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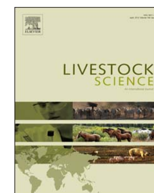

Estimation of the metabolic rate by assessing carbon-13 turnover in broiler tissues using the stable isotope technique

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ABSTRACT

The aim of this study was to estimate the metabolic rate of broiler tissues by assessing carbon turnover in different growth phases by using the stable isotope technique and by evaluating the natural changes in Carbon-13 (^{13}C) between C3 and C4 cycle plants. Six-hundred 1-day-old broiler chicks were divided into six groups and subjected to dietary changes containing different isotopic values at 7, 14, 21, 28, and 35 days of age. Birds of Group 1 received diets composed predominantly of C_3 cycle plants shortly after accommodation, while birds of other groups remained predominantly fed with C_4 diet. The diet of Group 2 was mainly replaced by C_3 diet on the 7th day of age. Group 3 started to receive C_3 diet on the 14th day of age, whereas the diet of Group 4 was replaced on the 21st, Group 5 on the 28th. In Group 6, the substitution occurred on the 35th day of age. Samples of the pectoral muscle, keel, tibia, legs muscle, intestinal mucosa, blood, plasma, and feathers were collected. Carbon isotope ratios were determined by mass-spectrometry of isotope ratios. To measure the speed of carbon substitution after a set interval (turnover), a time exponential function was employed and obtained by exponential equations of the first order calculated with Minitab® 16 software. The carbon half-life varied from 1.78 to 8.20 days for the pectoral muscle, from 1.91 to 12.24 days for the keel, from 2.32 to 10.71 days for the tibia, from 1.87 to 9.43 days for the leg muscle, from 0.8 to 1.58 days for the intestinal mucosa, from 0.64 to 1.71 days for the plasma, from 2.61 to 11.07 days for blood, and from 1.84 to 28.41 days for the feathers during the total growth phase. With the exception of tibia and blood, all other tissues presented a high metabolic rate in the first week of the broilers' life. The metabolic rate of tissues is faster in younger animals, and the metabolism slows down as the broilers get older. The metabolic rates in the pectoral muscle, keel, tibia, and feathers decreased with age, whereas those of the plasma and intestinal mucosa were high in all evaluated periods; thus, these tissues can be used to study traceability of feed at all stages of the broilers' life.

1. Introduction

The stable carbon isotopes technique was initially used in geological and archeological studies (Jones et al., 1979; Tieszen et al., 1979; Boutton et al., 1980). However, it has been lately increasingly and continuously applied in agricultural, ecological, and physiological research as an alternative technique in studies on nutrient digestion, absorption, and metabolism of humans and animals, as well as to identify and to determine the origin of plant and animal products (Gannes et al., 1998). This method is based on the fact that C_3 and C_4 plants possess distinctly different $^{13}C/^{12}C$ ratios due to fractionation during photosynthetic carbon fixation (Smith and Epstein, 1971; O'Leary, 1981). The values of isotope ratios were expressed in delta ($\delta^{13}C$). The $\delta^{13}C$ values of most C_3 plants range from −24‰ to −34‰ while most C_4 plants have $\delta^{13}C$ values which lie between −6‰ and −19‰ (Smith and Epstein, 1971). Thus, the stable carbon isotopes technique has been successfully used to test the authenticity and the quality of several products, such as orange juice (Bricout and Koziet, 1987; Koziet, 1993), wine (Martin et al., 1988), honey (Brookes et al., 1991; White et al., 1998) and dairy products (Rossmann et al., 2000; Manca et al., 2001). The difference of $\delta^{13}C$ values between these plants is large enough so that the relative amounts of C_3 and C_4 plants eaten by an animal can be determined from the $\delta^{13}C$ value of its carbon (DeNiro and Epstein, 1978), enabling analysis of turnover of stable isotopes in

Abbreviation: AOI, animal-origin ingredients; IRMS, isotope ratio mass spectrometer; PDB, PeeDee Belemnite

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animal tissues (Tieszen et al., 1983; Hobson and Clarck, 1992, 1993; Hobson, 1995; Hobson and Bairlein, 2003; Ayliffe et al., 2004; Buchheister and Latour, 2010; Oppel and Powell, 2010). More recently, this technique has been used in traceability studies of AOI in poultry feed via tissue analysis (Carrijo et al., 2006; Oliveira et al., 2010; Denadai et al., 2008; Sernagiotto et al., 2013) or identify the origin of a food (Rock, 2012). Furthermore, this technique has been used to evaluate the effect of natural additives, alternative to APE, on the structure of the intestinal mucosa. This was performed by assessing the tissue turnover, given the estimated speed of mucosal regeneration or development (Pelícia et al., 2011).

For traceability, the tissue chosen should reflect the recent diet of the animal and should be able to detect signs in isotopic assimilation of the ingredients to be traced, even if this was supplied during a single earlier growth phase of the animal only.

In the body, slow tissue metabolism can preserve the initial isotope signal from the diet for longer, thus enabling animal-origin ingredients (AOI) detection in poultry after slaughter, and sometimes when provided at an early phase. Conversely, tissues presenting a faster metabolism (in which the isotope signal from the diet is replaced more quickly) may enable AOI detection during the final phases of development, thus reflecting the importance of evaluating metabolic renewal rates or turnover of different tissues and stages of broilers growth. However, to develop these lines of research, further studies would be required to improve the knowledge of isotopic assimilation of organic matter in different bird' tissues and growth phases.

