



Barn vs. free-range chickens: Differences in their diets determined by stable isotopes

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ABSTRACT

We compared $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios of barn-raised and free-range chickens to determine if differences in their diets were reflected in the stable isotope composition of their tissues. We conducted a 120-day feeding trial with *Caipirinha* birds fed a corn–soybean based diet, milled-corn diet and free-range diet. Additionally, we analysed the stable isotope composition of barn-raised chickens bought in grocery stores and free-range homegrown chickens. In the feeding trials, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the barn-raised corn–soybean-fed *Caipirinha* chickens did not change with age, and their stable isotope composition reflected the composition of their diet. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of barn-raised corn-fed and free-range *Caipirinha* chickens changed with age toward a diet reflecting a predominance of C_4 carbon. The main difference between the free-range and the barn-raised chickens was the significantly higher $\delta^{15}\text{N}$ of the former in relation to the latter, probably due to ingestion of animal protein.

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1. Introduction

The growth in chicken production began mainly in the early 1970s with the initial recommendations that chicken meat was healthier than beef, and also due to the fact that chicken meat is less expensive than beef and pork (Bolis, 2002; Roberts, 2008). This significant increase in poultry production and consumption occurring in recent decades was only possible due to a series of changes in the poultry industry. These changes included, among others, changes in feed composition based on grains such as corn and soybean and some source of animal protein, and genetic improvements to increase feeding efficiency and the amount of breast meat (Roberts, 2008).

Regarding diet availability to chickens, there is on one side what are conventionally referred to as barn-raised chickens, in which the main nutritional characteristic is that this diet is provided exclusively by the producer; on the other side, there are the so-called free-range chickens, which roam freely through meadows and mimic their original foraging dietary habits, eating not only grass but also earthworms from the soil (Fanatico, 2006; Sossidou, Dal Bosco,

Elson, & Fontes, 2011; Zanusso & Dionello, 2003). The demand for such a product has been increasing in recent decades (Castellini, Mugnai, & Dal Bosco, 2002; Fanatico, Pillai, Emmert, & Owens, 2007).

Stable isotopic ratios of carbon and nitrogen have been largely used to infer diet sources of animals (Kelly, 2000; Newsome, del Rio, Bearhop, & Phillips, 2007). The first rationale justifying the use of stable isotopes for studying animal nutrition is related to the fact that carbon and nitrogen isotopes show relatively few and predictable changes when the atoms of these two elements pass through the food chain (Bearhop, Adams, Waldron, Fuller, & Macleod, 2004; Newsome et al., 2007). Therefore, the stable carbon and nitrogen isotopic composition of an animal will reflect approximately the same composition as their feed (e.g., Nardoto et al., 2006; Rogers, 2009). There is an extensive body of literature showing the usefulness of this technique, based on the pioneering work of DeNiro and Epstein (1978, 1981) and Tieszen, Hein, Qvortrup, Troughton, and Imbamba (1979), among others.

Stable isotopes have been especially useful in nutrition ecology of wild animals (Newsome et al., 2007). There is also a growing use of stable isotopes to investigate livestock in terms of authenticity and nutrition studies (e.g., Bahar et al., 2009; Guo, Wei, Pan, & Li, 2010; Harrison et al., 2010, 2011; Heaton, Kelly, Hoogewerff, & Woolfe, 2008; Osorio, Moloney, Schmidt, & Monahan, 2011; Schmidt et al., 2005). Several studies involving livestock are based on changing

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the original diet for another with different isotopic composition to track changes of isotopic composition of tissues over time (e.g., Ayliffe et al., 2004; Bahar et al., 2005; Harrison et al., 2011; West et al., 2004). Specifically in poultry, stable isotopic composition has been used to determine turnover time in tissues (Cruz et al., 2005; Hobson & Clark, 1992); nutrient routing (Cruz et al., 2004); to track the presence of animal protein in commercial poultry rations (Carrijo et al., 2006; Denadai, 2008; Mori et al., 2007), to detect the presence of corn in poultry diet (Rhodes et al., 2010), and to differentiate eggs laid by hens under different growth systems (Rogers, 2009). The latter was the only study designed to investigate differences between barn-raised and free-range chickens, and it was specifically designed for eggs and not for meat. Therefore, there is a need for studies that aim to investigate whether it is possible to differentiate barn-raised from free-range chickens by the use of stable isotopes. This is especially important because free-range chickens usually have a higher price than barn-raised chickens.

