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■Keywords

Carbon-13, quail production, stable isotopes, nitrogen-15, traceability.

The Use of Carbon and Nitrogen Stable Isotopes for the Detection of Poultry Offal Meal in Meat-Type Quail Feeds

ABSTRACT

The objective of the present study was to trace the inclusion of poultry offal meal (POM) in the diet of meat-type quails reared for a long period using the technique of stable isotopes. A number of 320 quails were randomly distributed into eight treatments: vegetable diet (T1), and a diet containing 8% POM were fed until the end of the experimental period (T2) or replaced by the vegetable diet on day 42 (T3), 56 (T4), 70 (T5), 84 (T6), 98 (T7), and 112 (T8). Breast muscle samples were collected from four birds randomly selected per treatment every 14 days. The obtained isotope results were submitted to multivariate analysis of variance (MANOVA) with the aid of the GLM procedure of statistical SAS program. Treatments were different from T1 when birds were sacrificed at least two weeks after the diet was changed. T2 results were different from T1 in all evaluated periods. It was concluded that it is possible to trace poultry offal meal inclusion in a strictly vegetable diet after the diet was changed for at least 14 days.

INTRODUCTION

Quail meat production has globally increased and it is an interesting option for poultry producers, as quails are able to transform common feedstuffs in a high-quality protein source for human consumption (Baumgartner, 1994). In addition, quail meat presents pleasant flavor and appearance, and it is easy to store.

The number of quails reared in Brazil was approximately 6.2 million in 2002, and has constantly increased thereafter, with an estimated production of 14.68 million in 2010. Two main varieties are reared: Japanese quails (*Coturnix coturnix japonica*), exclusively used for egg production and the common quail (*Coturnix coturnix coturnix*), used both for egg and meat egg production. Quails are produced in Brazil almost exclusively for egg production. Approximately 28% of the eggs are consumed as preserved eggs, 71% fresh, and only 1% in other forms (Bertechini, 2010).

The increasing demand for product quality has made farmers and food processing companies to rethink their production systems in order to offer provenly safe products to their customers. Food scares in the 1990s related to bovine spongiform encephalopathy (BSE), foot-and-mouth disease cases and the avian flu outbreak in Asia contributed to the adoption of more stringent food safety measures (Gottmann, 2010).

Traceability systems have been implemented to try to ensure the safety of animal products to the consumers in response to the requirements imposed by the European Union in Chapter II, section I, article 11 of the CE regulation n. 1069/2009 of the European Parliament and the Council, which banned the imports of products derived from animals fed by-products derived from animals of the same species.



According to Block Jr. (2002), several methods have been proposed for the identification of animal by-products in animal feeds, including DNA hybridization, ELISA, and PCR. Also, mass spectrometry, analyzing isotope ratios of the chemical element carbon, has been successfully applied to test the authenticity and quality of food products, such as fruit juice (Bricout & Koziat, 1987), wine (Martin *et al.*, 1988), dairy products (Rossmann *et al.*, 2000; Manca *et al.*, 2001), and vegetable oils (Kelly *et al.*, 1997).

Plant species with different photosynthesis cycles (C_3 and C_4) present different carbon isotope ratios ($^{13}C/^{12}C$), and these natural differences allow their use as markers in animal feeds. The advantages of this method is that it does not change animal natural conditions, no specifically marked compounds need to be synthesized, and particularly, human health and environmental safety issues associated to the use of radioactive isotopes are prevented (Jones *et al.*, 1979; Tieszen, 1978; Tyrrell *et al.*, 1984; Boutton *et al.*, 1988; Metges *et al.*, 1990). The utilization of stable isotopes as markers in different poultry tissues allows estimating the rate of replacement of tissue stable isotopes by those derived from the diet (Denadai *et al.*, 2007; Gottmann *et al.*, 2008; Pelícia *et al.*, 2011; Araujo *et al.*, 2011).

Carbon isotope ratios ($^{13}C/^{12}C$) combined with nitrogen isotope ratios ($^{15}N/^{14}N$) in the final product allow tracing the inclusion of animal by-product meals in broiler (Carrijo *et al.*, 2006; Gottmann *et al.*, 2008; Oliveira, 2010), layer (Denadai *et al.*, 2008), and quail (Móri *et al.*, 2007) feeds. However, this technique had not been employed yet in studies with poultry reared for long periods, where theoretically animal by-products could be included and withdrawn from the feeds without being detected by this method. Meat-type quails were used as a model in the present study because they are small and have low feed intake, and therefore, lower production cost and easier management.

