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Poultry Offal Meal Traceability in Meat Quail Tissues using the Technique of Stable Carbon ($^{13}C/^{12}C$) and Nitrogen ($^{15}N/^{14}N$) Isotopes

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ABSTRACT

Studies on the detection of animal by-products in poultry meat are rare, and non-existent on quail meat. This study aimed at detectiong increasing levels of poultry offal meal (POM) in quail meat, using carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) stable isotopes technique. Sixty four on-day-old male quails derived from a commercial farm were randomly distributed into seven different groups, which were fed experimental diets containing 0, 1.5, 3.0, 4.5, 6.0, 7.5, and 15% of POM. Diets were formulated to contain equal energy, protein, and amino acid levels. Four individuals per treatment were sacrificed at 42 days of age for breast muscle (*Pectoralis major*), keel, and tibia collection, which were subsequently submitted to analyses. Isotopic $\delta^{13}C$ and $\delta^{15}N$ enrichment was observed in all analyzed tissues, with the lowest detection level of 3% dietary inclusion of poultry offal meal.

INTRODUCTION

Increasing public demands for quality products, specifically for safe products, have required farmers and agribusinesses to change their production systems in order to supply products with proven safety, particularly after the outbreaks of Bovine Spongiform Encephalopathy (BSE) or "mad cow disease". The problems caused by this zoonosis in Europe, Japan, and Canada aroused the interest of consumers on aspects related to the origin of the consumed products and to production methods. This resulted in the development of new technologies and reinforced the concept of traceability, integrating all the links of the production chain, from farm to retail. The adoption of mechanisms that may ensure the quality of the final product through the identification and detailed knowledge on the different production processes is now an essential requirement, i.e., an inherent condition of the production process (Antunes, 2003). Moreover, traceability systems are the best tool to provide information on product quality to the consumers, allowing transparency of the different production chains (Bertolini *et al.*, 2006).

Some systems have allowed the identification of animal by-products, such as microchips, bar codes, labels, tags, etc. However, these systems rely on farmers' integrity, and are based on recorded information, but records are not sufficient to ensure product intrinsic quality. According to Ilbery *et al.* (2000), an independent meat certification technology needs to be developed to comply with consumers' demands.

The analysis of isotopic ratio for the chemical element carbon ($^{13}C/^{12}C$) by mass spectrometry has been successfully used to test the authenticity, the quality, and the geographic origin of several products, such as fruit juice (Bricout & Koziet, 1987), wine (Martin *et al.*, 1988), dairy products (Rossmann *et al.*, 2000; Manca *et al.*, 2001), beer (Sleiman, 2006), and orange beverages (Queiroz, 2005).

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CO_2 is the main chemical compound of terrestrial plants, with a value of $\delta^{13}C$ of approximately -7.7‰ (Kennedy & Krouse, 1990). As a function of their photosynthetic cycle, plants can be divided into two different groups: those belonging to C_3 photosynthetic cycle or to C_4 photosynthetic cycle. The first are called C_3 cycle plants because the first synthesized organic compound in their biochemical pathway contains three carbon atoms. On the other hand, those which first organic compound contains four carbon atoms are called C_4 cycle plants (Ducatti, 2004).

During photosynthetic assimilation, C_3 plants fix CO_2 from the atmosphere through the *Calvin-Benson* cycle, with $\delta^{13}C$ values between -22 and -34‰ (modal value = -26.7‰). CO_2 fixation by C_4 plants uses the *Hatch-Slack* cycle, and presents values between -9 and -16‰ (modal value = -12.6‰). As the concentration of C_3 and C_4 plants is lower than the international standard (Pee Dee Belemnit - PDB), their values are always negative. Therefore, C_3 and C_4 have different isotopic fingerprints due to the fractioning during photosynthetic fixation of carbon (Smith & Epstein, 1971; O'Leary, 1981; Kennedy & Krouse, 1990; Vogel, 1993; Ducatti, 2004). The natural differences in carbon isotopic ratio among plant species provides the opportunity to use part of these plants as tracers in animal, with no need to change their natural state or to synthesize specific markers, in addition to prevent human health and environmental safety problems related to the use of radioactive isotopes (Jones *et al.*, 1979; Tieszen, 1978; Tyrrell *et al.*, 1984; Boutton *et al.*, 1988; Metges *et al.*, 1990).

