items	Starter diets			
	CD	GD	GAD	ND
Ingredients (%)				
Rice, grits	64.25	63.25	63.25	63.25
Soybean meal 46%	30.00	30.00	30.00	30.00
Whey protein concentrate	-	-	-	-
Maltodextrin	-	-	-	-
Corn gluten meal 60%	1.30	1.30	1.30	1.30
Soybean oil	1.50	1.50	1.50	1.50
Glutamine (99%)	-	1.00	-	-
Glutamic acid (98.5%)	-	-	1.00	-
Nucleotides ¹ (97%)	-	-	-	1.00
Dicalcium phosphate	1.23	1.23	1.23	1.23
Limestone	0.83	0.83	0.83	0.83
Sodium chloride	0.46	0.46	0.46	0.46
L-lysine HCl (78.4%)	0.09	0.09	0.09	0.09
DL-methionine (99%)	-	-	-	-
L-threonine (98%)	-	-	-	-
L-tryptophan (99%)	-	-	-	-
L-valine (96.5%)	-	-	-	-
Zinc oxide (77%)	-	-	-	-
Choline chloride (60%)	0.07	0.07	0.07	0.07
BHT antioxidant ²	0.02	0.02	0.02	0.02
Mineral premix ³	0.10	0.10	0.10	0.10
Vitamin premix ⁴	0.15	0.15	0.15	0.15
Sweetener ⁵	0.02	0.02	0.02	0.02
Isotopic values δ ¹³ C (‰)	-27.46	-28.10	-28.87	-27.17
Calculated values				
Metabolizable energy (kcal/kg)	3,370	3,370	3,370	3,370
Crude protein (%)	19.9	19.90	19.90	19.90
Digestible lysine (%)	1.01	1.01	1.01	1.01
Digestible methionine (%)	0.31	0.31	0.31	0.31
Digestible valine (%)	0.20	0.20	0.20	0.20
Digestible threonine (%)	0.64	0.64	0.64	0.64
Digestible tryptophan (%)	0.23	0.23	0.23	0.23
Calcium (%)	0.72	0.72	0.72	0.72
Phosphorus (%)	0.34	0.34	0.34	0.34

CD, control diet (no glutamine, glutamic acid or nucleotides); GD, diets containing 1% glutamine; GAD, diets containing 1% glutamic acid; ND, diets containing 1% nucleotides.

¹ The nutritional values of the ingredients were considered as proposed by Rostagno et al. (2011)

² 5'-disodium inosinate and 5'-disodium guanylate;

³ BHT, Butylated hydroxy toluene.

⁴ Mineral premix supplied per kg of diet: Fe, 40 mg; Cu, 35 mg; Mn, 20 mg; Zn, 40 mg; Co, 0.36 mg; I, 0.84 mg; Se, 0.12 mg.

⁵ Vitamin premix supplied per kg of diet: Vit. A, 25,000 IU; Vit. D₃, 5,000 IU; Biotin, 5 mg; Niacin, 10 mg; Calcium pantothenate, 30 mg; Vit. B₁₂, 70 µg; Vit. B₂, 18 mg; Vit. E, 75 mg; Vit. K₃, 1 mg.

⁶ Composed by sodium saccharin, neohesperidin and silicon dioxide.

sample to the standard;

R = represents the ratio of the heavier (¹³C) to the lighter (¹²C) stable isotopes (dimensionless);

To evaluate the speed of the carbon substitution of the

samples, the following exponential function of time was employed (Ducatti et al., 2002):

$$\delta^{13}\text{C} (t) = \delta^{13}\text{C} (f) + [\delta^{13}\text{C}(i) - \delta^{13}\text{C}(f)] e^{-kt}$$

where:

δ¹³C (t) = isotopic enrichment of tissue at any time (t) (dimensionless);

δ¹³C (f) = isotopic enrichment of tissue at the equilibrium or final condition (dimensionless);

δ¹³C (i) = isotopic enrichment of tissue, at the beginning (dimensionless);

k = constant of turnover rate, in units of time⁻¹;

t = time (days) since the diet substitution.

