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Traceability of poultry offal meal in broiler feeding using isotopic analysis ($\delta^{13}C$ and $\delta^{15}N$) of different tissues*

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ABSTRACT

Our goal was to trace the inclusion of poultry offal meal (OM) in diets by using carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratios of different tissues in order to contribute for the development of an independent technology for the certification of the feeding of broilers reared on diets with no addition of animal ingredients. Eighty one-day-old chicks were randomly distributed into five experimental treatments, that is, diets containing increasing levels of OM inclusion (0, 2, 4, 8 and 16% OM), with four replicates of four birds each. At 42 days of age, four birds per treatment (n=4) were randomly selected, weighed, and sacrificed to collect breast muscle (*Pectoralis major*), keel and tibia samples to determine their isotopic ratios ($^{13}C/^{12}C$ e $^{15}N/^{14}N$). It was observed that ^{13}C and ^{15}N enrichment increased as a function of increasing OM inclusion in all diets. The analyses of the *Pectoralis major* showed that that only treatments with 8 and 16% OM dietary inclusion were different form those in the control group (0% OM). On the other hand, when the keel and tibia were analyzed, in addition to 8 and 16% OM), the treatment with 4% OM inclusion was also different from the control group. The use of isotopic ratios of stable carbon and nitrogen isotopes is an alternative to trace OM inclusion in broiler diets as it is capable of tracing OM levels below those usually practiced by the poultry industry in Brazil.

INTRODUCTION

Animal byproducts have been used in animal feeding for many years without much concern. However, the use of fish, meat, blood, and offal meals, blood plasma and milk by-products has been widely questioned, and were even banned in some country. This was partially due to consumers' scare after BSE (*Bovine Spongiform Encephalopathy* - mad cow disease) cases and problems of animal product contamination with *Salmonella* and *Escherichia coli*. In Brazil, to provide more safety to these protein sources, rendering plants that produce animal meals and fat must comply with the requirements established by Normative Instruction # 15 of October 29, 2003 of the Brazilian Ministry of Agriculture (MAPA, according to its acronym in Portuguese) to ensure their hygiene and health, as well as good manufacturing practices (GMP).

When consumers have reservations as to determined food categories, producers are consequently suffer pressure to replace their current production practices by methods accepted by the consumers. According to the regulation (CE) # 1774/2002 of the European Parliament and the European Union Council, Consolidated Text (CONSLEG, 2004), chapter 1, article 22, it is forbidden to feed an animal species with transformed animal proteins derived from bodies, or parts of bodies, of animals of the same species.

Many markets importing chicken meat from Brazil, such as the European Union and the Middle East, require that birds are not fed with animal feed ingredients and antibiotic growth promoters. Since the end of 2004, Brazil became the world leading chicken meat exporter, both in terms of revenues and volume, achieving record performance . According to ABEF (2010), Brazil exported in 2009 3.6 million tones of chicken meat, and the estimated export revenues was USD 5.8 billion, which represents a significant growth of 46% when the years of 2004 and 2009 are compared.

Moreover, the global organic market turns over approximately USD 23.5 billion dollars annually, and it is expected to grow 20% per year. This market includes fresh, processed, further-processed products, and even personal care articles, produced with raw materials derived from organic systems (Souza & Alcântara, 2005). In this scenario, the so-called organic, alternative, or label-rouge chickens, which, as well as export poultry, are not fed any animal byproducts, becomes increasingly important in the domestic and international markets.

Traceability is a key tool for the certification of meats and other products, which is essential for the survival and success of companies inserted in an increasingly competitive and demanding market. By definition, according to ISO norm 8402 (1994), traceability is the capacity to trace the history, application or location of an entity by means of previously recorded identification. However, recording data is not sufficient to ensure the specific characteristics of a given product. According to Ilbery *et al*. (2000), it is necessary to develop independent technologies of meat certification to placate consumers, protect regional designations and ensure fair competition.