The main objective of this study was to investigate temporal changes in the isotopic composition of chickens grown under controlled conditions receiving two different diets. One diet was a conventional grain-based ration used in commercial barn-raised chicken plants, and the other diet was typical of free-range chickens in Brazil, which is a mixture of corn, grass and earthworms from the soil. We further compared the stable isotopic composition of barn-raised chickens bought in grocery stores, produced by 15 different companies, with 27 homegrown free-range chickens obtained from local households.

2. Material and methods

2.1. Feeding trials

The *Caipirinha* is a slow-growing chicken developed by the Genetic Department of the Escola Superior de Agricultura “Luiz de Queiroz” (ESALQ) and its main characteristics are its resilience and adaptability as a free-range bird, especially developed for homegrown conditions. Seventy-five *Caipirinha* broilers received the same corn and soybean starter feed *ad libitum* for the first 28 days (Table 1). After this period, broilers were divided into three groups of 25 birds each and were fed *ad libitum* with three different diets. One group continued receiving a final corn and soy-based feed (*Caipirinha*-barn-raised corn-soybean-fed; Table 1), a second group was allowed free access to grass pasture areas and also received milled corn (*Caipirinha*-free-range), and, finally, a third cohort received only milled corn (*Caipirinha*-barn-raised corn-fed). Individuals for each diet-treatment were kept apart and those allowed to pasture had free access to grass areas.

At 28, 60, 90, and 120 days of age, five individuals randomly selected from each treatment were slaughtered and the breast muscle of each bird was analysed for carbon and nitrogen stable isotopes.

Samples of each type of diet (starter and final), five samples of grasses and surface soil (0–10 cm) randomly sampled from the pasture plots were also collected for stable isotope analysis.

Table 1
Composition of the corn-soybeans rations used in feeding trials.

Parameter	Start – 28 days	Finish – 28 to 120 days
Metabolisable energy (kcal kg ⁻¹)	2950	3050
Crude protein (%)	21.0	18.0
Crude fibre (%)	4.50	4.50
Ether extract (%)	3.00	3.50
Calcium (%)	1.20	1.20
Phosphorus (%)	0.65	0.60
Lysine (%)	1.19	0.97
Methionine + cystine (%)	0.89	0.76
Linoleic acid (%)	1.80	2.00
Xanthophylls (ppm)	9.50	1.10

2.2. Barn-raised and free-range homegrown chickens

We analysed the breast muscle of 32 barn-raised chickens bought in grocery stores, produced by 15 different companies, and 27 homegrown free-range obtained from local households in Brazil. Information about the diet composition of all barn-raised birds was provided on all commercial brand labels, being mostly composed of grains. However, the proportion of each grain was not divulged. The main grains of these feeds were milled corn, milled sorghum, wheat meal, soybean meal, cotton meal, and pearl rice; and the main animal protein sources were: bone meal, offal meal, fish meal, and feather meal. It is important to mention that we did not use these diets to feed chickens in our feeding trials. The household birds had free access to grass areas, and rations of milled corn and leftovers from homemade meals were also offered to them, such as cooked rice and beans, and greens from salads, such as lettuce, kale, arugula, etc.

2.3. Isotopic analyses

All chicken breasts were oven-dried at 65 °C until constant weight and then ground to a fine powder. We did not extract lipids from our samples because breast muscles of Brazilian chickens have a very low lipid content, varying from 0.5% to 1.5% (Assis et al., 2010). Although there are several studies showing that lipids tend to have a lower $\delta^{13}\text{C}$ ratio than tissues with low lipid content (Bahar et al., 2009), this amount of lipids would probably not affect our results.