The half-life values (T_{50%}) of the carbon in the organs, at t = T and the total time (T_{95%}) necessary for the initial atoms substitution by the final atoms were determined by the equation:

$$t = (-1/k) \ln (1 - F),$$

where:

t = time of the initial atoms substitution to the final substitution (days);

ln = Neperian logarithm (natural);

F = value of the atom exchange, which can vary between zero and 0.95;

k = constant of turnover rate (day⁻¹), (Ducatti et al., 2002).

Statistical analysis

Eleven piglets per treatment were used at each slaughter days 0, 1, 2, 4, 5, 7, 9, 13, 20, 27, and 49 post-weaning to determine the first order exponential equations by the software Minitab 16 and to express the carbon substitution values for each animal weight categories (light, medium and heavy) and also, to determine the half-life values (T_{50%}) and 95% substitution (T_{95%}) of the ¹³C stable isotopes. The data were analyzed by the variance test and the means were compared by Tukey's test (5%) using the general linear model procedure of SAS v.9.2 (SAS Institute Cary, NC, USA).

RESULTS AND DISCUSSION

Turnover of the ¹³C in organs

No differences (p>0.05) for the ¹³C turnover in the pancreas were found among treatments. However, there were a faster tendency of ¹³C incorporation of the ND in the pancreas (p = 0.094), and it required 9.34 days to achieve the half-life status and 31.04 days for 95% substitution of the ¹³C in this organ. The half-life values obtained for the piglets fed CD, GD, and GAD, respectively were: 13.91,

14.37, and 11.07 days; and for the 95% substitution of the ^{13}C were: 46.22, 47.73, and 36.79 days (Table 4).

The pancreas is related directly to the digestive system

and due to this fact, the dietary nucleotides are very important to the gut health maintenance and the immunomodulation (Ohyanagi et al., 1989), furthermore,

Table 4. Equations of the tissue isotopic enrichments by time, the half-life turnover values ($T_{50\%}$) and 95% substitution ($T_{95\%}$) of ^{13}C stable isotopes from piglets' pancreas and liver by weight categories and diets