The objective of the present study was to trace the inclusion of poultry offal meal (POM) in the diet and its subsequent replacement by a vegetable diet in meat-type quails reared for a long period using the technique of stable isotopes.

MATERIALS AND METHODS

The experiment was carried out at the facilities of the Poultry Nutrition Laboratory of the School of Veterinary Medicine and Animal Science, UNESP, Botucatu campus, Brazil, between April 10 and August 13, 2007, totaling 126 experimental days.

One-day-old quails ($n=320$) were housed in 16 (0.30m x 0.95m x 0.50m) cages at a density of 20 birds per cage, totaling 40 birds per treatment.

Each cage was initially equipped with mini cup drinkers and tray feeders. On day 21, this equipment was replaced by nipple drinkers and trough feeders. Birds were offered water and feed *ad libitum* during the entire experimental period.

A 24-h light lighting program was adopted during the first three weeks, using 100W incandescent bulbs, and thereafter, natural light was used.

Quails were randomly distributed into eight treatments: a vegetable diet (T1) based on corn and soybean meal, and a diet containing 8% POM were fed until the end of the experimental period (T2) or replaced by the vegetable diet on days 42 (T3), 56 (T4), 70 (T5), 84 (T6), 98 (T7), and 112 (T8) of the experimental period.

Feeds were formulated to supply quails' nutritional requirements. A commercial two-phase feeding schedule was adopted: starter (1-21 days of age) and grower (22-126 days of age). The experimental diets were based on corn and soybean meal and formulated to contain equal energy and protein levels (Table 1).

Four birds per treatment (one per replicate) were randomly selected and sacrificed by neck dislocation to collect *Pectoralis major* muscle samples per sampling period. Birds were sacrificed at 14-d intervals, that is on the days the POM-containing diet was changed to the vegetable diet (days 42, 56, 70, 84, 98, 112, and 126).

Samples were prepared according to Oliveira (2010) and Móri *et al.* (2007, 2008), placed in duly identified plastic bags and immediately frozen at $-20^{\circ}C$ for subsequent isotope analyses. Samples were dried in force-ventilation oven at $65^{\circ}C$ and then ground in freeze-mill until a very fine particle size homogenous material was obtained.

Isotopic analyses were conducted at the Environmental Stable Isotopes Center, Biosciences Institute (CIE/IB), UNESP, Botucatu campus, according to Oliveira (2006), Móri *et al.* (2007, 2008), Denadai *et al.* (2008) and Gottmann *et al.* (2008). In tin capsules, approximately 100 μg and 400 μg of sample were weighed for carbon and nitrogen analyses, respectively.

In order to determine carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotopic ratios, an isotopic ratio mass spectrometer (IRMS – DELTA-S, FINNIGAN MAT) coupled to an Elemental Analyzer (EA 1108 CHN) was used, according to the method described by Ducatti *et al.* (2004).



Table 1 – Feedstuff composition, calculated nutritional levels, and mean isotope values of the starter (1-21 d) and grower (22-126 d) experimental diets.

Ingredients (%)	Experimental diets			
	Starter		Grower	
	Vegetable	8% POM ¹	Vegetable	8% POM ¹
Ground corn	44.96	50.69	50.94	58.19
Soybean meal	48.50	37.29	40.82	29.26
Poultry offal meal	-	8.00	-	8.00
Crude soybean oil	2.87	0.90	4.82	2.34
Calcitic limestone	1.02	0.88	0.92	0.77
Dicalcium phosphate	1.80	0.74	1.66	0.60
DL-Methionine	0.10	0.07	0.09	0.06
L-lysine	-	-	-	0.03
Mineral and vitamin supplement	0.40 ²	0.40 ²	0.40 ³	0.40 ³
Salt	0.35	0.35	0.35	0.35
Inert material (kaolin)	-	0.68	-	-
Total	100.00	100.00	100.00	100.00
Calculated nutritional levels				
Metabolizable energy, kcal/kg	2900	2900	3100	3100
Crude protein, %	26.00	26.00	23.00	23.00
Calcium, %	1.00	1.00	0.90	0.90
Available phosphorus, %	0.45	0.45	0.42	0.42
Methionine, %	0.44	0.49	0.44	0.44
Methionine + cystine, %	0.90	0.90	0.81	0.81
Lysine, %	1.40	1.45	1.29	1.26
Analyzed mean isotope values ⁴				
δ ¹³ C	-19.48	-18.53	-19.17	-17.44
δ ¹⁵ N	1.34	2.26	1.88	2.45