Different from carbon, the $^{15}N/^{14}N$ isotopic ratio in plants does not depend on their photosynthetic cycle. In legumes that use atmospheric air as nitrogen fixation source, this isotopic ratio is similar to the international standard (N_2 atm). In other plants, such as grasses, the ratio varies as a function of the isotopic ratio of the specific nitrogen source in each soil, and depends on many other factors, such as weather and soil fertilization.

Similarly to carbon, the natural abundance of ^{15}N can be used as a tracer in animal nutrition studies. The abundance of naturally marked substrates, with no restriction to its use in terms of environmental contamination, indicates that these tracers can be used in studies on aquatic organisms (Schroeder, 1983), sharks (Domi *et al.*, 2005), oysters (Piola *et al.*, 2005), and seals (Zhao *et al.*, 2006). The team of the Center of Stable Environmental Isotopes (CIE/IB/UNESP Botucatu) suggests that these natural tracers can be successfully used in studies on layers (Denadai *et al.*, 2007; Carrijo *et al.,* 2000), broilers (Gottmann, 2007; Oliveira, 2005; Carrijo *et al.*, 2006; Cruz, 2002), fish (Zuanon *et al.*, 2006), and sheep (Silva, 2003).

Agribusinesses strive to comply with the requirements of modern consumers, who use their power of choice, and therefore certificated products, containing consistent information on food safety, nutritional quality, feeding regime, and origin, are essential.

Considering the scope of the use of stable isotope technique, and the need to differentiate Brazilian poultry products in the global market, the present study aimed at detecting the inclusion of poultry offal meal (POM) in the breast muscle, keel, and tibia of 42-day-old meat quails by analyzing $^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios in these tissues.

MATERIAL AND METHODS

The experiment was carried out in the facilities of the Poultry sector of Edgárdia Experimental Farm of the School of Veterinary Medicine and Animal Science of the State University of São Paulo (UNESP), Botucatu campus, in 2006. A total number of 56 one-day-old male European quails (*Coturnix coturnix coturnix*) was acquired from a commercial farm. Birds were housed from one to 42 days of age in a 15 x 4m rearing house, with asbestos tiles and lateral plastic curtains. Seven 100 cm x 80 cm x 35 cm metal cages, used for rearing laying quails, housed eight birds each. Each cage was equipped with a brooder with a 550watts infrared lamp. Chick cup drinkers, with 0.5L capacity, were used, and water was changed twice daily. These drinkers were replaced by a trough drinker, placed in back of the cage, when birds were 14 days of age. During the first 10 days, birds were fed in a pan feeder, covered with a 1cm plastic mesh, with the aim of reducing feed wastage. On day 11, feed was provided in trough feeders placed in the front of the cages. Feed and water were offered *ad libitum* during the entire experimental period. Lighting program applied during the first two weeks was 24 hours of light, using 100-watts incandescent light bulbs. Only natural light was provided thereafter.

The experimental treatments were T1, T2, T3, T4, T5, T6, and T7, which corresponded to the inclusions of 0, 1.5, 3.0, 4.5, 6.0, 7.5, and 15% POM, respectively. Feeding schedule was divided into starter (one to 21 days of age) and finished (22 a 42 days of age) phase. Feeds were formulated to supply the birds' nutritional

requirements (Tables 1 and 2). Both basal diets contained the equal energy, protein, phosphorus, and amino acid (methionine) levels. Each ingredient used for feed manufacturing derived from the same batch. According to chemical analysis, the poultry offal meal contained 96.14% dry matter (DM), 65.54% crude protein (CP), 12.47% ether extract (EE), 14.49% mineral matter (MM), and mean isotopic values of $\delta^{13}C$ = - 16.28 ± 0.07‰ and $\delta^{15}N$ = 4.30 ± 0.03 ‰.

Table 1 - Percentage composition of ingredients, calculated nutritional levels, and mean isotopic values of the starter experimental diets (one to 21 days of age), on fresh matter basis.