Diets	Weight category	Function	$T_{50\%}$ (d)	Average (d)	$T_{95\%}$ (d)	Average (d)
Pancreas						
Control	Light	$\delta^{13}\text{C} = -26.09 + 9.14e^{-0.047054t}$ $R^2 = 0.97$	14.73	13.91	48.93	46.22
	Medium	$\delta^{13}\text{C} = -26.23 + 9.57e^{-0.050768t}$ $R^2 = 0.97$	13.65		45.36	
	Heavy	$\delta^{13}\text{C} = -26.14 + 9.19e^{-0.05191t}$ $R^2 = 0.98$	13.35		44.36	
1% Glutamine	Light	$\delta^{13}\text{C} = -27.87 + 11.42e^{-0.040083t}$ $R^2 = 0.96$	17.29	14.37	57.45	47.73
	Medium	$\delta^{13}\text{C} = -26.00 + 10.00e^{-0.061705t}$ $R^2 = 0.95$	11.23		37.32	
	Heavy	$\delta^{13}\text{C} = -27.35 + 11.06e^{-0.047551t}$ $R^2 = 0.96$	14.58		48.42	
1% Glutamic acid	Light	$\delta^{13}\text{C} = -25.86 + 9.53e^{-0.059272t}$ $R^2 = 0.97$	11.69	11.07	38.85	36.79
	Medium	$\delta^{13}\text{C} = -25.81 + 9.21e^{-0.062754t}$ $R^2 = 0.99$	11.05		36.69	
	Heavy	$\delta^{13}\text{C} = -25.31 + 8.62e^{-0.066135t}$ $R^2 = 0.97$	10.48		34.82	
1% Nucleotides	Light	$\delta^{13}\text{C} = -25.42 + 7.77e^{-0.088811t}$ $R^2 = 0.85$	7.80	9.34	25.93	31.04
	Medium	$\delta^{13}\text{C} = -27.39 + 10.13e^{-0.063102t}$ $R^2 = 0.99$	10.98		36.49	
	Heavy	$\delta^{13}\text{C} = -26.55 + 13.34e^{-0.074986t}$ $R^2 = 0.95$	9.24		30.71	
Liver						
Control	Light	$\delta^{13}\text{C} = -26.56 + 9.87e^{-0.07878t}$ $R^2 = 0.89$	8.80	9.12 ^{ab}	29.23	30.31 ^{ab}
	Medium	$\delta^{13}\text{C} = -27.33 + 10.67e^{-0.069536t}$ $R^2 = 0.95$	9.97		33.11	
	Heavy	$\delta^{13}\text{C} = -26.67 + 8.57e^{-0.080572t}$ $R^2 = 0.73$	8.60		28.58	
1% Glutamine	Light	$\delta^{13}\text{C} = -26.87 + 9.92e^{-0.062844t}$ $R^2 = 0.96$	11.03	10.15 ^a	36.64	33.74 ^a
	Medium	$\delta^{13}\text{C} = -26.63 + 9.71e^{-0.075975t}$ $R^2 = 0.99$	9.12		30.31	
	Heavy	$\delta^{13}\text{C} = -27.20 + 10.66e^{-0.067168t}$ $R^2 = 0.98$	10.32		34.28	
1% Glutamic acid	Light	$\delta^{13}\text{C} = -25.87 + 9.02e^{-0.0103515t}$ $R^2 = 0.96$	6.70	7.83 ^{ab}	22.24	26.03 ^{ab}
	Medium	$\delta^{13}\text{C} = -26.63 + 9.99e^{-0.078747t}$ $R^2 = 0.95$	8.80		29.24	
	Heavy	$\delta^{13}\text{C} = -26.58 + 9.70e^{-0.086526t}$ $R^2 = 0.99$	8.01		26.61	
1% Nucleotides	Light	$\delta^{13}\text{C} = -26.46 + 9.74e^{-0.08887t}$ $R^2 = 0.97$	7.80	7.36 ^b	25.91	24.47 ^b
	Medium	$\delta^{13}\text{C} = -26.36 + 10.01e^{-0.101396t}$ $R^2 = 0.97$	6.84		22.71	
	Heavy	$\delta^{13}\text{C} = -26.80 + 10.26e^{-0.092912t}$ $R^2 = 0.98$	7.46		24.78	

Mean values in the same column followed by different lowercase letters differ by Tukey's test at 5% probability (n = 11).

they can act as immunonutrients and growth promoters (Rossi et al., 2007). However, no differences ($p > 0.05$) were found for the carbon incorporation in the pancreas when the exogen nucleotides were supplemented in the diets.

The pancreas is involved in glutamine synthesis and degradation. In this biochemical process, the glutaminase enzyme catalyzes the glutamine hydrolysis and leads to the synthesis of ammonia and glutamate (Meister, 1980) or, the glutamine synthase enzyme catalyzes the conversion of glutamine from glutamate and ammonia in presence of ATP (Borges et al., 2008). However, no influence of these enzymes on the ^{13}C turnover was registered in the GD and GAD.

Burrin and Stoll et al. (2009) found that glutamine effectively suppressed glucose oxidation in the enterocytes while glucose had little effect on oxidation of glutamine. Although glutamine and glutamate were directed preferably to the mitochondrial oxidation, most of the glucose was used for the purpose of biosynthetic metabolism (Frigerio et al., 2008). This fact might explain the lower enzymatic activity in the pancreas when the piglets were fed GD and GAD.