1 POM = poultry offal meal. **2** Vitamin and mineral supplement (Nutrona) for the starter phase per kg product: Mn = 18,750mg; Zn = 17,500mg; Fe = 11,50mg; Cu = 2,000mg; I = 187.5mg; Se = 75mg; Vit. A = 1,680,00IU/kg; Vit. D3 = 400,000IU/kg; Vit. E = 3,500mg; Vit. K3 = 360mg; Vit. B1 = 436.5mg; Vit. B2 = 1,200mg; Vit. B6 = 624mg; Vit. B12 = 2,400mcg; folic acid = 200mg; pantothenic acid = 3,120mg; niacin = 8,400mg; biotin = 10mg; choline = 75.700mg; coccidiostat = 25,000mg; growth promoter = 20,000mg. **3** Vitamin and mineral supplement (Nutrona) for the grower phase per kg product: Mn = 18,750mg; Zn = 17,500mg; Fe = 11,250mg; Cu = 2,000mg; I = 187,5mg; Se = 75mg; Vit. A = 1,400,00IU/kg; Vit. D3 = 300,000IU/kg; Vit. E = 2,500mg; Vit. K3 = 300mg; Vit. B1 = 388mg; Vit. B2 = 1,000mg; Vit. B6 = 520mg; Vit. B12 = 2,000mcg; folic acid = 162.5mg; pantothenic acid = 2,600mg; niacin = 7,000mg; choline = 65,250mg; coccidiostat = 15,000mg; growth promoter = 20,000mg. **4** Mean isotope values expressed as δ ¹³C relative to the Pee Dee Belemnite standard (PDB) and δ ¹⁵N relative to atmospheric N₂ standard.

The results of the analyses were expressed in delta (δ) per thousand of the sample isotope ratio relative to the international standards Pee Dee Belemnite (PDB) and atmospheric nitrogen (N₂) for the elements carbon and nitrogen, respectively, according to the expression:

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

Where:

δ X = enrichment of the heaviest isotope of the chemical element X of the sample relative to the respective international standard.

R = ratio between the least and the most abundant isotope (¹³C/¹²C and ¹⁵N/¹⁴N).



The obtained isotope results were submitted to multivariate analysis of variance (MANOVA) with the aid of the GLM procedure of SAS (2002) statistical program. Based on the data generated by error matrices, 95% confidence regions were determined to detect possible isotope differences ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) between the vegetable treatment (T1) and treatments with POM inclusion during different times of the rearing period (T2, T3, T4, T5, T6, T7, T8).

In order to be considered different from the T1 (vegetable diet), the confidence region of any determined treatment cannot overlap any axis of the graph. When an ellipse overlaps one of the axes, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differences among treatment means is equal to zero, and therefore, there are no differences among the evaluated treatments ($p > 0.05$).

RESULTS AND DISCUSSION

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope results of the breast muscle of meat-type quails obtained in the present study are shown in Table 2, and the ellipses generated

from the data obtained in the statistical analysis are presented in Figure 1.

Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope enrichment of the breast muscle was detected in birds that were fed the diets containing POM sometime during the rearing period. This ^{13}C and ^{15}N enrichment when poultry were fed animal by-product meals was also observed by Oliveira (2010), Carrijo *et al.* (2006), Móri *et al.* (2007, 2008), Denadai *et al.* (2008) and Gottmann *et al.* (2008).

According to DeNiro & Epstein (1976, 1978), the isotope values of animal meals used as feedstuffs reflect the isotope signal of the animal from which they derive, and are $\pm 2\text{‰}$ for $\delta^{13}\text{C}$ and $\pm 3\text{‰}$ for $\delta^{15}\text{N}$, thereby allowing the detection of the inclusion of poultry offal meal in the feed of meat-type quails.

The longer ellipses in Figure 1 indicate that the amplitude of the points generated by the error matrix was wider, possibly due to the high standard deviations observed. According to Murakami & Ariki (1998), Garcia & Pizzolante (2004) and Móri *et al.* (2007), higher than expected standard deviations are

Table 2 – Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ means and standard deviations of the breast muscle ($n = 4$) of meat-type quails.