The measurement of the stable isotopes of the chemical element carbon ($^{13}C/^{12}C$) has been successfully used to test the authenticity, quality and geographical origin of several products, such as fruit juices (Bricout & Koziet, 1987; Koziet *et al*., 1993), wines (Martin *et al*., 1988), honeys (Brookes *et al*., 1991; Reniero *et al*., 1997; White *et al*., 1998), dairy products (Rossmann *et al*., 1998; Rossmann *et al*., 2000; Manca *et al*., 2001), vegetable oils (Kelly *et al*., 1997), and to characterize and differentiate Iberian swine diets, allowing animal classification according to the kind of feed received during the fattening period (Gonzáles-Martín *et al*., 1999; Gonzáles-Martín *et al*., 2001). Coupled with the isotopic ratio of the chemical element nitrogen ($^{15}N/^{14}N$), it allowed the certification of geographic region and cattle and sheep feeds (Piasentier *et al*., 2003; Renou *et al*., 2004; Schimidt *et al*., 2005).

Each tissue or biochemical fraction has its own isotopic "memory", which is a function of the isotopic ratio of the food used for its synthesis, of subsequent foods, or biochemical turnover rate of that tissue (Tieszen, 1978). Isotopic ratios of various biochemical components of food fractionate or change when they are incorporated into consumers' tissues. The direction and magnitude of this change depend on the analyzed tissue (Tieszen *et al*., 1983). Therefore, the isotopic ratios of biochemical fractions of a specific dietary ingredient may be better evidenced in certain tissues than in others.

According to Carrijo *et al*. (2006), carbon and nitrogen isotope ratios can be used as a tool to trace the inclusion of animal byproducts in broiler diets by analyzing the isotopic ratio of the breast muscle (*Pectoralis major*). However, other tissues should be studied for that purpose, since no papers in literature on this topic were found.

As the technique of stable isotopes for traceability and certification purposes of broiler diet patterns developed, it became necessary to trace the use of poultry offal meal (OM) in broiler feeding, which led to this study, which aim was to verify the possibility of tracing OM inclusion in broiler diets using the technique of carbon and nitrogen stable isotopes in different tissues.

MATERIALS AND METHODS

The experiment was conducted in the bioclimatic chambers of Avian Nutrition Laboratory, FMVZ, Universidade Estadual de São Paulo (UNESP), Botucatu, SP, Brazil.

Eighty one-day old male Cobb chicks, derived from a flock of 43-week-old broiler breeders and vaccinated i against Infectious Bursal Disease, Marek's disease, and fowl pox, were obtained from a commercial hatchery. Chicks were housed in 0.50 m high, 0.50 m wide and 0.60 m deep metal cages equipped with individual feeders and nipple drinkers. Each cage housed four birds. .

Immediately before housing, a representative sample of ten chicks was removed directly from the transport crates in order to determine the initial average isotopic values of the evaluated tissues (breast muscle, keel, and tibia) in $\delta^{13}C$ (-18.74 ± 0.14; -17.78 ± 0.10;

and -16.57±0.42‰, respectively) and $\delta^{15}N$ (5.43±0.31; 5.74±0.27; and 6.08±0.24‰; respectively).

A 24-hour lighting program was adopted, and room temperature was controlled in order to maintain birds at thermoneutral temperature for the entire rearing period, as shown in Table 1. Both feed and water were provided *ad libitum*.

Table 1 - Layout of room temperature control to maintain a thermoneutral environment for broilers during the experimental period.

Age, days	Temperature of Bioclimatic Chamber, °C
1 to 4	35
5 to 7	32
8 to 14	30
15 to 16	28
17 to 21	26
22 to 42	24

Sartori (2000).

Birds were randomly distributed into five treatments with four replicates of four birds each. Experimental treatments are shown in Table 2. Birds were fed the experimental diets during the entire experimental period (1 to 42 days of age). Feed was formulated to meet the nutritional requirements recommended by Rostagno *et al*. (2000) for a two-phase feeding program: from 1 to 21 (starter diets) and from 22 to 42 days of age (grower diets). All diets contained equal energy, protein, calcium, phosphorus, methionine + cystine, and lysine levels.

Tables 3 and 4 show ingredient percentage composition, calculated nutritional values, and isotopic values of the starter and growers diets, respectively.

Table 2 - Identification of experimental treatments.

