Animal Nutrition 3 (2017) 225–231

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dietary glutamine, glutamic acid and nucleotide supplementation accelerate carbon turnover ($\delta^{13}C$) on stomach of weaned piglets

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ARTICLE INFO

Article history:
Received 13 February 2017
Received in revised form
7 April 2017
Accepted 13 April 2017
Available online 20 April 2017

Keywords:
Additives
Biochemistry
Isotopic dilution
Mass spectrometry
Nutrition
Swine

ABSTRACT

The use of stable isotope analysis as a tool for characterization of carbon turnover ($\delta^{13}C$) in piglet's tissues by tracing its feeding system has drawn attention. Thus, this study aimed at evaluating the influence of dietary glutamine, glutamic acid and nucleotides supplementation on carbon turnover in fundic-stomach region of weaned piglets at an average age of 21 days. The diets consisted of additive-free diet – control (C); 1% glutamine (G); 1% glutamic acid (GA) and 1% nucleotides (Nu). At weaning day (day 0: baseline), 3 piglets were slaughtered to quantify the $\delta^{13}C$ of stomach. The remaining 120 piglets were blocked by weight and sex, randomly assigned to pens with 3 piglets slaughtered per treatment at days 1, 2, 4, 5, 7, 9, 13, 20, 27 and 49 after weaning in order to verify the fundic-stomach isotopic composition by treatments. Samples were analyzed in terms of $^{13}C/^{12}C$ ratio by mass spectrometry and converted to relative isotopic enrichment values ($\delta^{13}C$ ‰) used to plot the first order exponential curves over time using OriginPro 8.0 software. The inclusion of glutamine, glutamate and nucleotides in piglet's diets has accelerated the carbon turnover in stomach during the post-weaning period, demonstrating also that glutamate has guaranteed fastest ^{13}C incorporation rate on fundic-stomach region and pH-lowering. Besides that, stable isotopes technique ($\delta^{13}C$) has proved to be an important methodology to determine the time-scales at which piglets shift among diets with different isotopic values, characterizing the trophic effects of additives and the phenotypic flexibility of stomach.

1. Introduction

Nutrition management is very important in post-weaning period, due to the change to solid feed that was basically milk, before weaning (Lallès et al., 2007). This change causes nutritional stress to early-weaned piglets, being characterized by lower feed intake and frequent diarrhea status (Dong and Pluske, 2007; Quadros et al., 2002). An alternative to compensate the loss of digestive activity is the usage of complex diets, which reduce the incidence and severity of post-weaning diarrhea (Van Der Peet-Schwering and Binnendijk, 1998), with reduction in piglet mortality.

As a way of reducing negative weaning results, high digestibility ingredients (Trindade Neto et al., 2002) and antimicrobials have been added to the diets, which improve the growth rate, feed:gain ratio and reduce the susceptibility of piglet to clinical and subclinical infections (Lovatto et al., 2005).

However, the use of antibiotics for this purpose has been banned with the main claim that they can cause bacterial resistance. Due to this, several researches have been done to find alternative substances to antibiotics, as performance-enhancing additives, that stimulate the growth and cell differentiation of intestinal tract and immune system of piglets, causing no bacteria resistance (Yu et al., 2002).

Moreover, these alternative antimicrobial sources, such as glutamine, glutamate and nucleotides have been used in animal

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

http://dx.doi.org/10.1016/j.aninu.2017.04.006

nutrition being involved in gastric emptying (Toyomasu et al., 2010), stimulation of saliva, gastric and pancreatic juices' secretion (Halpern, 2000), umami taste (Halpern, 2000), growth of visceral organs (Lackeyram et al., 2001), trophic action in faster growing tissues (Mateo, 2005), and carbon turnover-decreasing in digestive organs (Amorim, 2012; Saleh, 2016). Therefore, these additives are important at post-weaning phase due to the maintenance of digestive organs development and high turnover rate of cells, improving piglet performance in this critical period (Domeneghini et al., 2004; Liu et al., 2002; Sauer et al., 2012; Wu et al., 2010).