	Experimental diets						
Ingredients (%)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Ground corn	44.81	46.00	47.21	48.50	49.50	50.34	53.32
Soybean meal	48.60	46.45	44.35	42.20	40.10	38.05	27.98
Poultry offal meal	-	1.50	3.00	4.50	6.00	7.50	15.00
Soybean oil	2.93	2.52	2.10	1.66	1.32	1.03	-
Calcitic limestone	1.03	0.98	0.98	0.94	0.90	0.90	0.61
Dicalcium phosphate	1.79	1.60	1.38	1.18	1.00	0.77	-
DL-Methionine	0.05	0.05	0.04	0.04	0.03	0.03	-
L-Lysine	-	-	-	-	-	-	-
Kaolin	-	0.15	0.19	0.23	0.40	0.63	2.34
Salt	0.39	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin-mineral premix[1]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100	100	100
Calculated nutritional levels							
Metabolizable energy (kcal/kg)	2900	2900	2900	2900	2900	2900	2900
Crude protein (%)	26.00	26.00	26.00	26.00	26.00	26.00	26.00
Crude fiber (%)	3.94	3.84	3.75	3.65	3.56	3.46	2.97
Calcium (%)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Avail. phosphorus (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.49
Methionine (%)	0.44	0.45	0.44	0.45	0.44	0.45	0.44
Methionine + Cystine (%)	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Lysine (%)	1.50	1.49	1.48	1.47	1.46	1.45	1.41
Mean Isotopic Values[2]							
$\delta^{13}C$	-21.11	-20.58	-20.12	-19.90	-19.80	-18.71	-17.41
$\delta^{15}N$	0.73	0.83	1.20	1.30	1.39	1.57	2.28

1 - Composition of the vitamin-mineral premix from Nutron®/kg feed: folic acid 200 mg; pantothenic acid 3,120 mg; choline 75,500 mg; biotin 10,000 mcg; niacin 8,400 mg; Vit. A 1,680 UI; Vit. B1 436.50 mg; Vit. B12 2,400 mcg; Vit B2 1,200 mg; Vit. B6 624 mg; Vit. D3 400,000 UI; Vit. E 3,500 mg; Vit. K3 360 mg; Cu 2,000 ppm; Fe.12,500 ppm; I. 187.50 ppm; Mn.18,750 ppm; Zn. 17,500 ppm; Se 75.00 ppm. 2 - Mean isotopic values expressed as d ^{13}C relative to the standard *Peedee Belemnite (PDB)* and d ^{15}N relative to the standard atmospheric N_2.

On day 42, four birds per treatment (n = 4) were randomly selected and sacrificed by neck dislocation to collect breast muscle, keel, and tibia samples for isotopic analyses. Breast muscles samples were collected by cutting a section of approximately 20 g in transversal direction of the intermediate longitudinal third of the muscle. In order to collect keel samples, the cartilage of the sternum was dissected, and its insertion in the bone was transversally cut in a right angle to the dorsal surface. Bone samples were obtained by collecting the intermediate longitudinal third of the right tibia. Bone marrow was removed by washing with distilled water. All tissue samples were duly identified and frozen at –20°C. At the time of analysis, tissue samples were thawed, washed in distilled water, placed on Petri dishes, and dried in a force-ventilation oven (Marconi – model MA 035) at 55°C for 48 hours. After drying, samples were ground in a cryogenic mill (Spex – model 6700 *freezer/mill*) at –196°C at maximum frequency for three minutes in order to obtain homogenous material with very fine particle size, with the appearance of talcum (Licatti, 1997; Ducatti, 2004).

Table 2 - Percentage composition of ingredients, calculated nutritional levels, and mean isotopic values of the finisher experimental diets (22 to 42 days of age), on fresh matter basis.

