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Traceability of Animal by Product Meals in Broilers Fed Sugarcane Yeast Using Stable Isotopes

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Animal by product meals, broilers, isotopes, sugarcane yeast, traceability.

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ABSTRACT

The objective of this study was to verify if the dietary inclusion of sugarcane yeast at levels commonly used in broiler diets influences the traceability of cattle meat meal and poultry offal meal, using the technique of stable carbon and nitrogen isotopes in the breast muscle of chickens. A number of 325 one-d-old male broilers were randomly distributed into 13 treatments with 25 birds each. Treatments consisted of a control diet based on corn and soybean meal, and the inclusion of 1, 2, 4, or 6% meat and bone meal, poultry offal meal or sugarcane yeast. At 42 days of age, six birds per treatment were randomly selected, sacrificed, and their breast muscle was collected for isotopic ration analysis. The isotopic ratio of birds fed the diet with inclusion of 6% sugarcane yeast was different from those fed the control treatment, but not from those fed diets with the inclusion of 2, 4 and 6% meat and bone meal or 4 and 6% poultry offal meal. The inclusion of 6% sugarcane yeast in broiler diets based on corn and soybean meal may affect the traceability of animal by product meals.

INTRODUCTION

According to the Brazilian Association of Broiler Producers and Exporters (ABEF, 2009), Brazil is the third largest broiler producer and the world largest chicken meat exporter. However, the markets importing Brazilian chicken meat has imposed several restriction relative to rearing, feeding, and trade practices.

The increasing consumer concerns with food safety have led the industry to propose methods to provide the certification of origin and of the quality of animal and plant products around the world (Denadai, 2009). One of them is traceability, a tool used in the meat production system that has been critical for the survival and success of meat companies in an increasingly competitive and demanding market (Iba *et al*., 2003), and which still requires much research and development, particularly in terms of methods.

Many importers of Brazilian chicken meat, such as countries of the European Union and Middle East, require that broilers are not fed any animal by of product feedstuff (Bellaver *et al*., 2005). One way to detect the presence of animal feedstuffs in broilers feeds is to use stable isotopes, because the dietary isotopic signature can be detected in the bird's body in up to ± 2.0‰ for $\delta^{13}C$ and up to ± 3.0‰ for $\delta^{15}N$, according to DeNiro & Epstein (1976, 1978).

The isotopic ratio of carbon to nitrogen allows identifying the geographic origin and feeding method in sheep (Piasentier *et al*., 2003), characterizing and differentiating dietary regimes in Iberian pigs (González-Martin *et al*., 1999) and tracing animal feedstuffs in broiler diets (Oliveira, 2005; Carrijo *et al*., 2006). However, some feedstuffs,

such as sugarcane yeast and wheat bran, may interfere in the traceability of animal by product meals when the technique of stable isotopes due to its high protein content and consequent Nitrogen-15 and Carbon-13 enrichment (Gottmann, 2007).

Poultry production in Brazil uses a wide range of sugarcane yeast inclusion levels in feeds because it is usually applied as a feed additive. However, according to Grangeiro *et al*. (2001), levels of up to 7.5% yeast can be used as protein source in broiler diets without any negative effect on broiler performance.

The objective of the present study was to determine if the inclusion of sugarcane yeast (*Saccharomyces cerevisiae*) at the levels commonly used by the broiler industry interferes in the traceability of animal by product meals when the carbon and nitrogen stable isotopes method is applied.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Nutrition Laboratory of the School of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP, Brazil.

A total number of 325 one-day-old male Cobb® broiler chicks, vaccinated at the hatchery against Infectious Bursal Disease and Marek's Disease, was used. Birds were housed in an experimental broiler house, where birds were randomly distributed in 13 pens measuring 2.5 m^2, corresponding to 13 treatments, with 25 birds/pen at a density of 10 birds /m^2. There were six replicates per treatment, and each slaughtered bird was considered one replicate.

Water and feed were supplied *ad libitum* during the entire experimental period. Feeds were based on corn, soybean meal, soybean oil, dicalcium phosphate, calcitic limestone, salt, DL-methionine, L-lysineand vitamin and mineral supplements, as adapted from the recommendations of Rostagno *et al*. (2005). Treatments consisted of: vegetable or control diet (VEG), vegetable diet with the inclusion of cattle meat meal at 1% (1FC), 2% (2FC), 4% (4FC) or 6% (6FC); vegetable diet with the inclusion of poultry offal meal at 1% (1FV), 2% (2FV), 4% (4FV) or 6% (6FV); and vegetable diet with the inclusion of sugarcane yeast at 1% (1LC), 2% (2LC), 4% (4LC) or 6% (6LC).