Soil and grass samples were air-dried, sieved using a 2-mm mesh and homogenised. A smaller sub-sample was collected, handpicked to remove fine roots and other debris and then ground in a mortar and pestle. A 1.5–2 mg sub-sample of ground chicken and leaf material or 15–20 mg sub-sample of ground soil were placed and sealed in a tin capsule and loaded into a ThermoQuest-Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan-MAT; San Jose, CA) in line with an Elemental Analyser (Model 1110; Carlo Erba, Milan, Italy). Stable isotope ratios of C and N were measured relative to recognised international standards. Internal working standards (sugarcane leaves and tropical surface soil) were included in every run, as a standard laboratory procedure. Stable isotope values are reported in “delta” notation, as δ values in parts per thousand (‰), so that $\delta\text{‰} = (R \text{ sample}/R \text{ standard} - 1) \times 1000$, in which R is the molar ratio of the rare to abundant isotope (¹⁵N/¹⁴N; ¹³C/¹²C) in the sample and the standard. The precision of the measurements was $\pm 0.3\%$, 0.1% , 0.3‰ and 0.5‰ for C, N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

2.4. Statistical analyses

The Shapiro–Wilk test was used to test the normality of the data. As the data followed a normal distribution, the analyses were performed using parametric tests (ANOVA). A *post hoc* Tukey test was used to assess differences between stable isotopic compositions of *Caipirinha* chicken fed with different diets. All statistical analyses were performed using the software STATISTICA, Version 9.0 for Windows (StatSoft, Tulsa, OK, 2010). Differences at the 0.05 level were reported as significant.

In order to estimate the turnover rate of breast tissue, we used the following equation:

$$\delta_t = \delta_n + (\delta_0 - \delta_n) * e^{-(ct)} \quad (1)$$

where δ is the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of the breast muscle at time t after the diet change; δ_0 is the initial $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of the breast muscle before the diet change at time $t = 28$ days; δ_n is the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of the breast muscle in equilibrium with the new diet; c is the total turnover; and t is the time in days since the start of the

new diet (Hesslein, Hallard, & Ramlal, 1993; Hobson & Clark, 1992). Half-lives ($t_{1/2}$) of breast muscle were estimated by the following equation:

$$t_{1/2} = -\ln(2)/c \quad (2)$$

The time to reach 99% of the turnover in the tissue is given by the following equation:

$$t_{99} = \ln(0.01)/c \quad (3)$$

The observed temporal change of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values after the diet change followed an exponential model. Accordingly, generated $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were adjusted in a non-linear regression equation using the software STATISTICA Version 10.

3. Results

3.1. Stable isotopic composition of feeds, grass and soils of pasture areas

The $\delta^{13}\text{C}$ values of milled corn used as a diet component in this study were those typical of C_4 plants and similar to the average value found for grass samples (Table 2). The diet for barn-raised chickens used in the trials was basically composed of corn and soybean without any kind of animal protein added. Their $\delta^{13}\text{C}$ values reflect the relative proportion of C_3 (soybean) and C_4 (corn) plants. The starter diet (given up to 28 days) had a proportion of 65% C_4 (corn), while the final diet (given after 28 days) had a proportion of 52% (Table 2). The remaining 35% and 48%, respectively, was composed of C_3 (soybean).

The $\delta^{15}\text{N}$ values of our starter and final diets were lower than the milled corn because the presence of soybean in significant proportions and the absence of animal protein (Table 2). The highest $\delta^{15}\text{N}$ values were observed in grass and soil samples. Generally these high values are due to ammonia volatilisation from animal faeces, which is a highly fractionating process, leading to an N-15 enrichment of the substrate (Choi, Ro, & Hobbie, 2003).

3.2. Feeding trials

3.2.1. Barn-raised trials

The average $\delta^{13}\text{C}$ values of barn-raised corn–soybean-fed *Caipirinha* chicken diet did not change with chicken ages and was similar to the $\delta^{13}\text{C}$ of their diet (Fig. 1). However, we observed variable diet–tissue fractionation during the trial. During the first 28 days, the $\delta^{13}\text{C}$ of the tissue was lower than the diet and the fractionation was -0.1‰ . After the initial period, the $\delta^{13}\text{C}$ of the tissue became higher than the diet and the fractionation increased to 1‰ at 60 days, decreasing to 0.6‰ at 90 days and increasing again to 1.1‰ at 120 days.