	Experimental diets						
Ingredients (%)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
Ground corn	50.42	52.38	53.45	54.19	54.50	55.19	60.70
Soybean meal	40.91	38.63	36.52	34.50	32.53	30.50	19.83
Poultry offal meal	0	1.50	3.00	4.50	6.00	7.50	15.00
Soybean oil	4.99	4.32	3.95	3.70	3.59	3.35	1.46
Calcitic limestone	0.90	0.86	0.87	0.82	0.82	0.79	0.78
Dicalcium phosphate	1.67	1.47	1.26	1.08	0.85	0.65	0.01
DL-Methionine	0.10	0.09	0.09	0.08	0.08	0.07	0.05
L-Lysine	-	-	-	-	-	-	0.07
Kaolin	0.26	-	0.11	0.38	0.88	1.20	1.35
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin-mineral premix[1]	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Total	100	100	100	100	100	100	100
Calculated nutritional levels							
Metabolizable energy (kcal/kg)	3100	3100	3100	3100	3100	3100	3100
Crude protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Crude fiber (%)	3.54	3.45	3.35	3.26	3.16	3.06	2.55
Calcium (%)	0.90	0.90	0.90	0.90	0.90	0.90	1.03
Avail. phosphorus (%)	0.42	0.42	0.42	0.42	0.42	0.42	0.48
Methionine (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Methionine + Cystine (%)	0.82	0.82	0.82	0.82	0.82	0.82	0.82
Lysine (%)	1.29	1.28	1.27	1.26	1.25	1.25	1.25
Mean Isotopic Values[2]							
$\delta^{13}C$	-20.25	-19.80	-19.40	-19.15	-18.42	-18.15	-16.77
$\delta^{15}N$	0.96	1.19	1.28	1.36	1.60	1.80	2.47

1 - Composition of the vitamin-mineral premix from Nutron®/kg feed: folic acid 162.50 mg; pantothenic acid 2,600 mg; choline 65,250; niacin 7,000 mg; Se 75.00 ppm; Vit. A 1,400 UI; Vit. B1 388.00 mg; Vit. B12 2,000 mcg; Vit B2 1,000 mg; Vit. B6 520 mg; Vit. D3 300,000 UI; Vit. E 2,500 mg; Vit. K3 300 mg; Cu 2,000 ppm; Fe.12,500 ppm; I. 187.50 ppm; Mn.18,750 ppm; Zn. 17,500 ppm. 2 - Mean isotopic values expressed as d ^{13}C relative to the standard *Peedee Belemnite (PDB)* and d ^{15}N relative to the standard atmospheric N_2.

Isotopic analyses of feed ingredients, feeds, and tissues were carried out at the Center of Stable Environmental Isotopes of the Biosciences Institute (CIE/IB), UNESP, Botucatu campus. Isotopic carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) ratios were determined in a isotopic ratio mass spectrometer (IRMS) type DELTA – S (Finnigan Mat) coupled to an Elementary Analyzer

(EA 1108 CHN), according to the method described by Ducatti (2004). Analyses results were expressed as *delta per thousand* of the sample isotopic ratio relative to the international standards *PeeDee Belemnite* (PDB) and atmospheric nitrogen (N_2), for the elements carbon and nitrogen, respectively, according to the formula:

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

where R represents the ratio between the heaviest and the lightest isotope, specifically $^{13}C/^{12}C$ and $^{15}N/^{14}N$, of the sample and the standard.

The obtained isotopic results were submitted to multivariate analysis of variance (MANOVA) using GLM (General Linear Model) procedures of SAS (1999) statistical software. Data were generated by the error matrices for each tissue, which were later graphically distributed in regions (ellipses) with 95% confidence of observing possible differences between experimental treatment means and control treatment means. This software allows to verify if the values of the isotopic pair ($\delta^{13}C$ and $\delta^{15}N$) of the control treatment (vegetable feed), are statistically different from the values of the isotopic pair of the treatments which included animal protein. In order to determine the estimated inclusion percentage of poultry offal meal (POM) in breast muscle, keel, and tibia composition, the model of isotopic dilution of two sources and two isotopes in product synthesis (Ducatti, 2004). This model allows measuring the index of participation of each source based on the analyses of the breast muscle, keel, and tibia of the quails fed exclusively plant ingredients (source 1) and of the quails fed increasing POM levels (source 2). The following equation system was applied:

Product = Source 1 + Source 2

$$\delta^{13}C = a\delta F_1 + b\delta F_2 \quad (1)$$

$$\delta^{15}N = a\delta' F_1 + b\delta' F_2 \quad (2)$$

where:

$\delta^{13}C$ and $\delta^{15}N$ = relative isotopic enrichment value of the product (breast muscle, keel, or tibia) for carbon-13 and nitrogen-15, respectively.

δF_1 e δF_2 = relative isotopic enrichment value of carbon of source 1 (vegetable) and source 2 (animal), respectively.