Treatments	Inclusion of offal meal (OM) in diet, %
T0	0
T2	2
T4	4
T8	8
T16	16

Similar small amounts of broken rice, a photosynthetic cycle C_3 plant, with a value of $\delta^{13}C$ = -29.69±0.02 ‰, were added to the grower diets to obtain carbon isotopic values similar those of the starter diets in order to avoid any variation in $\delta^{13}C$ values among diets, which could lead to possible miscalculation of carbon isotopic fractionation factor of tissues relative to their respective diets (Hobson & Clark, 1992b). All ingredients used in diet manufacturing derived from the same batch. The offal meal used in this experiment was donated by a poultry processing plant located in Tietê, SP, Brazil. This ingredient contained 95.5% dry matter (DM); 65.4% crude protein (CP); 14% ether extract (EE); 13.5% mineral matter (MM), and average values of $\delta^{13}C$ = -16.60±0,05 ‰ and of $\delta^{15}N$ = 4.25±0.05 ‰.

Table 3 - Ingredient percentage composition, calculated nutritional values, and average isotopic values in the starter diets (1 to 21 days of age).

Ingredients, %	Offal meal, %				
	0	2	4	8	16
Corn, grain	57.91	60.34	62.77	66.48	67.44
Soybean, meal	35.45	32.04	28.62	22.10	10.35
Poultry offal, meal	-	2.00	4.00	8.00	16.00
Soybean, oil	2.57	1.83	1.10	-	-
Limestone	0.99	0.95	0.91	0.84	0.54
Dicalcium phosphate	1.82	1.57	1.31	0.80	-
DL-Methionine	0.24	0.23	0.23	0.22	0.21
L-Lysine	0.17	0.21	0.25	0.29	0.37
Kaolin	-	-	-	0.50	4.40
Salt	0.45	0.43	0.41	0.37	0.29
Vitamin-mineral supplement[1]	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100
Calculated composition					
ME, kcal/kg	3,000	3,000	3,000	3,000	3,000
CP, %	21.40	21.40	21.40	21.40	21.40
CF, %	3.23	3.10	2.97	2.72	2.15
Ca, %	0.96	0.96	0.96	0.96	0.96
Available P, %	0.45	0.45	0.45	0.45	0.45
Met, %	0.57	0.56	0.57	0.56	0.57
Met + Cys, %	0.90	0.90	0.90	0.90	0.90
Lys, %	1.26	1.26	1.26	1.26	1.26
Average isotopic values[2]					
$\delta^{13}C$, ‰	-18.08	-17.77	-16.61	-15.82	-14.59
$\delta^{15}N$, ‰	1.20	1.53	1.79	2.29	3.05

Starter vitamin-mineral supplement Vaccinar® (levels per kg of diet): vitamin A, 14,000 IU; vitamin D_3, 2,500 IU; vitamin E, 25 mg; vitamin K_3, 3 mg; thiamine, 2 mg; riboflavin, 5 mg; pyridoxine, 4 mg; vitamin B_{12}, 25 mcg; niacin, 35 mg; pantothenic acid, 12 mg; biotin, 0.10 mg; folic acid, 1 mg; choline, 800 mg; antioxidant, 2 mg; selenium, 0.18 mg; iron, 50.10 mg; manganese, 78 mg; iodine, 0.70 mg; copper, 10 mg; zinc, 55 mg.[2]Average isotopic values of $\delta^{13}C$ and $\delta^{15}N$ relative to the international standard Peedee Belemnite (PDB) and atmospheric nitrogen (N_2), respectively.

At 42 days of age, four birds per treatment (n = 4) were randomly selected, weighed, and sacrificed by neck dislocation for collection of breast muscle, keel and tibia samples to carry out isotopic analyses.

Breast muscle samples were collected removing a cross-sectional slice of approximately 5 mm from the middle longitudinal third of the left *Pectoralis major*. In order to obtain keel samples, the cartilaginous projection of the sternum was dissected, and its insertion in the bone was transversally cut, determining a right angle with its dorsal surface. Bone samples were obtained by collecting the middle longitudinal third of

the left tibia, which had its marrow content removed by washing with distilled water. All tissue samples were duly identified and frozen at -20°C.

Tissue samples were subsequently thawed, washed in distilled water, and dried in a forced-ventilation oven (Marconi - model MA 035) at 55°C for 48 hours. Sample were then ground in a cryogenic mill (Spex - model 6750 freezer/mill) at -196°C for three (tissues) or five (diets) minutes at maximum frequency in order to obtain homogeneous material with very thin talcum-like particle size (Licatti, 1997; Ducatti, 2004).