The average $\delta^{15}\text{N}$ of barn-raised corn–soybean-fed *Caipirinha* chickens did not differ between the 28-day and 60-day old chickens, being similar to the $\delta^{15}\text{N}$ of their diet. However, these values

Table 2

$\delta^{13}\text{C}$, $\delta^{15}\text{N}$ values (‰) and relative proportion (%) of C_4 and C_3 contents of milled corn samples used in the feeding trials, and corn–soybean diets used in the feeding trials; and grass and soil samples of the free-range *Caipirinha* chickens feeding trials.

Sample	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Feeding trials milled corn ^a	-11.2	3.6
Feeding trials milled corn ^a	-11.4	4.1
Feeding trials starter diets (up to 28 days) ^a	-16.7	0.6
Feeding trials final diets (after 28 days) ^a	-18.8	0.1
Grass	-13.0	8.9
Soil	-16.3	8.8

^a Diets used in this study.

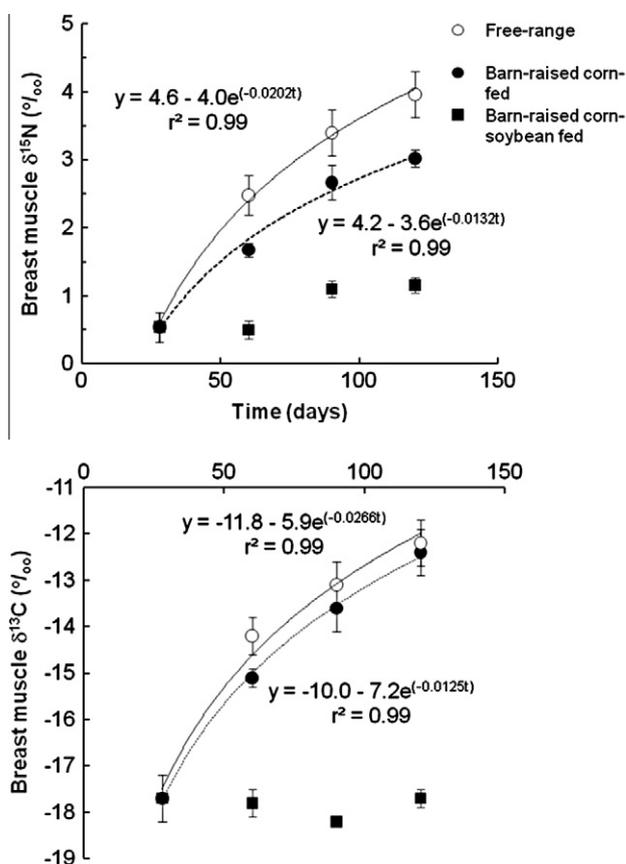


Fig. 1. Age variability of the $\delta^{15}\text{N}$ (upper panel) and $\delta^{13}\text{C}$ (lower panel) of *Caipirinha* chickens with the following diets: free-range, barn-raised corn-fed and corn-soybean-fed.

became significantly higher for 90-day and 120-day old chickens (Fig. 1). As for carbon isotopes, the fractionation tissue–diet changed over time. As the chickens became older, the fractionation increased from -0.1‰ at 28 days, to 0.4‰ at 60 days, 1.0‰ at 90 days, and 1.1‰ at 120 days.

The carbon isotopic composition of the barn-raised corn-fed *Caipirinha* chickens showed significant changes as chickens aged (Fig. 1). At the end of the trial (120 days), the $\delta^{13}\text{C}$ values of these chickens tend to be similar to the isotopic values of the milled corn used as feed in our experiment (Fig. 1). However, it is clear from the curve that the isotopic equilibrium with the new diet was not achieved (Fig. 1). The $t_{1/2}$ was equal to approximately 55 days, and the $\delta^{13}\text{C}_n$ derived from Eq. (1) was equal to -10.0‰ demonstrating the fact that isotopic equilibrium was not achieved (Table 3).

The $\delta^{13}\text{C}$ average values of 120-day old barn-raised corn-fed *Caipirinha* chickens were significantly higher ($p = 0.001$) than the average $\delta^{13}\text{C}$ of the 120-day old barn-raised corn–soybean-fed *Caipirinha* chickens (Table 3).