$\delta' F_1$ e $\delta' F_2$ = relative isotopic enrichment value of nitrogen of source 1 (vegetable) and source 2 (animal), respectively.

By isolating value (b) of equation (1) and equation (2), and making them equal, value (a) is obtained, i.e., the index of participation of the vegetable source.

$$\frac{\delta^{13}C - a\delta F_1}{\delta F_2} = \frac{\delta^{15}N - a\delta' F_1}{\delta' F_2}$$

$$a = \frac{\delta^{13}C\delta' F_2 - \delta^{15}N\delta F_2}{\delta F_1\delta' F_2 - \delta' F_1\delta F_2} \quad (3)$$

By replacing value (a) in equation (1) or equation (2), value (b) is obtained, i.e., the index of participation of the animal source.

$$b = \frac{\delta^{15}N\delta F_1 - \delta^{13}C\delta' F_1}{\delta F_1\delta' F_2 - \delta' F_1\delta F_2} \quad (4)$$

By summing equation (3) with equation (4), and making it equal to one, the following equation is obtained:

$$\frac{\delta^{13}C\delta' F_2 - \delta^{15}N\delta F_2}{\delta F_1\delta' F_2 - \delta' F_1\delta F_2} + \frac{\delta^{15}N\delta F_1 - \delta^{13}C\delta' F_1}{\delta F_1\delta' F_2 - \delta' F_1\delta F_2} = 1$$

$$(\delta^{13}C\delta' F_2 - \delta^{15}N\delta F_2) + (\delta^{15}N\delta F_1 - \delta^{13}C\delta' F_1) = (\delta F_1\delta' F_2 - \delta' F_1\delta F_2)$$

or:

$$a(\delta F_1\delta' F_2 - \delta' F_1\delta F_2) + b(\delta F_1\delta' F_2 - \delta' F_1\delta F_2) = (\delta F_1\delta' F_2 - \delta' F_1\delta F_2)$$

The result is $a + b = 1$, as there are only two sources of participation and only one product.

In order to avoid a possible fractioning factor between the diet (source) and the studied tissue (product), and different carbon/nitrogen ratios, the same tissue is used when applying the equation system (source: tibia/product: tibia).

For source 2, isotopic values of the breast muscle, keel, and tibia derived from the treatment with 15% inclusion (maximum inclusion level) were used, where *b* values were corrected by multiplying the obtained values by 0.15.

RESULTS AND DISCUSSION

The results of the isotopic analyses ($\delta^{13}C$ and $\delta^{15}N$) in the starter and finisher diets used in the present study are presented in Tables 1 e 2, respectively. As POM inclusion percentage increased in the experimental diets, an enrichment of $\delta^{13}C$ and $\delta^{15}N$ values was observed. This enrichment possibly occurred as a function differences in diet composition: diets were formulated to contain equal energy and protein levels in both rearing phases, but as POM dietary levels increased, soybean meal and oil percentages increased, whereas corn percentage decreased.

$\delta^{13}C$ and $\delta^{15}N$ means for the studied tissues of 42-day-old quails are presented in Table 3. Mean values reveal the isotopic enrichment of the breast muscle, keel, and tibia of the birds fed increasing POM levels as compared to those that received the diet with no POM inclusion (control treatment). Although each tissue has its own isotopic value, fractioning factor, and isotopic turnover (Hobson & Clark, 1992b; 1992a), the animal is what it isotopically consumes, i.e., ±1‰ ^{13}C and ±3‰ ^{15}N (DeNiro & Epstein, 1976; 1978). This behavior is shown in Figures 1, 2, and 3, where one can observe that the confidence ellipses are placed in a linear and increasing form as a function of POM dietary level. This is only possible to visualize when diets are carefully formulated and analyzed, and allows its use for the certification process of meat quails.

In the breast muscle, with POM inclusion levels equal or above 3.0% (treatments T4, T5, T6 e T7), confidence ellipses increased the distance from the graph axes, which represent the control treatment (Figure 1). Confidence regions of treatments T4, T5, T6 (POM inclusions of 4.5, 6.0, and 7.5%) present overlapping, demonstrating that these treatments were not different, and reinforcing the notion that any inclusion level above this interval can be isotopically detected. There was a slight dislocation of T2 confidence region towards the carbon axis. This behavior probably indicated the low efficiency of the breast muscle of quails in the detection of dietary POM inclusion levels below 3% T3).