Treatamentos ¹		Time of sacrifice (days)						
		42	56	70	84	98	112	126
T1	$\delta^{13}\text{C}$	-20.64 \pm 0.48	-21.43 \pm 0.40	-21.53 \pm 0.18	-21.32 \pm 0.43	-21.70 \pm 0.31	-21.81 \pm 0.13	-21.20 \pm 0.29
	$\delta^{15}\text{N}$	3.03 \pm 0.06	3.00 \pm 0.20	2.99 \pm 0.09	3.09 \pm 0.15	3.13 \pm 0.12	3.28 \pm 0.13	3.44 \pm 0.27
T2	$\delta^{13}\text{C}$	-19.43 \pm 0.38	-19.37 \pm 0.36	-19.90 \pm 0.13	-19.86 \pm 0.17	-19.94 \pm 0.20	-20.13 \pm 0.43	-19.34 \pm 0.29
	$\delta^{15}\text{N}$	3.54 \pm 0.08	3.78 \pm 0.20	3.89 \pm 0.16	3.99 \pm 0.09	3.77 \pm 0.12	3.80 \pm 0.19	4.49 \pm 0.29
T3	$\delta^{13}\text{C}$		-20.34 \pm 0.37	-20.74 \pm 0.35	-20.99 \pm 0.19	-21.42 \pm 0.26	-21.47 \pm 0.04	-21.32 \pm 0.08
	$\delta^{15}\text{N}$		3.40 \pm 0.24	3.01 \pm 0.20	3.14 \pm 0.15	3.14 \pm 0.07	3.28 \pm 0.04	3.88 \pm 0.55
T4	$\delta^{13}\text{C}$			-20.61 \pm 0.34	-20.93 \pm 0.23	-21.37 \pm 0.18	-21.56 \pm 0.36	-20.61 \pm 0.34
	$\delta^{15}\text{N}$			3.53 \pm 0.07	3.46 \pm 0.15	3.39 \pm 0.08	3.32 \pm 0.11	4.33 \pm 0.14
T5	$\delta^{13}\text{C}$				-20.60 \pm 0.07	-20.89 \pm 0.23	-20.91 \pm 0.23	-21.28 \pm 0.28
	$\delta^{15}\text{N}$				3.40 \pm 0.09	3.49 \pm 0.16	3.34 \pm 0.28	3.62 \pm 0.25
T6	$\delta^{13}\text{C}$					-20.66 \pm 0.36	-21.26 \pm 0.08	-20.93 \pm 0.27
	$\delta^{15}\text{N}$					3.70 \pm 0.18	3.38 \pm 0.08	4.05 \pm 0.23
T7	$\delta^{13}\text{C}$						-20.41 \pm 0.26	-20.99 \pm 0.10
	$\delta^{15}\text{N}$						3.53 \pm 0.16	3.48 \pm 0.10
T8	$\delta^{13}\text{C}$							-20.51 \pm 0.24
	$\delta^{15}\text{N}$							4.49 \pm 0.21

1T1: diet based on corn and soybean meal until d 126; T2: diet with 8% poultry offal meal inclusion (POM) until d 126; T3: diet with 8% POM inclusion until d 42, and T1 diet thereafter; T4: diet with 8% POM inclusion until d 56, and T1 diet thereafter; T5: diet with 8% POM inclusion until d 70, and T1 diet thereafter; T6: diet with 8% POM inclusion until d 84, and T1 diet thereafter; T7: diet with 8% POM inclusion until d 98, and T1 diet thereafter; T8: diet with 8% POM inclusion until d 112, and T1 diet thereafter.