The technique currently used to quantify the trophic action of the dietary additives on tissues and organs of production animals is the stable isotopes (Manetta and Benedito-Cecilio, 2003) that measures the carbon turnover rate (Criss, 1999; Gannes et al., 1998) and track the metabolic pathway and the composition of a given tissue, because they vary in their elemental turnover rates, due to their different metabolic activities which govern the time frame over which dietary information is captured and integrated by each tissue (O'Brien, 2015).

Thus, considering the aforementioned, this study aimed at evaluating the carbon turnover ($\delta^{13}C$) on stomach of early-weaned piglets fed glutamate, glutamine and nucleotides by stable isotopes ratio spectrometry (IRMS).

2. Material and methods

The experiment was conducted at São Paulo State University (UNESP), Faculty of Veterinary Medicine and Animal Science, Botucatu Campus with the approval by the Animal Ethics Committee from this institution (protocol number 159/2013) and, in accordance with the directive 2010/63/EU.

A total of 123 weanling piglets, females and castrated males of crossbred commercial lineage (Landrace × Large White) were housed in a nursery facility with a ceiling height of 3.5 m, side curtains and suspended metal pens of 1.75 m^2 that were equipped with one feeder, one nipple-type drinker, and one heater. The pens had a partially slatted plastic flooring and compact concrete floor under the heater. The internal temperature of nursery facility was controlled by adjustment of side curtains and management of the heaters.

The piglets were fed *ad libitum* within a feeding program to attend its nutritional requirements in accordance with Rostagno et al. (2011) in the following phases: pre-starter 1 (21 to 35 days), pre-starter 2 (36 to 49 days), and starter (50 to 70 days) diets. The evaluated treatments were additive-free diet − control (C), diet containing 1% glutamine (G), diet containing 1% glutamate (GA), and diet containing 1% nucleotides (Nu) showed in Tables 1 and 2.

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

At weaning day (the baseline: experimental day 0), 3 piglets were slaughtered after a manual electrical stunning and exsanguination, in order to express the isotopic composition of tissue, which was a function of diets fed sows in gestation and lactation phases. The remaining 120 animals were blocked by weight and sex, randomly assigned to pens, and diets with 3 piglets per treatment at days 1, 2, 4, 5, 7, 9, 13, 20, 27, and 49 after weaning.

After slaughter, samples of fundic-stomach region were removed, washed with de-ionized water, and placed into Eppendorf tubes of 1.50 mL (Eppendorf A.G., Hamburg, Germany), identified and immediately frozen (−18 °C) for further analyses. The sampling procedures were concentrated in the first days of experimental trial due to the higher speed of ^{13}C isotopic dilution in the tissue (Hobson and Clark, 1992).

The previously frozen samples were dried in a forced-circulation air oven (Marconi, MA 035-5, Piracicaba, SP, Brazil) at 56 °C for 24-h for isotopic analyses of stomach. Since the lipid fraction may cause isotopic fractionation at up to 5‰ on ^{13}C values (Piasentier et al., 2003), samples were degreased with ethyl ether C.P. (chemically pure) at 65 °C for 4-h in a Soxhlet apparatus (Tecnal, TE-044, Piracicaba, SP, Brazil) and then stored in plastic flasks and milled at constant rotation (21,076 × *g* at −190 °C for 5 min) in a cryogenic mill (SPEX Sample Prep, Geno/Grinder 2010, Metuchen, NJ, USA) to obtain a homogenous material (<60 μm). 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, Thermo Scientific Inc., Waltham, MA, USA) coupled with an elemental analyzer (EA 1108-CHN-Fisions Instruments, Thermo Scientific Inc., Waltham, MA, USA) were used at the center of Environmental Stable Isotopes − UNESP Biosciences Institute. The data were expressed in $\delta^{13}C$ 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:

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

$\delta^{13}C$ is the enrichment of the isotopic ratio $^{13}C/^{12}C$ of the sample to the standard; R = represents the ratio of the heavier (^{13}C) to the lighter (^{12}C) stable isotopes.

To evaluate the speed of carbon substitution in stomach, the following exponential function of time was employed (Ducatti et al., 2016):

$$\delta^{13}C_{(t)} = \delta^{13}C_{(f)} + \left[\delta^{13}C_{(i)} - \delta^{13}C_{(f)}\right] e^{-kt}, \text{ where:}$$

$\delta^{13}C_{(t)}$ = isotopic enrichment of tissue at any time (t); $\delta^{13}C_{(f)}$ = isotopic enrichment of tissue at the equilibrium or final condition; $\delta^{13}C_{(i)}$ = isotopic enrichment of tissue at the beginning; k = turnover constant, in units of time^{-1}; t = time (days) since the diet substitution.