Isotopic analyses of tissue, ingredient, and diet samples were conducted at the Stable Isotopes Center, IB, UNESP, Botucatu, SP, Brazil. In order to determine carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratios, an isotopic ratio mass spectrometer (IRMS) was used (DELTA-S, FINNIGAN MAT) coupled to an Elemental Analyzer (EA 1108 CHN), according to the method described by Ducatti *et al*. (1979). Carbon and nitrogen analyses were carried out in duplicate in all samples.

The results of the analyses were expressed in parts per thousand (‰) relative to the international standard Peedee Belemnite (PDB) and atmospheric nitrogen (N_2), for carbon and nitrogen elements, respectively, according to the expression:

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

Where R represents the ratio between the least and the most abundant isotope, in particular $^{13}C/^{12}C$ and $^{15}N/^{14}N$.

The results obtained in the isotopic analyses of carbon and nitrogen were submitted to multivariate analysis of variance (MANOVA) with the aid of the GLM procedure (*General Linear Model*) of the statistical program SAS (1999). Based on the data generated by the error matrices for each tissue, regions (ellipses) were defined within 95% confidence limits to assess differences between experimental treatment averages and control group average (strictly-vegetable diet).

Data on live weight immediately before slaughter were analyzed by one-way ANOVA, using the GLM procedure of the statistical program SAS (1999).

RESULTS AND DISCUSSION

The results of the isotopic analyses ($\delta^{13}C$ and $\delta^{15}N$) of the diets used in this study are shown in Tables 3 and 4. Owing to the increasing OM percentage in diets, $\delta^{13}C$ and $\delta^{15}N$ were enriched in these diets. This was probably due to the variations in the percentage composition of the dietary ingredients. Because all diets within rearing phase (starter and grower) were formulated to contain equal energy and protein levels, the inclusion of soybean meal and oil decreased and corn inclusion increased as dietary OM percentage increased ($\delta^{13}C$ = -16.60‰).

Table 4 - Ingredient percentage composition, calculated nutritional values, and average isotopic values in grower diets (22 to 42 days of age).

Ingredients, %	Offal meal, %				
	0	2	4	8	16
Corn, grain	59.74	62.20	64.67	69.27	70.50
Soybean, meal	29.73	26.32	22.91	16.20	4.30
Poultry offal, meal	-	2.00	4.00	8.00	16.00
Rice, broken	3.69	3.68	3.63	3.77	3.94
Soybean, oil	3.05	2.31	1.58	0.14	-
Limestone	0.93	0.89	0.85	0.78	0.35
Dicalcium phosphate	1.63	1.37	1.12	0.60	-
DL - Methionine	0.22	0.22	0.21	0.20	0.19
L – Lysine	0.22	0.25	0.29	0.34	0.43
Kaolin	-	-	-	-	3.67
Salt	0.39	0.36	0.34	0.30	0.22
Vitamin-mineral supplement[1]	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100
Calculated composition					
ME, kcal/kg	3,100	3,100	3,100	3,100	3,100
CP, %	19.30	19.30	19.30	19.30	19.30
CF, %	2.94	2.82	2.69	2.44	1.87
Ca, %	0.88	0.88	0.88	0.88	0.88
Available P, %	0.41	0.41	0.41	0.41	0.41
Met, %	0.52	0.52	0.52	0.52	0.52
Met + Cys, %	0.83	0.83	0.83	0.83	0.83
Lys, %	1.16	1.16	1.16	1.16	1.16
Average isotopic values[2]					
$\delta^{13}C$, ‰	-18.14	-17.64	-16.97	-15.85	-14.47
$\delta^{115}N$, ‰	2.11	2.27	2.45	3.13	4.29

[1]Grower vitamin-mineral supplement Vaccinar® (levels per kg of diet): vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 4 mg; pyridoxine, 4 mg; vitamin B_{12}, 20 mcg; niacin, 30 mg; pantothenic acid, 10 mg; biotin, 0.06 mg; folic acid, 1 mg; choline, 600 mg; antioxidant, 2 mg; selenium, 0.18 mg; iron, 50.10 mg; manganese, 78 mg; iodine, 0.70 mg; copper, 10 mg; zinc, 55 mg. [2]Average isotopic values of $\delta^{13}C$ and $\delta^{15}N$ in regards to the international standard *Peedee Belemnite* (PDB) and atmospheric nitrogen (N_2), respectively.