The average $\delta^{15}\text{N}$ values of barn-raised corn-fed *Caipirinha* chickens also increased with the chickens' age (Fig. 1). However, as for carbon, the isotopic equilibrium for nitrogen was not achieved either (Fig. 1). The $t_{1/2}$ was equal to approximately 53 days and the $\delta^{15}\text{N}_n$ also derived from Eq. (1) was equal to 4.6‰ (Table 3).

The average $\delta^{15}\text{N}$ values of barn-raised 120-day old corn-fed chickens were significantly higher ($p = 0.001$) than the average $\delta^{15}\text{N}$ value of the 120-day old barn-raised corn–soybean-fed *Caipirinha* chickens.

3.2.2. Free-range chickens

The carbon isotopic composition of free-range *Caipirinha* chickens showed significant changes with chicken ages (Fig. 1). At the

Table 3

Values of turnover (c), half-life ($t_{1/2}$), time to reach isotopic equilibrium (t_{99}), δ ratios at the beginning of the feeding trials (δ_{28}), δ ratios at the end of the feeding trials (δ_{99}), δ ratios at the isotopic equilibrium (δ_n), and coefficient correlation of the exponential decay curve (r^2).

Parameter	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	Corn	Free-range	Corn	Free-range
c	-0.0125	-0.0266	-0.0132	0.0202
$t_{1/2}$ (days)	55	26	53	34
t_{99} (days)	368	173	349	228
δ_{28} (‰)	-17.7	-17.7	0.5	0.5
δ_{120} (‰)	-12.4	-12.2	3.0	4.0
δ_n (‰)	-10.0	-11.8	4.2	4.6
r^2	0.99	0.99	0.99	0.99

end of the 120 days, the $\delta^{13}\text{C}$ values of these chickens tended to be similar to the $\delta^{13}\text{C}$ ratio of grasses sampled in the pasture plot (Table 3). However, like the barn-raised corn-fed chicken, the isotopic equilibrium was not achieved (Fig. 1). In this case, the $t_{1/2}$ was equal to approximately 26 days, shorter than the $t_{1/2}$ found for barn-raised corn-fed chicken, and the $\delta^{13}\text{C}_n$ derived from Eq. (1) was equal to -11.8‰ (Table 3). The $\delta^{13}\text{C}$ average values of 120-day old free-range *Caipirinha* chickens were also significantly higher ($p = 0.0001$) than the average $\delta^{13}\text{C}$ of 120-day old barn-raised corn-soybean-fed *Caipirinha* chickens.

The average $\delta^{15}\text{N}$ values of free-range *Caipirinha* chickens also increased with the chickens' age like the $\delta^{13}\text{C}$ values (Fig. 1). In this case, the increase of $\delta^{15}\text{N}$ values of free-range *Caipirinha* chickens was significantly higher ($p = 0.0001$) than the values found of barn-raised corn-fed *Caipirinha* chickens (Fig. 1). Again it seems that the isotopic equilibrium was not achieved: $t_{1/2}$ was equal to 34 days and the $\delta^{15}\text{N}_n$ derived from Eq. (1) was equal to 4.6‰ .

The average $\delta^{15}\text{N}$ values of 120-day old free-range *Caipirinha* chickens were significantly ($p = 0.0001$) higher than the average $\delta^{15}\text{N}$ value of the 120-day old barn-raised corn-soybean-fed *Caipirinha* chickens.

3.2.3. Barn-raised chickens bought in grocery stores (commercial)

The average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of commercial barn-raised chickens were similar to the barn-raised corn-soybean-fed *Caipirinha* chickens (Fig. 2). However, it is difficult to make a more detailed comparison between these two groups because we have no information about the diet of commercial chickens. Therefore we cannot establish the fractionation between the diet and the tissue, and this fact is important in such comparisons (Cruz et al., 2005). Additionally, commercial chickens are slaughtered when they are 42 days old, and the barn-raised corn-soybean-fed *Caipirinha* chickens used in the feeding trials were slaughtered at different ages.