The carbon half-life ($T_{50\%}$) in stomach (Table 3), at $t = T$ and the total time ($T_{90\%}$) necessary for initial atoms substitution by final atoms was determined by the equation:

$$T_{50\%} = \ln 2/k, \text{ where:}$$

ln = Napierian logarithm (natural); k = turnover constant (day^{-1}), defined as incorporation rate of carbon isotopes (Ducatti et al., 2002, 2016).

To determine the percentage of atom substitution (F) at the end of experiment (Table 3), the following equation was applied:

$$F = 1 - e^{-kt}, \text{ where:}$$

F = value of atomic substitution, which can vary from 0.0 to 0.99, considering the stabilized system between 0.90 and 0.99; k = turnover constant (day^{-1}); t = time of the initial atoms substitution to the final substitution (days), in this case, 49 experimental days.

The diet pH on stomach was evaluated according to the following standard methodology (AOAC, 1990) by a portable pH meter (Tecnopon, model mPa-210P, Piracicaba, Brazil) (Table 4).

Table 1
Percentage composition and isotopic enrichment of the pre-starter 1, pre-starter 2, and starter diets.

Item	Pre-starter 1				Pre-starter 2				Starter			
	C	G	GA	Nu	C	G	GA	Nu	C	G	GA	Nu
Ingredients, %												
Rice grits	57.41	56.41	56.41	56.41	60.51	59.51	59.51	59.51	64.25	63.25	63.25	63.25
Soybean meal	20.00	20.00	20.00	20.00	25.00	25.00	25.00	25.00	30.00	30.00	30.00	30.00
WPC	6.80	6.80	6.80	6.80	3.70	3.70	3.70	3.70	–	–	–	–
Maltodextrin	6.66	6.66	6.66	6.66	3.17	3.17	3.17	3.17	–	–	–	–
Corn gluten meal	2.60	2.60	2.60	2.60	1.69	1.69	1.69	1.69	1.30	1.30	1.30	1.30
Soybean-oil	1.48	1.48	1.48	1.48	1.53	1.53	1.53	1.53	1.50	1.50	1.50	1.50
Glutamine (99%)	–	1.00	–	–	–	1.00	–	–	–	1.00	–	–
Glutamate (98.5%)	–	–	1.00	–	–	–	1.00	–	–	–	1.00	–
Nucleotides[1]	–	–	–	1.00	–	–	–	1.00	–	–	–	1.00
Dicalcium phosphate	1.25	1.25	1.25	1.25	1.50	1.50	1.50	1.50	1.23	1.23	1.23	1.23
Limestone	1.03	1.03	1.03	1.03	0.90	0.90	0.90	0.90	0.83	0.83	0.83	0.83
NaCl	0.59	0.59	0.59	0.59	0.62	0.62	0.62	0.62	0.46	0.46	0.46	0.46
L-Lys·HCl (78%)	0.77	0.77	0.77	0.77	0.55	0.55	0.55	0.55	0.09	0.09	0.09	0.09
DL-Met (99%)	0.23	0.23	0.23	0.23	0.21	0.21	0.21	0.21	–	–	–	–
L-Thr (98%)	0.31	0.31	0.31	0.31	0.22	0.22	0.22	0.22	–	–	–	–
L-Trp (99%)	0.06	0.06	0.06	0.06	0.02	0.02	0.02	0.02	–	–	–	–
L-Val (96%)	0.11	0.11	0.11	0.11	0.03	0.03	0.03	0.03	–	–	–	–
ZnO (77%)	0.34	0.34	0.34	0.34	–	–	–	–	–	–	–	–
Choline chloride	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
BHT	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sweetener[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
$\delta^{13}C$, ‰	−26.86	−26.44	−26.76	−29.91	−27.11	−27.76	−26.14	−27.30	−27.46	−28.10	−28.87	−27.17