Although the ingredients used in diet manufacturing were not isotopically analyzed for carbon or nitrogen, it is known that plants of the C_3 photosynthetic cycle (e.g., soybeans, rice, wheat) have a $\delta^{13}C$ modal value of -27.6‰ relative to the PDB standard, whereas the plants of the C_4 photosynthetic cycle (e.g., corn, sorghum, sugarcane) present a $\delta^{13}C$ modal value of -12.6‰ (Vogel, 1993).

Grower diets should be richer in ^{13}C as compared to starter diets, because they contained more energy and less protein, changing dietary ingredient percentage composition; however, no significant differences were observed. This would have required

the inclusion of more corn and less soybean meal in the grower diet, but instead, small amounts of broken rice were included in the experimental grower diets to minimize the possible variation in $\delta^{13}C$ relative to the starter diets to allow proper calculation of isotopic carbon fractionation factors in tissues and their respective diets, without changing $\delta^{13}C$ value of the diet as a whole.

Likewise, variations in dietary ingredient percentages should have been directly responsible for nitrogen isotopic enrichment (Tables 3 and 4) since, as dietary OM inclusion increased ($\delta^{15}N$ = 4.25‰), lower soybean meal and higher corn needed to be added. Soybean meal $\delta^{15}N$value is close to the standard atmospheric N_2 value ($\delta^{15}N$ 0.0 ± 1.0‰), because it fixes air nitrogen and has a ^{15}N fractionation factor around one unit, usually below 1.003‰ (Kohl & Shearer, 1980; Handley & Raven, 1992; Werner & Schmidt, 2002). The $\delta^{15}N$ value of plants that are not able to fix atmospheric nitrogen depends on the abundance of that isotope in the soil and in the manure used in the crop, such as the case of corn (Choi *et al*., 2002), and probably, rice. Sleiman *et al*. (2004), evaluating corn and rice samples in some Brazilian states, found average values of 4.77±1.16‰ and 9.23± 0.72‰ for $\delta^{15}N$, and -11.74±0.40 and -28.87±0.42 for $\delta^{13}C$, respectively.

Average values of $\delta^{13}C$ and $\delta^{15}N$ in the *Pectoralis major*, keel, and tibia of broilers in the end of the experimental period are shown in Table 5.

Figures 1A, 1B, and 1C show the regions (ellipses) within 95% confidence limits to assess differences between isotopic pair ($\delta^{13}C$ and $\delta^{15}N$) averages in each tissue according to experimental treatment as compared to the control group (strictly-vegetable diet).

No significant differences were found in broiler average live weight immediately before slaughter.

It was observed that ^{13}C and ^{15}N enrichment increased as a function increasing OM dietary inclusion. Although each tissue within the same animal may have specific isotopic signature, fractionation factor (Hobson & Clark, 1992b), and isotopic turnover (Hobson & Clark, 1992a), the animal is what it isotopically consumes, according to DeNiro & Epstein (1976, 1978), or up to ±2.0‰ ^{13}C and up to ±3.0‰ ^{15}N. As the isotopic signature of the diets changed with OM inclusion (Tables 3 and 4) and that was reflected in the broilers' tissues (Table 5, and Figures 1A, 1B and 1C), considerations on diet composition are essential in studies carried out to detect animal byproducts in broiler feeding.

In the *Pectoralis major*, it was observed that only T8 and T16 average values (8 and 16% OM) were different from that of the control group (0% OM), as their confidence regions did not overlap any axis of the graph. On the other hand, the analyses of the keel (Figure 1B) and the tibia (Figure 1C) showed that, in addition to treatments T8 and T16, treatment T4 (4% OM) was also different from the control group.

In order to be considered different from the control group, the confidence region of any determined treatment cannot overlap any axis of the graph. The figures show that the minimum detectable limit of OM inclusion level in broiler diets was slightly below 8%, when the tissue analyzed was *Pectoralis major*, because its confidence region was closer to $\delta^{15}N$ zero value. When the keel was used, that limit would probably be between 2% and 4% of OM inclusion. Using the tibia, the lower end of the confidence region of the 2% OM dietary level overlapped the $\delta^{13}C$ axis ($\delta^{15}N$ = zero). Nevertheless, to define that ellipsis of confidence, 1437 isotopic pair ($\delta^{13}C$ and $\delta^{15}N$) data were generated by tibia error matrix, and for only 5.2% of those pairs, ^{15}N values were null or negative ($\delta^{15}N$ = -0.01‰). Therefore, the minimum detectable limit of OM inclusion in this experiment must be immediately above 2% of OM inclusion in the diet.