Ducatti et al. (2014) assessed ^{13}C turnover in pectoral muscle, keel, tibia, and liver to obtain physiological information that would be essential for traceability studies. However, the time of assessment and tissue harvest was not sufficient for these tissues to achieve a balanced level of carbon isotope exchange. The authors suggested that new studies should be carried out that increased the evaluation time to confirm the half-lives of stable isotopes. Therefore, the aim of this study was to estimate the metabolic rate via carbon turnover in pectoral muscle, legs muscle, keel, tibia, intestinal mucosa, blood, plasma, and feathers from broiler wings at different growth stages, using natural differences in ^{13}C existing between plants with distinct photosynthetic cycles (C_3 and C_4).

2. Material and methods

2.1. Birds, diets and experimental design

The survey was conducted at the Laboratory of Avian Nutrition of Faculdade de Medicina Veterinária e Zootecnia, UNESP, Botucatu Campus, São Paulo, Brazil, and all studies were approved by the Ethics Committee for Animal Experimentation (Protocol No 78/2009 - CEEA).

Six-hundred 1-day-old male Cobb broiler chicks were distributed in a completely random design into six groups (named G1, Group 1; G2, Group 2; G3, Group 3; G4, Group 4; G5, Group 5 and G6, Group 6). Each experimental unit comprised 25 birds (8.3 birds/m^2). Birds were housed in an experimental shed divided into twenty-four floor pens of 3 m^2. Pens were equipped with hanging drinkers and tubular feeders. Feed and water were offered ad libitum during the entire experimental period. Temperature and ventilation were controlled manually by managing the rearing house lateral curtains.

The rearing period was divided into five phases (pre-starter: 0–7 days, starter: 8–21 days, grower: 22–35 days, finisher 1: 36–62 days, and finisher 2: 63–98 days of age). The food was formulated and adapted in accordance with the tables provided by Rostagno et al. (2011) for nutritional requirements. To reduce variation in the isotopic signal of diets supplied in each growth phase, their concentrations were adjusted where necessary with the addition of rice or corn (Table 1).

The chicks were obtained from breeder hens that received diets predominantly composed by grains of photosynthetic cycle C_4 plants (based on corn and soybeans). At birth, they presented isotopic values of Carbon-13 in tissues similar to those found in the diets (close to −19‰). To assess their turnover in tissues, birds of G1 received diets composed predominantly of C_3 cycle plants (based on rice and soybean) shortly after accommodation, while birds of other groups remained predominantly fed with C_4 diet (based on corn and soybeans). The diet of G2 was mainly replaced by C_3 diet (based on rice and soybeans) on the 7th day of age. G3 started to receive C_3 diet on the 14th day of age, whereas the diet of G4 was replaced on the 21st, G5 on the 28th. In G6, the substitution occurred on the 35th day of age (Table 2).

2.2. Sample collection and preparation

Weekly replacement of the diet allowed the evaluation of tissue turnover during several stages of bird growth, since broiler metabolism differs with age, being faster at the beginning due to the full exchange of isotopic signals in the first week of life that may occur according to the tissue analyzed. The first collection of each group was always performed prior to the exchange of the diets to detect the baseline isotopic signal of the tissues. The collection time of each group was estimated, due to the decrease of metabolism with advancing age, to allow the total isotopic signal exchange of the analyzed tissues. During collection days, six birds per group were randomly selected and sacrificed by cervical dislocation, and each bird was considered a repetition. Blood samples, blood plasma, pectoral muscle, keel, tibia, leg muscle (thigh/drumstick complex), intestinal mucosa, and wing feathers were harvested.

Five-centimeter thick slices of the pectoral muscle were obtained from 5 cm from the longitudinal middle third of left Pectoralis major. To obtain keel samples, the cartilaginous extension was removed from the sternum and the bone insertion was trimmed by a transversal cut, forming a right angle with its dorsal surface. Leg muscle samples were taken from the more lateral peroneal iliotibial. Tibial bone samples were obtained from the longitudinal middle third of the left tibia, excluding spinal content through scraping and rinsing with distilled water. Mucosal samples were collected from small intestine (in the duodenum) by means of scraping with a glass cover slip. Blood samples were collected in 1-mL plastic tubes and in test tubes pre-treated with heparin, which were subsequently centrifuged to obtain plasma. Feather samples were taken from bird's wings. All samples were labeled accurately and immediately frozen at −20 °C until further use.

Sample preparation and isotope analyses were performed at the Centro de Isótopos Estáveis of Instituto de Biociências, UNESP, Botucatu Campus, São Paulo, Brazil. Samples of pectoral muscle, keel, tibia, leg muscle, and feather were thawed, washed in distilled water, and dried in a forced-ventilation oven (MA035, Marconi, Piracicaba, Brazil) at 55 °C for 48 h. Samples of blood, plasma, and mucosa were thawed and dried under a vacuum for 48 h in a lyophilizer (L108, Liotop, São Carlos, Brazil). Approximately 2.0 g each of the pectoral muscle, keel, tibia, and leg muscles samples, and each experimental diet was ground using a cryogenic mill (2010 Geno/Grinder, SPEX SamplePrep, Metuchen, USA) at −196 °C for 3 min to obtain homogeneous material with fine granulometry (less than 60 µm), which were packed in plastic tubes as previously identified.

2.3. Isotopic and analytical measurements

Approximately 50–70 µg of samples were weighed and packed into tin capsules, and introduced by means of an automatic viewer in an elemental analyzer (Flash 2000 Organic Elemental Analyzer, Thermo Fisher Scientific, Waltham, USA) for isotope ratio mass spectrometer (IRMS), in which, oxygen (O2) and copper oxide (CuO) were reacted to obtain CO2. The gases formed were separated in a gas chromatographic column and analyzed by mass-spectrometry of isotope ratios (Delta V Isotope Ratio Mass Spectrometer, Thermo Fisher Scientific, Waltham, USA).

The values of isotope ratios were expressed in delta ($\delta^{13}C$) relative to PeeDee Belemnite (PDB) international standard with an analysis

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