C = control diet; G = glutamine diet; GA = glutamic acid diet; Nu = nucleotides diet; WPC = whey protein concentrate; BHT = butylated hydroxytoluene.
[1] Nucleotides is composed by 97% of 5′-disodium inosinate and 5′-disodium guanylate.
[2] Mineral and vitamin 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; vitamin A, 25,000 IU; Vitamin D_3, 5,000 IU, biotin, 5 mg, niacin, 10 mg, calcium pantothenate, 30 mg, Vitamin B_{12}, 70 μg, Vitamin B_2, 18 mg; Vitamin E, 75 mg; Vitamin K_3, 1 mg.
[3] The sweetener is composed by sodium saccharin, neohesperidin, and silicon dioxide.

Table 2
Calculated nutritional composition (%) of the pre-starter 1, pre-starter 2, and starter diets.[1]

Item	Pre-starter 1				Pre-starter 2				Starter			
	C	G	GA	Nu	C	G	GA	Nu	C	G	GA	Nu
ME, kcal/kg	3,400	3,400	3,400	3,400	3,383	3,383	3,383	3,383	3,370	3,370	3,370	3,370
Crude protein	19.00	19.00	19.00	19.00	19.55	19.55	19.55	19.55	19.90	19.90	19.90	19.90
Digestible Lys	1.45	1.45	1.45	1.45	1.33	1.33	1.33	1.33	1.01	1.01	1.01	1.01
Digestible Met	0.52	0.52	0.52	0.52	0.50	0.50	0.50	0.50	0.31	0.31	0.31	0.31
Digestible Val	1.00	1.00	1.00	1.00	0.92	0.92	0.92	0.92	0.20	0.20	0.20	0.20
Digestible Thr	0.91	0.91	0.91	0.91	0.84	0.84	0.84	0.84	0.64	0.64	0.64	0.64
Digestible Trp	0.26	0.26	0.26	0.26	0.24	0.24	0.24	0.24	0.23	0.23	0.23	0.23
Lactose-equivalent	10.00	10.00	10.00	10.00	5.01	5.01	5.01	5.01	–	–	–	–
Total Ca	0.82	0.82	0.82	0.82	0.83	0.83	0.83	0.83	0.72	0.72	0.72	0.72
Digestible P	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.34	0.34	0.34	0.34

C = control diet; G = glutamine diet; GA = glutamic acid diet; Nu = nucleotides diet; ME = metabolizable energy.
[1] Nutritional values of the ingredients are proposed by Rostagno et al. (2011).

2.1. Statistical analysis

Three piglets per treatment were used at following slaughter days: 0, 1, 2, 4, 5, 7, 9, 13, 20, 27 and 49 after weaning to determine with each set of data (slaughter day versus $\delta^{13}C$, ‰ of stomach from each piglet) and adjusted it to the first-order equation by a nonlinear regression analysis using the NLIN of SAS (SAS Institute Cary, NC, USA) procedure, in order to establish the formula for the stomach $\delta^{13}C$ over time (Fig. 1). The exponential graphics were plotted by OriginPro software v. 8.07 (OriginLab Corporation, Northampton,

Table 3
Stomach half-life ($T_{50\%}$), total substitution ($T_{90\%}$), and substituted atoms ($F_{\%}$) values during trial period ($n = 3$).

Item	$T_{50\%}$, day	$T_{90\%}$, day	$F_{\%}$[1]
C	16.27	54	95
G	13.91	46.21	96
GA	10.18	33.82	99
Nu	11.72	38.94	99

C = control diet; G = glutamine diet; GA = glutamic acid diet; Nu = nucleotides diet.
[1] Carbon isotopes substitution at end of trial.

Table 4
Values of piglets' stomach-pH according to experimental diets.

Item	Stomach pH				CV, %	*P*-value
	C	G	GA	Nu		
Pre-starter 1	6.12^{b}	6.21^{ab}	5.22^{c}	6.28^{a}	0.66	<0.001
Pre-starter 2	6.07^{b}	6.06^{b}	5.25^{c}	6.18^{a}	0.67	<0.001
Starter	6.07^{a}	6.08^{a}	5.26^{b}	6.17^{a}	1.64	<0.001
Mean	6.08	6.12	5.24	6.21		

C = control diet; G = glutamine diet; GA = glutamic acid diet; Nu = nucleotides diet; CV = coefficient of variation.
[a,b] Within a row, means with the same lowercase letter are not significantly different ($P > 0.05$).