In all tissues, $\delta^{15}N$ differences between treatments and control (Figures 1A, 1B, and 1C) were lower than $\delta^{13}C$ differences, generating confidence regions in the graph that are more distant from the carbon axis than from the nitrogen axis. Therefore, this indicates that

Table 5 - Average $\delta^{13}C$ and $\delta^{15}N$ values and their respective standard deviations in different tissues of 42-day-old broilers.

Inclusion of offal meal (OM) in diet, %	Tissue					
	Pectoralis major		Keel		Tibia	
	$\delta^{13}C$, ‰	$\delta^{15}N$, ‰	^{13}C, ‰	$\delta^{15}N$, ‰	$\delta^{13}C$, ‰	^{15}N, ‰
0	-19.37 ± 0.16	2.55 ± 0.05	-16.25 ± 0.18	2.66 ± 0.13	-15.46 ± 0.19	2.63 ± 0.31
2	-18.91 ± 0.13	2.60 ± 0.20	-15.74 ± 0.20	2.71 ± 0.28	-15.03 ± 0.36	2.93 ± 0.25
4	-18.23 ± 0.11	2.68 ± 0.24	-15.27 ± 0.05	3.08 ± 0.17	-14.49 ± 0.06	3.23 ± 0.20
8	-17.04 ± 0.04	3.04 ± 0.20	-14.51 ± 0.15	3.60 ± 0.14	-13.52 ± 0.09	3.82 ± 0.27
16	-15.44 ± 0.08	3.82 ± 0.13	-13.11 ± 0.04	5.14 ± 0.17	-11.84 ± 0.14	5.09 ± 0.12

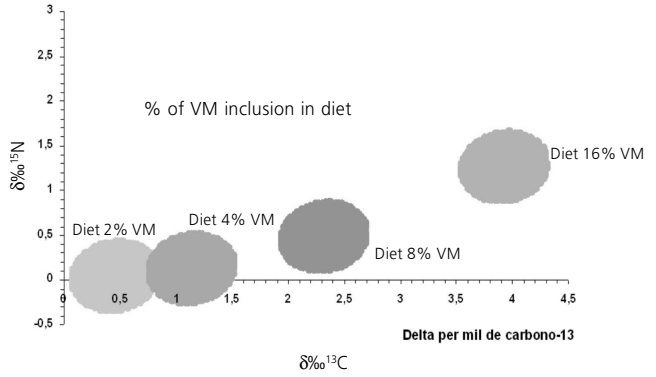

Figure 1A - Regions with 95% confidence limits of isotopic pairs ($\delta^{13}C$ and ^{15}N) average values in the *Pectoralis major* of 42-day-old broilers submitted to different treatments (2, 4, 8 and 16% of OM dietary inclusion). The axis values measure the differences between treatments averages and control group average (fed a strictly-vegetable diet).

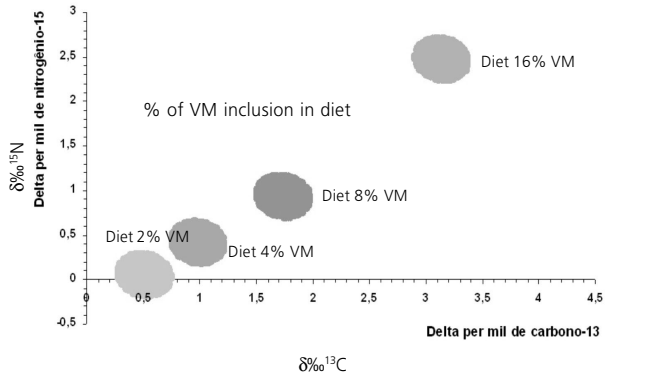

Figure 1B - Regions with 95% confidence limits of isotopic pair ($\delta^{13}C$ and ^{15}N) average values in the keel of 42-day-old broilers submitted to different treatments (2, 4, 8 and 16% of OM dietary inclusion). The axis values measure the differences between treatments averages and control group average (fed a strictly-vegetable diet).