A three-phase feeding program was adopted: starter (1 to 21 days), grower (22 to 35 days) and finisher (36 to 42 days), as shown in Table 1.

On day 42, six birds (n = 6) per treatment were randomly selected and individually identified by a numbered leg band. These birds were slaughtered at the experimental processing plant of FMVZ/UNESP – Botucatu. Birds were electrically stunned, bled, plucked and eviscerated, and the carcasses were cut up for the removal of the breast muscle (*Pectoralis major*).

Breast muscle samples were collected by cutting a slice measuring approximately 5 mm transversal to the longitudinal middle third of the left *Pectoralis major* (Gottmann, 2007). Samples were duly identified, placed in plastic bags and frozen at -20 °C until analysis. Isotopic analyses were carried out at the Center Stable Isotopes of the Biosciences Institute, UNESP, Botucatu, SP, Brazil.

Samples for isotopic analyses were thawed, rinsed in distilled water, and dried in a forced-ventilation oven (Marconi – Ma 035) at 56 °C for 48 hours. Then, samples were placed in a tube and ground in a liquid-nitrogen cryogenic mill (Spex freezer/mill – model 6750), at - 190 °C for 3 minutes (tissue) and at maximal frequency to obtain a homogenous material with very fine particle size and microscopic aspect (Rosa *et al*., 2002).

Samples of approximately 50-60 µg and 500-600 µg were weighed in tin capsules to determine carbon and nitrogen isotopic ratios, respectively. The capsules were introduced by means of an automatic sampler in the element analyzer (EA 1108 - CHN - Fisons Instruments, Rodano, Italy), where, in the presence of oxygen (O_2) and copper oxide (CuO), samples were quantitatively burnt to obtain CO_2 and NOx, which was then reduced to N_2 in the presence of copper. The obtained gases were separated in gas chromatography column, and analyzed in isotopic ratio mass spectrometer (Delta S – Finnigan MAT, Bremen, Germany).

Isotopic ratio values were expressed as delta per thousand (‰) relative to the international standard PeeDee Belemnite (PDB) for $\delta^{13}C$ and atmospheric air nitrogen for $\delta^{15}N$ (Ducatti, 2004), according to the following general equation:

$$\delta X_{(sample,\ standard)} = [(R_{sample} - R_{standard}) / R_{standard}] \times 1000,$$

where δX represents the enrichment of the heaviest isotope of the chemical element X (C or N) of the sample relative to the international standard and R represents the ratio between the least and the most abundant isotope, specifically $^{13}C/^{12}C$ and $^{15}N/^{14}N$.

The obtained isotopic results were submitted to multivariate analysis of variance (MANOVA) using the GLM procedure of SAS statistical program (2004). Based on the data generated by error matrices, the

Table 1 - Ingredient and calculated nutritional compostition of the starter (1 to 21 days) and finisher (22 to 42 dayss) experimental diets.