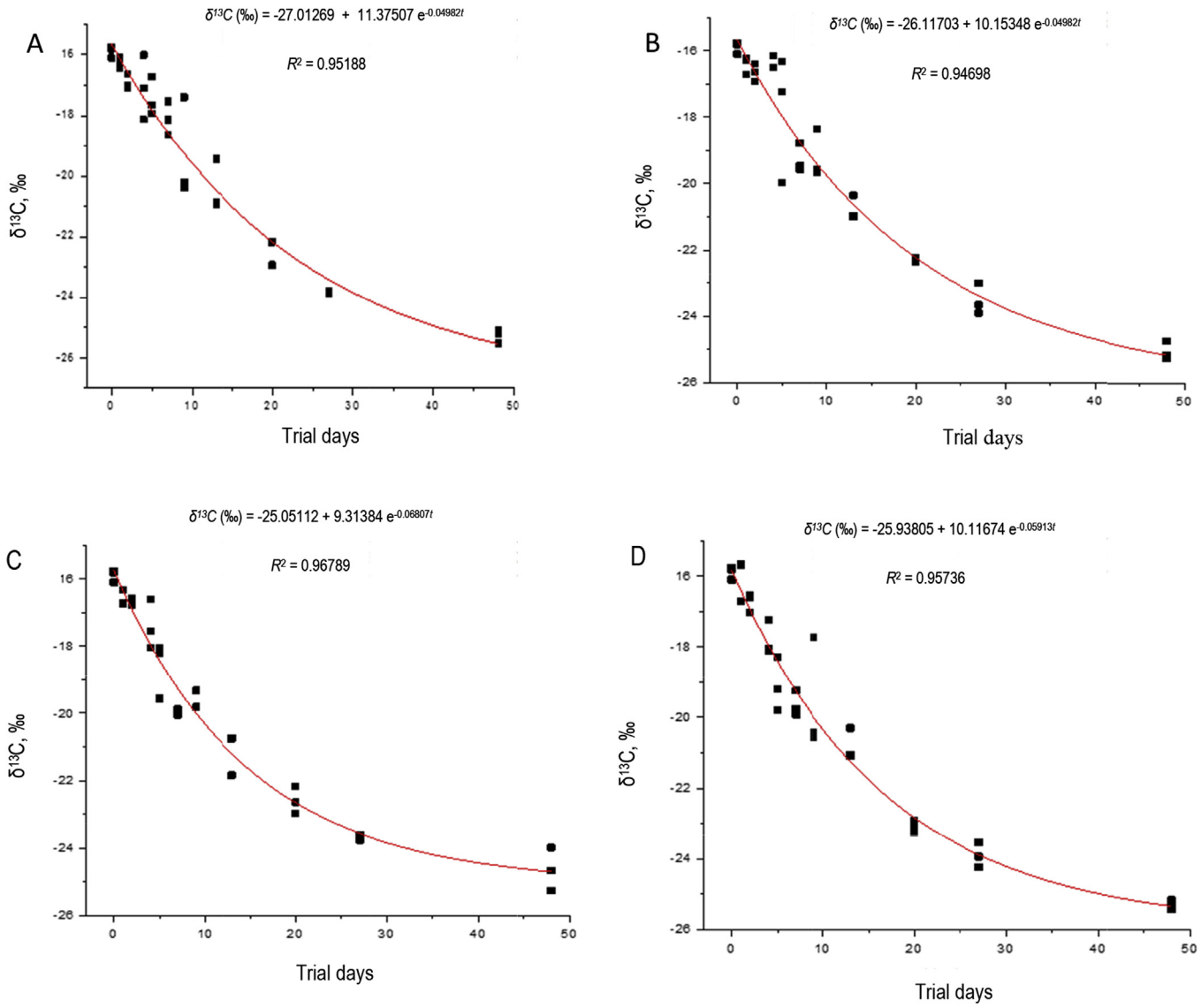

Fig. 1. Non-linear adjustment of exponential curves ($\delta‰^{13}C_{V\text{-}PDB}$) of piglets' stomach: (A) control diet, (B) 1% glutamine, (C) 1% glutamic acid, (D) 1% nucleotides.