In the liver, differences were found ($p < 0.05$) among the treatments studied. The ND showed faster half-life values and 95% substitution of the ^{13}C values ($T_{50\%} = 7.36$ and $T_{95\%} = 24.47$ days) than GD ($T_{50\%} = 10.15$ and $T_{95\%} = 33.74$ days), although the CD ($T_{50\%} = 9.12$ and $T_{95\%} = 30.31$ days) and GAD ($T_{50\%} = 7.83$ and $T_{95\%} = 26.03$ days) did not differ from the other diets.

Even though no differences ($p > 0.05$) were found between the CD and the GAD, the faster ^{13}C turnover was observed in the liver of the piglets fed ND, followed by the piglets fed the GAD and the CD. Carver (1994) reported that inclusion of nucleotides in diet promoted hepatocytes growth and regeneration, and played an important role in glycogen synthesis (Grimble, 1994; Rodwell and Kennelly, 2003).

The nucleotides are more required for rapid turnover tissues, which demand a high production of nucleic acids for cell replication, synthesis of purine and pyrimidine which increases the DNA pool and the protein synthesis for tissue regeneration, such as in the liver (Rudolph, 1994; Carver, 1999). The weaning piglets undergo a series of challenges that may jeopardize the functioning of some organs, such as the liver. For this reason, the supplementation of nucleotides in the present study might have influenced the acceleration of the ^{13}C turnover in the liver, favoring the regeneration of this organ.

Caldara et al. (2008) studied the influence of glutamine on ^{13}C turnover in the pancreas and liver of weaned piglets and, reported the increase on carbon substitution as a function of supplementation with glutamine in both organs (anabolic stimulus). However, this action was not observed in the present study and, GD showed similar results as the

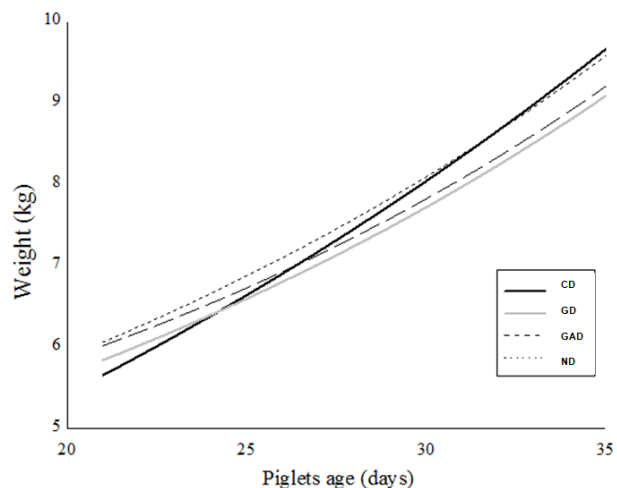


Figure 1. Graphical representation of the exponential equations of piglets' weight from 21- to 35-day-old fed the control diets (CD), the diets containing 1% glutamine (GD), the diets containing 1% glutamic acid (GAD) or the diets containing 1% nucleotides (ND) ($n = 33$ for each curve).

CD and the GAD. Furthermore, piglets fed ND had longer incorporation of ^{13}C .

Piglet development

The ^{13}C turnover value in the liver of piglets fed the GD was the slowest ($T_{50\%} = 10$ days and $T_{95\%} = 34$ days), suggesting that a greater preservation of hepatocytes or even lower growth rate of this organ might have occurred, once the growth rhythm of the piglets fed GD was lower than other treatments at post-weaning weeks (Figure 1).

Glutamine, glutamate and nucleotides triggered higher responses of fast turnover in animal tissues (Van Buren and Rudolph, 1997; Rhoads and Wu, 2009) and for animals in catabolic states such as the weaned piglets. Therefore, these additives might become important dietary components for the maintenance and regeneration of these organs (Fox et al., 1988; Carver, 1999). In addition, these additives also might have trophic action in the pancreas and in the liver of weaned piglets, favoring the increase of the development of these organs, which are fundamental to the digestive process. The technique of ^{13}C isotopic dilution demonstrated the trophic action of nucleotides in the liver, increasing its cell regeneration.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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