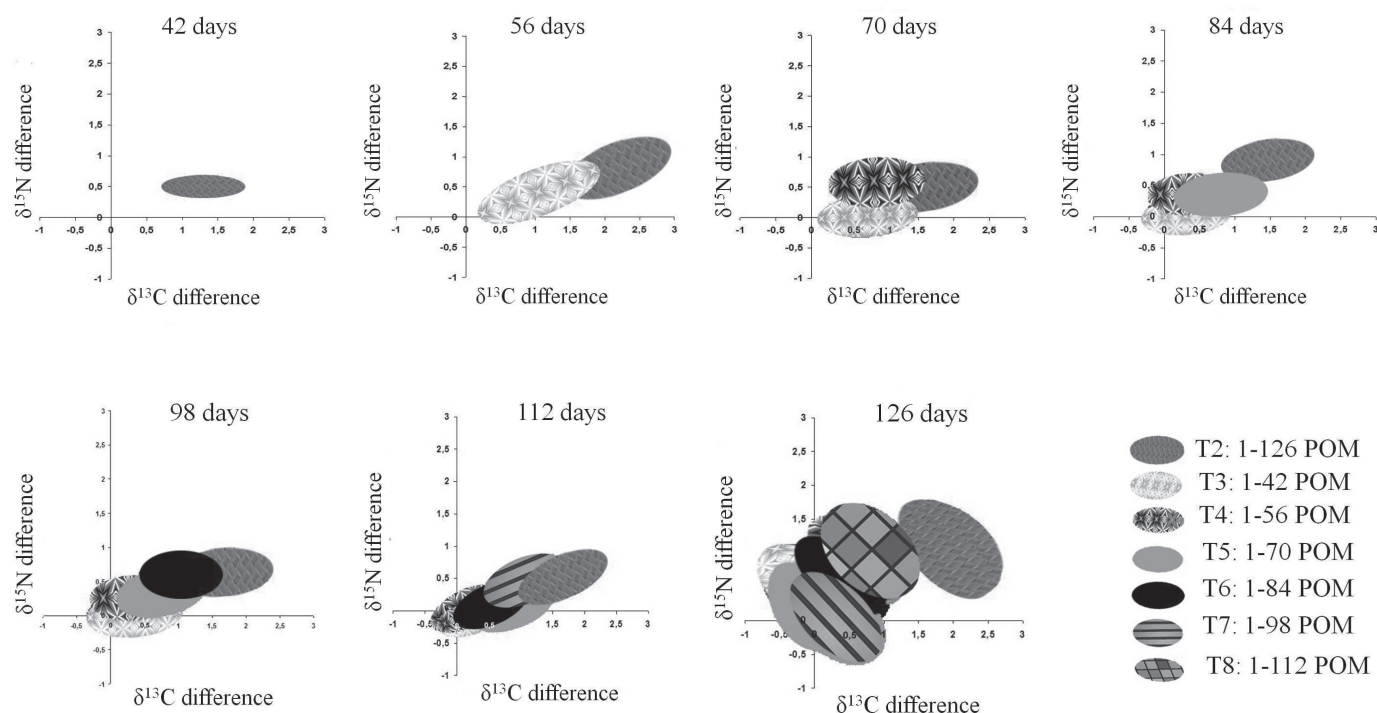


Figure 1 – Confidence regions¹ of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the breast muscle of meat-type quails fed poultry offal meal compared with those of quails fed the purely vegetable diet (T1), according to day of sacrifice.

¹ Overlapping ellipses representing the treatments are not different ($p > 0.05$).

frequent in meat-type quails, as these birds have not been submitted to significant genetic improvement.

Differences in $\delta^{15}\text{N}$ between birds fed POM sometime during the rearing period and those fed only the vegetable diet (T1) were lower than $\delta^{13}\text{C}$ differences, placing confidence regions closer to the carbon axis than to the nitrogen axis. According to Oliveira (2010), this behavior is due to the carbon and nitrogen isotope differences between the vegetable diet and that containing poultry offal meal.

Figure 1 shows that T2 was different from T1 on all evaluation days. This is attributed to the fact that T1 birds were fed the vegetable diet and T2 the diet with POM inclusion, both for the entire rearing period. The different isotope signals of these diets allowed differentiating the treatments by the use of the breast muscle, in agreement with Móri *et al.* (2007, 2008). However, T3 birds were not significantly different from the vegetable-diet treatment (T1). T1 was different from T4 between d 56 and 70, from T5 between d 70 and 84, from T6 between d 84 and 98, and from T7 between d 98 and 112, and from T8 between d 112 and 126.

The treatments with the dietary inclusion of POM were different from the vegetable treatment (T1) when birds were sacrificed two weeks after the diet was

changed, and thereafter presented similar behavior to T1, except for T3, which was similar to T1 14 days after the diet was changed, and for T2, which was different from T1 at all evaluated times.

It was possible to trace POM in the breast muscle when the diet was changed to the vegetable diet two weeks before sacrifice possibly because the growth curve of the experimental birds was close to the stabilization of the isotope signal. Therefore, their metabolism was probably slower than that of the broilers evaluated by Oliveira (2010), which presented similar isotope signal 14 days after the change of a diet containing poultry offal meal to a vegetable diet.

However, Oliveira (2010) found that dietary POM can be detected in the keel and the tibiotarsus two weeks after the diet is changed to a purely vegetable diet.

Considering these differences among tissues, further studies are needed to evaluate the possibility of tracing dietary poultry offal meal at different inclusion levels and using tissues with slower metabolism.

CONCLUSIONS

The use of the technique of carbon and nitrogen stable isotopes for tracing poultry offal meal inclusion



in the diet of meat-type quails reared for a long period can only detect its total absence in the breast muscle for intervals longer than 14 days of feeding.

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