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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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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.

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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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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 1 feeder, 1 nipple-type drinker, and 1 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 for 5 min at constant rotation (9,700 rpm) in a cryogenic mill (SPEX Sample Prep, Geno/Grinder 2010, Metuchen, NJ, USA) at −190 °C 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:

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

$\delta^{13}C$ 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).

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