Ingredients, %	**Starer experimental diet**												
	VEG	**1FC**	**1FV**	**1LC**	**2FC**	**2FV**	**2LC**	**4FC**	**4OM**	**4LC**	**6FC**	**6FV**	**6LC[1]**
Ground corn	60.50	61.34	61.45	60.02	61.90	62.46	59.61	63.54	64.60	59.00	63.92	64.61	58.41
Soybean meal 45	33.82	32.77	32.38	33.42	32.03	30.78	32.80	29.89	27.41	31.41	27.99	24.39	29.96
Soybean oil	1.36	1.09	1.03	1.41	0,90	0.70	1.45	0.37		1.46	0.26		1.46
Meat & bone meal - 40			1.00			2.00		4.00			6.00		
Poultry offal meal				1.00			2.00		4.00			6.00	
Sugarcane yeast					1.00			2.00		4.00			6.00
Calcitic limestone	1.00	0.82	0.98	0.98	0.66	0.98	0.99	0.36	0.98	1.00		0.97	1.00
Dicalcium phosphate	1.80	1.48	1.75	1.78	1.15	1.70	1.77	0.49	1.62	1.75		1.55	1.75
DL-Methionine	0.24	0.24	0.22	0.24	0.23	0.21	0.24	0.23	0.18	0.24	0.23	0.18	0.26
L-Lysine	0.33	0.33	0.25	0.21	0.21	0.24	0.19	0.23	0.29	0.20	0.25	0.35	0.23
Kaolin											0.50	1.05	
Salt	0.46	0.44	0.44	0.45	0.42	0.43	0.45	0.39	0.42	0.44	0.35	0.40	0.43
Mineral & vitamin supplement[2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition													
ME, kcal/kg	2950	2950	2950	2950	2950	2950	2950	2950	2950	2950	2950	2950	2950
CP, %	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04	21.04
Ca, %	0.96	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.97	0.95	0.95
Available P, %	0.45	0.45	0.44	0.44	0.45	0.44	0.44	0.45	0.44	0.44	0.48	0.45	0.45
Met, %	0.56	0.55	0.54	0.56	0.55	0.52	0.56	0.55	0.50	0.56	0.55	0.49	0.57
Met + Cys, %	0.89	0.89	0.89	0.89	0.89	0.88	0.88	0.89	0.88	0.88	0.89	0.90	0.89
Lys, %	1.35	1.34	1.27	1.27	1.25	1.25	1.26	1.25	1.25	1.27	1.25	1.26	1.30
Ingredients, %	**Grower experimental diet**												
Ground corn	62.40	63.82	63.83	62.64	64.65	65.29	62.49	66.33	67.39	61.70	67.28	69.54	61.00
Soybean meal 45	29.90	28.70	28.15	29.03	27.65	26.26	28.29	25.47	22.99	27.08	23.49	19.62	25.78
Soybean oil	3.40	2.94	2.93	2.03	2.67	2.45	3.18	2.13	1.75	3.24	1.83	1.04	3.28
Meat & bone meal - 40		1.00			2.00			4.00			6.00		
Pouktry offal meal			1.00			2.00			4.00			6.00	
Sugarcane yeast				1.00			2.00			4.00			6.00
Calcitic limestone	0.94	0.78	0.93	0.94	0.63	0.94	0.95	0.32	0.94	0.95		0.93	0.96
Dicalcium phosphate	1.62	1.31	1.60	1.62	0.97	1.54	1.61	0.32	1.45	1.60		1.38	1.58
DL-Methionine	0.23	0.22	0.21	0.22	0.22	0.20	0.23	0.22	0.19	0.23	0.21	0.17	0.23
L-Lysine	0.23	0.25	0.26	0.27	0.25	0.34	0.28	0.29	0.34	0.22	0.30	0.38	0.20
Kaolin	0.40	0.10	0.22	0.16	0.10	0.20	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.39	0.38	0.38	0.39	0.36	0.38	0.38	0.33	0.36	0.38	0.29	0.34	0.37
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition													
ME, kcal/kg	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100
CP, %	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31	19.31
Ca, %	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.95	0.88	0.88
Available P, %	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.47	0.41	0.41
Met, %	0.53	0.51	0.50	0.52	0.51	0.49	0.52	0.51	0.48	0.53	0.50	0.46	0.53
Met + Cys, %	0.84	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.85	0.83	0.82	0.85	0.82
Lys, %	1.16	1.17	1.17	1.20	1.16	1.21	1.21	1.18	1.17	1.17	1.17	1.16	1.17

1 - VEG: Control diet based on corn and soybean meal; 1MM: 1% cattle meat and bone meal; 1OM: 1% poultry offal meal; 1SY: 1% sugarcane; 2MM: 2% cattle meat and bone meal; 2 OM: 2% poultry offal meal; 2SY: 2% sugarcane yeast; 4MM: 4% cattle meat and bone meal; 4OM: 4% poultry offal meal; 4SY: 4% sugarcane yeast; 6MM: 6% cattle meat and bone meal; 6OM: 6% poultry offal meal; 6SY: 6% sugarcane yeast. 2 - Mineral and vitamin supplement (levels per kg product): folic acid - 0,9 mg, Co - 0.4 mg, Cu - 6 mg, Choline - 350 mg, Fe - 50 mg, I -1 mg, Mn - 60 mg, Methionine - 1750 mg, Niacin - 40 mg, Se - 2,5 mg, Vit. A - 80,000 IU, Vit B1 - 2 mg, Vit B2 - 6 mg, Vit B12 - 15 mcg, Vit B6 - 4 mg, Vit D3 - 2,000 IU, Vit E - 18 IU, Vit H - 0.1mg, Vit K3 - 2 mg, Zn - 70 mg. 3 - Mineral and vitamin supplement (levels per product): folic acid -0.9 mg, Co - 0,4 mg, Cu - 6 mg, Choline - 350 mg, Fe - 50 mg, I - 1 mg, Mn - 60 mg, Methionine - 1750 mg, Niacin - 40 mg, Se - 2.5 mg, Vit. A - 80,000 IU, Vit B2 - 6 mg, Vit B12 - 15 mcg, Vit B6 - 4 mg, Vit D3 - 2,000 IU, Vit H - 0,1 mg, Vit K3 - 2 mg, Zn - 70 mg.

regions with 95% confidence were determined to identify possible differences among treatment means.