USA, 2007) in order to obtain the $T_{50\%}$-, $T_{90\%}$- and F-values. In regard to pH-values of stomach content, data were submitted to analysis of variance by PROC GLM procedure of SAS (SAS Institute Cary, NC, USA), and means compared by Tukey's test ($P < 0.05$).

3. Results

The $\delta^{13}C$ values in stomach of piglets at the weaning day (−15.89‰) determined before experimental diet switching was similar to that observed on the lactation diet (−16.14‰) fed sows and piglets at farrowing facility which had received corn as an energy source (a C_4 photosynthetic plant: −17.40‰). Thus, the piglet's stomach isotopic signals at weaning have reflected the diets from sows, as expected.

Moreover, the average carbon isotopic value in fundic region of 70-day-old piglets was −25.07‰, which was similar to the average value of diets provided to piglets after weaning (−27.50‰) that had contained rice as an energy source (a C_3 photosynthetic plant: −30.14‰).

In addition, the experimental period was adequate for the isotopic dilution to occur (Fig. 1), due to the stomach $\delta^{13}C_{V\text{-}PDB}$ values of piglets at initial condition (day 0 of trial) reflected isotopic signal that, on average, was −15.89‰, characteristic signal of corn (C_4-plant) present in diet provided to sows and suckling piglets in farrowing facilities (−16.14‰) and, due to the stomach $\delta^{13}C_{V\text{-}PDB}$ values verified at the end of the experiment (49 days) on average were −25.26‰, −25.05‰, −24.64‰ and −25.32‰, characteristic signal of rice (C_3-plant), respectively in the C-diets; G-diets; GA-diets and, Nu-diets provided to animals from 21 to 70 days of age (0 to 49 trial days), which mean values of these diets $\delta^{13}C_{V\text{-}PDB}$ were respectively −27.14‰, −27.43‰, −27.26‰ and, −28.13‰.

Regarding Table 3, the stomach of piglets fed GA diet had the fastest turnover (6-day lesser than C-diet), and 4% more ^{13}C-atom substituted than C-diet at same trial period, followed by the Nu-diet that presented a half-life of 4.55-day lesser than C-diet and the same ^{13}C-atom substitution of GA-diet. The $T_{50\%}$ of G-diet was 2.4-day lesser than C-diet with 1% more ^{13}C-atoms substituted at period, being the $T_{50\%}$ of C-diet the slowest carbon turnover value as well as the lowest percentage of ^{13}C-atom substitution. The $T_{90\%}$-values of all diets show that trial period was enough for carbon turnover and to achieve the isotopic stabilization in stomach.

Table 4 shows the pH values of stomach contents according experimental diets. The GA-diet has promoted the smallest pH-lowering value ($P < 0.001$) in all phases, whereas Nu-diet has promoted the highest pH-value at pre-starter 2 phase ($P < 0.001$).

4. Discussion

The changes observed during the experimental period indicated that 49 days were enough for stomach tissue to reach the $\delta^{13}C$ isotopic equilibrium plateau and to isotopically reflect the new diet (after diet switching), corroborating the findings of Smith and Epstein (1971); as well as to reflect the isotopic discrimination between cereals (corn versus rice), according to the findings of Park and Epstein (1961). Besides that, after observations of all results obtained in this study (prior and after diet switching), the principles of isotopic dilution were achieved (Hobson and Clark, 1992; Jones et al., 1979; Tieszen et al., 1983).

The data obtained from experimental diets and stomach tissue corroborate the reported by DeNiro and Epstein (1978), as well as by Phillips and Eldridge (2006), because stomach tissue presented isotopic signal similar to diet's isotopic signal and also due to the carbon stable isotope ratios of this organ versus the carbon stable isotope ratios of diet depend on the type of tissue (its metabolism) as much as the diet's isotopic nature (Ducatti et al., 2016).

Therefore, the association of diet's composition information and the isotopic signature of stomach tissue over time are representative of the truly assimilated diet ("I am what I eat") and reflect changes occurred over time, being 2 inseparable ways to integrate diet variations in time and, more generally, to study and monitor the feed usage by the piglets (Cresson et al., 2014; Post, 2002).