The differences between $\delta^{13}C$ and $\delta^{15}N$ means (Figures 1, 2, and 3) obtained in the error matrices are negative, and therefore the ellipses are located in the third quadrant. Confidence regions that overlap on any of the graph axes possibly present some positive values, and are not adequate to detect POM in quail tissues.

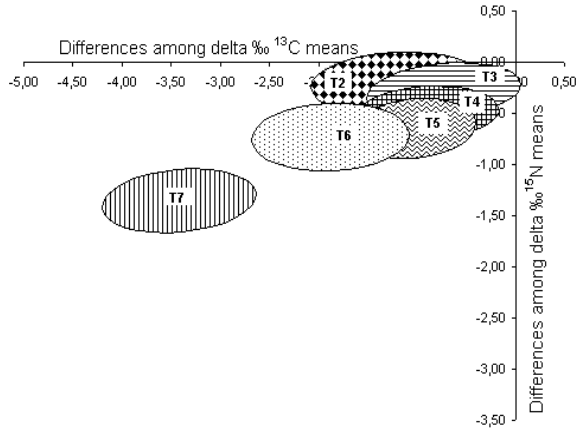

Figure 1 – Confidence regions for differences between delta ‰ ^{13}C and ‰^{15}N means of the breast muscle of 42-day-old meat quails in treatments T2, T3, T4, T5, T6, and T7.

In the keel of 42-day-old meat quails, dietary POM inclusions of 3.0% or more of POM were also different from the control treatment (Figure 2). Confidence regions in this region were very distinct as compared to the breast muscle. Confidence ellipses were "flatter", presenting higher amplitude of the points generated by the error matrix, which is possibly a result of the higher standard deviations. Higher than expected standard error are common in meat quails, as these birds were not submitted to high genetic improvement.

The detection level of POM in the tibia was similar to those found in the breast muscle and keel. In the tibia, ellipses presented visible distance and a more linear behavior (Figure 3).

Table 3 - Mean $\delta^{13}C$ and $\delta^{15}N$ isotopic values and respective standard deviations in the breast muscle, keel, and tibia of 42-day-old quails, according to different poultry offal meal inclusion levels in the diet.

Dietary POM inclusion, %	Sampled tissue					
	Breast muscle		Keel		Tibia	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
0.0	-21.70 ± 0.25	2.53 ± 0.07	-19.35 ± 0.31	3.57 ± 0.22	-18.61 ± 0.06	2.71 ± 0.07
1.5	-21.41 ± 0.13	2.70 ± 0.09	-18.82 ± 0.15	3.68 ± 0.03	-18.16 ± 0.40	2.90 ± 0.16
3.0	-20.99 ± 0.44	2.81 ± 0.08	-18.35 ± 0.65	3.80 ± 0.14	-17.91 ± 0.23	3.00 ± 0.10
4.5	-20.72 ± 0.38	3.04 ± 0.08	-18.17 ± 0.27	3.96 ± 0.13	-17.62 ± 0.40	3.20 ± 0.13
6.0	-20.52 ± 0.51	3.17 ± 0.15	-17.46 ± 0.44	4.13 ± 0.11	-17.12 ± 0.15	3.31 ± 0.18
7.5	-19.86 ± 0.50	3.21 ± 0.22	-16.59 ± 0.46	4.25 ± 0.20	-16.25 ± 0.50	3.52 ± 0.16
15.0	-18.27 ± 0.46	3.87 ± 0.15	-15.56 ± 0.42	4.9 0± 0.23	-14.84 ± 0.28	4.16 ± 0.18

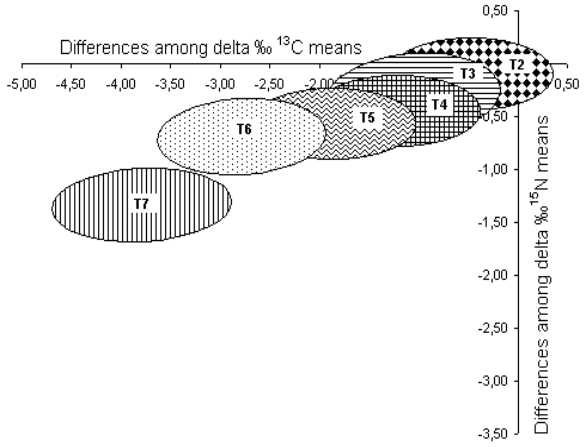

Figure 2 – Confidence regions for differences between delta ‰ ^{13}C and ‰^{15}N means of the keel of 42-day-old meat quails in treatments T2, T3, T4, T5, T6, and T7.