RESULTS AND DISCUSSION

Because sugarcane has the isotopic signals $\delta^{13}C$ = -10.49 ‰ and $\delta^{15}N$ = 4.36‰, its inclusion in the diet may lead to the dietary enrichment of carbon-13 and nitrogen-15, which also happens with the inclusion of poultry offal meal ($\delta^{13}C$= -16.28‰ and $\delta^{15}N$ = 4.29‰) and cattle meat meal ($\delta^{13}C$ = -12.82‰ and $\delta^{15}N$ = 7.72‰) in broiler diets (Gottmann *et al.*, 2008). The carbon-13 and nitrogen-15 enrichment when animal by product meals are included in broiler diets was also observed by Oliveira (2005) and Carrijo *et al*. (2006).

It is possible to determine the presence of animal by products in broiler feeds by detecting their isotopic signature in the body of broilers. This can be applied to other animal species, as the animal is what it isotopically eats, or up to ± 2.0‰ for $\delta^{13}C$ and up to ± 3.0‰ for $\delta^{15}N$, according to DeNiro & Epstein (1976, 1978).

The mean isotopic signals ($\delta^{13}C$ and $\delta^{15}N$) in the breast muscle of broilers showed that there was carbon-13 and nitrogen-15 enrichment with increasing dietary inclusion levels of meat meal, poultry offal meal and yeast (Table 2). Mean isotopic signals ($\delta^{13}C$ and $\delta^{15}N$) in the breast meat samples of each treatment were statistically compared with the means of the control treatment (Figure 1) and of the treatments with different yeast levels (Figure 2), generating regions of 95% confidence level.

Table 2 - Mean and standard deviation value of $\delta^{13}C$ and $\delta^{15}N$ in the breast muscle of 42-d-old broilers.

Treatment[1]	$\delta^{13}C$	$\delta^{15}N$
VEG	-18.93 ± 0.17	2.33 ± 0.17
1FC	-18.86 ± 0.18	2.40 ± 0.13
1FV	-18.81 ± 0.21	2.30 ± 0.12
1LC	-18.73 ± 0.15	2.39 ± 0.17
2FC	-18.83 ± 0.17	2.51 ± 0.21
2FV	-18.50 ± 0.20	2.23 ± 0.13
2LC	-18.63 ± 0.15	2.29 ± 0.10
4FC	-18.10 ± 0.13	2.67 ± 0.08
4FV	-18.14 ± 0.17	2.43 ± 0.14
4LC	-18.55 ± 0.15	2.39 ± 0.16
6FC	-17.55 ± 0.23	2.75 ± 0.21
6FV	-17.73 ± 0.22	2.68 ± 0.16
6LC	-18.18 ± 0.17	2.65 ± 0.12

1 - VEG: Control diet based on corn and soybean meal; 1FC: 1% cattle meat meal; 1FV: 1% poultry offal meal; 1SY: 1% sugarcane yeast; 2FC: 2% cattle meat meal; 2FV: 2% poultry offal meal; 2LC: 2% sugarcane yeast; 4FC: 4% cattle meat meal; 4FV: 4% poultry offal meal; 4LC: 4% sugarcane yeast; 6FC: 6% cattle meat meal; 6FV: 6% poultry offal meal; 6LC: 6% sugarcane yeast.

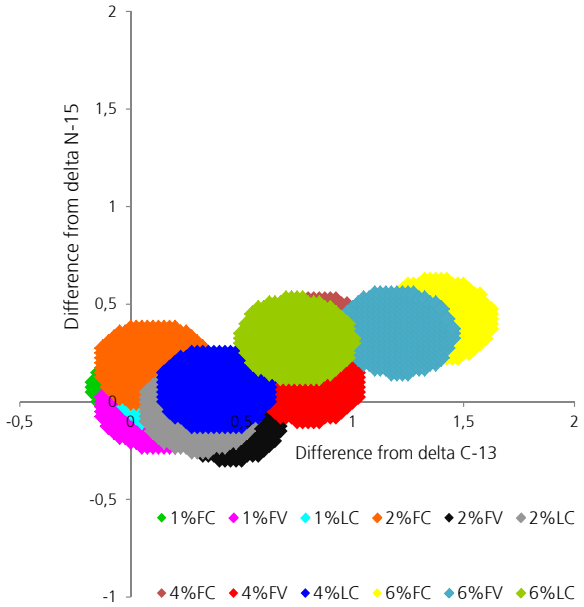

Figure 1 - Confidence regions established by the difference among $\delta^{13}C$ and $\delta^{15}N$ isotopic values in the muscle *Pectoralis major* of 42-d-old broilers of each treatment compared with the control treatment (Vegetable).