Another advantage of stable isotopes technique is that it is applied *in situ*, the closest condition to reality (Hyslop, 1980), they occur naturally and can be used as dietary tracers. The carbon stable isotopes technique present an alternative to study the digestive, absorption and metabolism of nutrients because the carbon atoms are part of nutrients and, capable to generate energy, also representing almost 50% of all chemical components of piglet's body (Murray, 1990).

Studies assessing the piglet stomach turnover by IRMS are scarce in the literature, being only available ecology studies with wild animals that have tracked their stomach contents throughout the seasons aiming to evaluate their food regimen (Connan et al., 2014; Jaeger et al., 2010). Thus, in order to compare the findings of piglet turnover of stomach tissue, we have found studies with mice obtained by auto radiographic method with ^{3}H-thymidine (a radioactive method) which is the most widely used for obtain the turnover of cells, as it usually labels the nuclei of mitotic cells. Creamer et al. (1961) reported results of turnover in the fundus of stomach in albino mice by this method of about 5 days, whereas Helander (1993) reported the turnover time of muco-zymogenic cells in mouse of 78 days after intravenous infusion of ^{3}H-thymidine.

The findings of Fry and Arnold (1982) have revealed tissue-specific isotopic incorporation rates that corroborate the results of present study. These authors verified that the internal organs and blood plasma tend to have high rates of isotopic incorporation compared to blood cells and muscles. In this way, the analysis of tissues can provide information about the temporal dynamics of diet usage (nutrients integration at different time-scales) and help the interpretation of the isotopic data of tissues.

Studies evaluating the turnover of other internal organs reported the same general conclusion that tissues of high metabolic activity tend to present lower carbon turnover values. Caldara et al. (2008) evaluating the effect of glutamine on carbon turnover of weaned piglets at 21-day-old obtained a liver-$T_{50\%}$ of 9.2 days (control diet) and 7.9 days (1% glutamine diet) throughout 46 days; whereas Amorim (2012) studying the effects of supplementation of additives in weaned piglets' organs reported liver-$T_{50\%}$ of 8 days (control diet), 10 days (1% glutamine diet), 7.1 days (1% glutamate diet) and 7.4 days (1% nucleotides diet); pancreas-$T_{50\%}$ of 14 days (control diet), 13.4 days (1% glutamine diet), 12 days (1% glutamate diet) and 10.5 days (1% nucleotides diet); kidneys-$T_{50\%}$ of 15.3 days (control diet), 15.2 days (1% glutamine diet), 13.1 days (1% glutamate diet) and 12.6 days (1% nucleotides diet). Besides that, the author has obtained spleen-$T_{50\%}$ of 15.1 days (control diet), 16.6 days (1% glutamine diet), 15 days (1% glutamate diet) and 14 days (1% nucleotides diet) throughout 49-day trial.

Another studies have shown that glutamine inclusion in the piglets' diet in the first week after weaning has improved feed:gain ratio, avoiding the villi atrophy and providing some resistance to *Escherichia coli* infection (Lopez et al., 1997; Wu et al., 1996), an increase on body weight gain, small intestine weight (increased villi), growth of visceral organs in early-weaned piglets (Lackeyram et al., 2001), being supportive in improving digestion, absorption and retention of nutrients by affecting tissue anabolism, stress and immunity (Lallès et al., 2009). According to Alverdy et al. (1992) the presence of L-glutamine in the diet increases the metabolic activity of enterocytes and their proliferation; maturation and migration of crypt cells, as well as, the lymphocytes function (Dugan et al., 1994).

Furthermore, Rezaei et al. (2013) reported that dietary supplementation with monosodium glutamate (MSG) is safe and improves growth performance in post-weaning pigs and stated also that in comparison to control diet, the dietary supplementation with 1%, 2% and 4% MSG dose-dependently increased plasma concentrations of glutamate, glutamine, and other amino acids (including lysine, methionine, phenylalanine and leucine), daily weight gain, and feed efficiency in post-weaning pigs. At first week post-weaning, dietary supplementation with 1% up to 4% MSG increased jejunal villus height, DNA content, antioxidative capacity, and reduced the incidence of diarrhea.