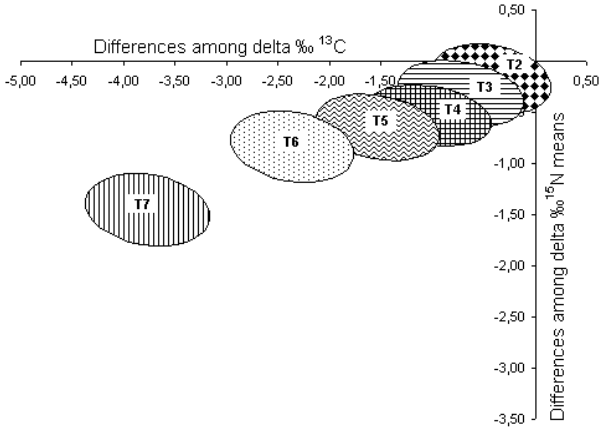

Figure 3 – Confidence regions for differences between delta ‰ ^{13}C and ‰^{15}N means of the tibia of 42-day-old meat quails in treatments T2, T3, T4, T5, T6, and T7..

Isotopic ^{13}C and ^{15}N enrichment was observed in the three studied tissues relative to control treatment means and treatments with increasing inclusions of POM. This similar enrichment of both isotopes in the breast muscle, keel, and tibia is probably explained by the high rates of basal metabolism of quails (Hobson & Clark, 1992a), as these birds present high activity, and consequently high turnover rate. Although isotopic enrichment was similar in the studied tissue, the keel of 42-day-old quails presented higher ^{15}N enrichment. This may be due to differences in the composition of essential and non-essential amino acids of these tissues. According to Moran Jr. (1999), most of the amino acids in the breast muscles are essential amino acids, which exhibit little change in their isotopic ratio when incorporated in the tissue (Pinnegar & Polunin, 1999). On the other hand, type-I collagen, which makes up approximately 95% of the organic bone matrix (Pizauro Jr., 2002), and therefore is the largest nitrogen source of the bone, consists mainly of non-essential amino acids. In this sense, diets containing POM supply a higher amount of ^{15}N for the endogenous synthesis of non-essential amino acids, as well as intact non-essential amino acids in the dietary protein. Some authors report that the primary sources responsible for the isotopic fractioning of nitrogen are metabolic reactions involved in the processes of deamination and transamination of amino acids (Gaelber *et al.*, 1996; Minagawa & Wada, 1984).

The variations found among tissue are still not well understood. According to Tiezen *et al.* (1983), the main biochemical fractions are isotopically different, and the isotopic differences in the body may be a reflex of their different biochemical composition. Tissue with lower lipid content would probably have a higher $\delta^{13}C$ level as compared to a tissue with higher lipid content, which is relatively poor in carbon-13 (Tiezen *et al.*, 1983; Piasentier *et a*l., 2003).

The results obtained by the equation system are shown in Table 4. These results suggest that the error in the comparison between experimental (used in the diet) and calculated values (equation system) are lower than 1%.

Table 4 - Dietary inclusion level of poultry offal meal determined by the equation system in the different tissues of 42-day-old meat quails.

POM dietary inclusion level (%)	Inclusion level (equation system)		
	Sampled tissues		
	Breast meat	Keel	Tibia
0	0.0	0.0	0.0
1.5	1.7	1.5	1.9
3.0	3.1	3.0	2.9
4.5	5.4	4.5	4.6
6.0	6.7	6.5	6.1
7.5	7.7	8.7	8.8
15.0	15.0	15.0	15.0

CONCLUSIONS

The technique of stable isotopes is able to detect the inclusion of poultry offal meal in the breast muscle, keel, and tibia of 42-day-old meat quails, when inclusion levels are equal or higher than 3%.

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