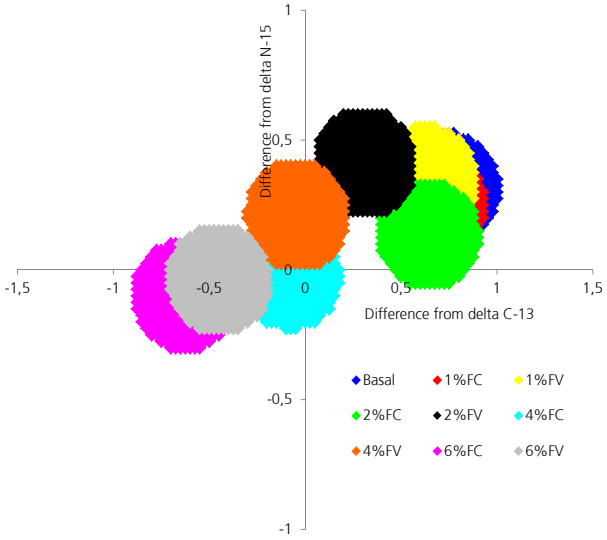

Figure 2 - Confidence regions established by the difference among $\delta^{13}C$ and $\delta^{15}N$ isotopic values in the muscle *Pectoralis major* of 42-d-old broilers of each treatment compared with the treatment containing 6% sugarcane yeast.

The compared treatments are different when the difference among their $\delta^{13}C$ and $\delta^{15}N$ means is different from zero, and therefore, the confidence region should not overlap any of the graph axes. The fact that the ellipsis overlaps one of the axis shows that the difference among $\delta^{13}C$ and $\delta^{15}N$ means of the

compared treatments is equal to zero, indicating that the treatments are not different.

The treatment with sugarcane yeast inclusion was different from the control treatment only after 6% inclusion; poultry off al meal after 6% and meat meal after 4%, as their ellipses did not overlap the delta C or delta N axes (Figure 1).

The treatments 1FC, 1FV, 1LC, 2FC, 2FV 2LC, 4FV and 4LC were not different from control treatment, possibly to the low inclusion levels of cattle meat meal, poultry offal mealand sugarcan eyeast, respectively. As to the other treatments, it was observed that there were differences among different inclusion levels of animal by product meals and sugarcane yeast relative to the control treatment. Oliveira (2005), Carrijo *et al*. (2006) and Gottmann (2007) also reported differences when comparing diets containing poultry offal meal and cattle meat meal with a standard diet based on corn and soybean meal.

Gottmann (2007) showed that the traceability of animal byproduct meals in the breast muscle of broilers, as determined by the technique of stable isotopes, was compromised by the presence of yeast and wheat bran. Poultry production in Brazil uses a wide range of sugarcane yeast inclusion levels in feeds because it is usually applied as a feed additive. However, according to Grangeiro *et al*. (2001), levels of up to 7.5 % yeast can be used as protein source in broiler diets without any negative effect on broiler performance.

The ellipses of treatments 2FC, 4FC, 6FC, 4FV and 6FV overlapped the axes of the differences in $\delta^{13}C$ and/or $\delta^{15}N$ and, therefore, these treatments were not different from the treatment containing 6% sugarcane yeast (Figure 2). This may have been due to the fact that the isotopic signal of sugarcane yeast ($\delta^{13}C$ = -10.49 ‰ and $\delta^{15}N$ = 4.36‰) is very close to the isotopic signals of poultry offal meal ($\delta^{13}C$= -16.28‰ and $\delta^{15}N$ = 4.29‰) and cattle meat meal ($\delta^{13}C$ =-12.82‰ and $\delta^{15}N$ = 7.72‰), demonstrating sugarcane yeast may affect the traceabilit yof animal by product meals as determined by the technique of the carbon to nitrogen isotopic ratio in broiler carcasses. In the present study, the dietary addition of sugarcane yeast affected the traceability of broiler carcasses, as after 6% inclusion level, its isotopic signature could no longer be differentiated from those of meat meal and off al meal.

The inclusion of 6% sugarcane yeastin broiler diets based on corn and soybean meal may interfere in the traceability of animal by product meals in broiler feeds when using the technique of stable carbon and nitrogen isotopes.

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