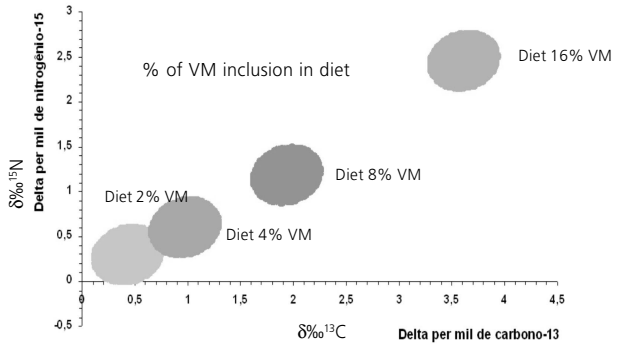

Figure 1C - Regions with 95% confidence limits of isotopic pairs ($\delta^{13}C$ and ^{15}N) average values in the tibia of 42-day-old broilers submitted to different treatments (2, 4, 8 and 16% of OM dietary inclusion). The axis values measure the differences between treatments averages and control group average (fed a strictly-vegetable diet).

$\delta^{15}N$ is more important in the detection of OM inclusion in broiler feeds because, to differentiate these feeds from those not containing OM, the confidence region of the tested inclusion level must present values higher than zero for all isotopic pairs.

As compared to the Pectoralis major, the keel and tibia were shown to be more adequate for the detection of low OM inclusion levels in broiler diets because they provided clearer evidence of the difference between treatments and the control group due to higher ^{15}N enrichment.

These significant ^{15}N enrichment differences among the studied tissues may be partially related to the differences in essential and non-essential amino acid tissue composition. Breast muscle tissue consists mainly of essential amino acids (Moran Jr., 1999), which, when incorporated into the tissues, do not considerably change their isotopic ratio (Pinnegar & Polunin, 1999). On the other hand, collagen, which accounts for approximately 90% of the bone organic matrix composition (Knott & Bailey, 1998) and, therefore, it is the main of nitrogen in the bone, contains more non-essential amino acids (Hobson *et al*., 1993). Hence, diets containing OM could provide a larger amount of ^{15}N available for the endogenous synthesis of non-essential amino acids, in addition to providing these intact in the dietary protein. The proteins in the keel cartilage also consists mostly of non-essential amino acids and/or similarly, the representative contents of glycosaminoglycan molecules in that tissue (Luo *et al*., 2002) have more ^{15}N available for their synthesis when broilers are fed OM-containing diets. It is believed that the primary sources of nitrogen isotopic fractionation are the metabolic reactions involved in the process of amino acid deamination and transamination (Gaelber *et al*. 1966; Minagawa & Wada, 1984; Hobson *et al*., 1993).

The variations observed among $\delta^{13}C$ tissue values may be partially related to the differences in the biochemical fractions, such as lipids, carbohydrates and proteins, of the feed ingredients; lipid fraction particularly may present ^{13}C depletion as compared to the other fractions (DeNiro & Epstein, 1978).

In the present study, OM dietary inclusion remained constant throughout the experimental period. However, in practical settings, OM could be used in broilers diet only during specific periods of the production cycle with the aim of conferring an isotopic signature similar to that resulting from a strictly-vegetable diet. In that aspect, the application of this technique has also shown promising results (Oliveira, unpublished data) as to the purpose of detecting OM feeding.

The levels of OM inclusion in broiler diets, as well as of other animal byproducts practiced by the Brazilian poultry industry are quite variable. However, the information gathered from several poultry companies allowed us to estimate that the average percentage of OM inclusion in broiler diets is around 8%, ranging from 4% to 12%. Considering this information, the results of the present study allow us to conclude that, under experimental conditions, the technique of stable isotopes is capable of tracing OM inclusion in broiler diets, even in levels lower than those usually practiced in commercial settings.

Therefore, the use of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratios is a potential alternative tool to be used in the certification process of the carcasses of broilers fed diets with no animal products (OM) to comply with the requirements of specific markets. However, further studies should be conducted to improve the application of this technique. In addition, studies on the variation of carbon and nitrogen isotopic ratios in the different ingredients used in broiler diets, as well as on the isotopic signature of those diets in different broiler tissues, are suggested. Furthermore, research collaboration is desirable to attempt to standardize the isotopic ratios (C and N) of each one of those ingredients.

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