Monosodium glutamate facilitates gastric emptying of a protein-rich meal, plays an important role in protein digestion (Zai et al., 2009) and, is added as a flavor enhancer to foods, being responsible for the fifth basic taste, referred to as umami (Halpern, 2000), what suggests the effects of MSG on the stomach physiology depends on nutritional conditions (Feng et al., 2014). Oral intake of MSG stimulates secretion from exocrine system (saliva, gastric and pancreatic juices) as well as binds to receptors on taste cells in oral cavity, activating taste nerves that elicit the umami taste (Halpern, 2000), activates contractile action in gastric fundus and ileum, possibly via cholinergic neurons (Niijima, 2000).

Besides that, the intragastric glutamate infusion accelerated gastric emptying and stimulated upper gut motility via vagus nerve in dogs (Toyomasu et al., 2010), whereas in rats, the intragastric infusion of free glutamate stimulated afferent gastric vagal nerves via serotonin secretion and nitric oxide production (Uneyama et al., 2006), although when added to a partial enteral diet for preterm pigs, it has slowed gastric emptying (Bauchart-Thevret et al., 2013).

In the other hand, nucleotides are important in providing nitrogen bases and nucleosides for tissues deficient on nucleotide synthesis, and have trophic action in faster growing tissues (Mateo, 2005), specially at periods as weaning, when the need for nucleotides is greater, but the availability of precursors required for their synthesis (energy and glutamine) is reduced (Rodwell, 2000; Rossi et al., 2007).

The supplementation of nucleotides in diets of post-weaning piglets is necessary to maintain the integrity of the

gastrointestinal tract, because studies have shown that 2% to 5% of the nucleotides in diet are retained in the small intestine, liver and muscles (Savaiano and Clifford, 1978; Uauy, 1994) and occurs at increased levels in young animals who fit into specific physiological conditions such as rapid growth, low immunocompetence or subject to stressors, common characteristics to the piglets' post-weaning phase (Gross and Savaiano, 1991).

According to Teixeira et al. (2001) verified that piglets from 21 to 28 days of age fed the mixture of 1% glutamine and glutamic acid had higher daily weight gain and daily feed intake and less diarrhea compared to control diet and, also Teixeira et al. (2014) observed that the mixture of 1% glutamine and glutamic acid in diet improved performance and morphophysiology of weaned piglets at 21 days of age.

Ferreira et al. (1992) reported that the supply of complex diets, from the 7th or 10th days after birth, stimulated the development of digestive enzymatic system, favoring the best usage of diets. Another study concluded that early-weaned piglets fed up to 200 mg/kg of nucleotides in the diet improved the performance without damage to their health, and also reduced the presence of *Salmonella* spp., which represents a reduction in diseases' incidence (Andrade, 2013).

Abreu et al. (2010) evaluated the effect of glutamine, nucleotides and swine plasma supplementation in diets on performance, intestinal morphology and immune response of weaned piglets at 21 days of age, and stated that the presence of nucleotides in diets has favored the weight gain of animals, what according to Mateo and Stein (2004) is explained by the fact that piglets in periods of fast growth require more quantity of nucleotides to express their genetic potential.

Concerning the stomach pH values, as digestion of dietary protein starts in gastric lumen, the pH-lowering promoted by glutamic acid may have contributed to the greater activation of gastric proteases and protein hydrolysis in comparison to other treatments, corroborating the findings of Bayley (2013) and the greater feed intake of piglets (Lescano et al., 2013).

In addition, Teixeira et al. (2014) reported that a decreased stomach pH value as animals get old is due to piglets' increasing capacity of adjusting gastric-pH by the hydrochloric acid production. Other study observed that low pH is beneficial to the development of the non-pathogenic bacteria and/or to inhibit the development of pathogenic bacteria (Wu et al., 2011).

5. Conclusion

Glutamine, glutamic acid or nucleotides dietary supplementation have accelerated the carbon turnover in piglets' stomach during post-weaning period. To date, carbon dilution technique ($\delta^{13}C$) is an important methodology that should be further independently investigated (stable isotope labeling for obtaining biomolecules as biomarkers for animal nutrition studies).

Conflict of interest statement

We wish to confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgments

The authors acknowledge the financial support of this work (grant number: 2014/13518-0) provided by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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