3.2.4. Free-range homegrown chickens

We observed that with few exceptions, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of barn-raised chickens were much more clustered to each other with much less variability in relation to the isotope composition of free-range homegrown chickens (Fig. 2). Additionally, $\delta^{15}\text{N}$ ratios of homegrown chickens are higher than barn-raised chickens and higher than the free-range *Caipirinha* chickens (Fig. 2).

A more rigorous comparison between homegrown chickens and others is difficult because the diet of homegrown chickens is quite variable in terms of composition and virtually unknown. Therefore, neither the isotopic fractionation between diet-tissue is known, nor whether chickens are in isotopic equilibrium with a particular type of diet. Another factor is that we do not know the ages that chickens were slaughtered, and as we observed, stable isotope composition may change with chicken age.

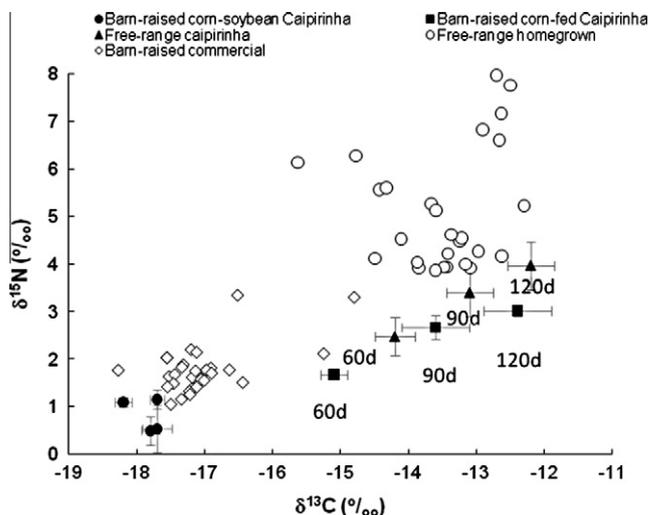


Fig. 2. $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ plot of feeding trials *Caipirinha* chickens, including barn-raised corn-soybean and corn-fed chickens, and free-range chickens; and barn-raised commercial chickens and free-range homegrown chickens.

4. Discussion

4.1. Turnover rates in barn-raised and free-range chickens

The turnover rates estimated using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios were similar (Table 3). Ogden, Hobson, and Lank (2004) and Bahar et al. (2009) found similar results, working with captive dunlin and with bovine muscles, respectively. Both authors concluded that this similarity in turnover rates suggests a protein molecule from the diet being quickly incorporated in body tissues. This is especially true for muscle tissues as suggested by Gannes, del Rio, and Koch (1998), and shown by Cruz et al. (2004), who studied chickens receiving diets with different protein and energy contents.

Consequently the $t_{1/2}$ were shorter in free-range (26–34 days) in relation to corn-fed chickens (53–55 days) (Table 3). We could not find values of $t_{1/2}$ for chickens of the same age as the ones used in this study for comparative purposes. Cruz et al. (2005) worked with 1-day to 30-day old chickens, and found a much shorter $t_{1/2}$ value (5–8 days) than ours. On the other extreme, Bahar et al. (2009), working with two types of bovine muscles found a much longer $t_{1/2}$, varying from 133 to 151 days. Intermediate between chickens and bovine, lamb muscle had an estimated $t_{1/2}$ varying from 76 to 92 days (Harrison et al., 2011). The shorter $t_{1/2}$ was associated with animals receiving a diet with higher energetic content than others that produced a longer $t_{1/2}$ (Harrison et al., 2011). Therefore, it is reasonable to speculate that free-range chickens had a shorter $t_{1/2}$ basically because besides corn, these chickens may take in additional energy and protein from grass and earthworms (Fanatico, 2006; Ipek, Karabulut, Sahan, Canbolat, & Yilmaz-Dikmen, 2009; Mattocks, 2002). Although, we did not have growth rates for our feeding trial animals, there are two studies showing that free-range chickens grew faster than chickens fed with only a grain-based diet (Buchanan, Hott, Kimbler, & Moritz, 2007; Ipek et al., 2009).

4.2. Stable isotope ratios to differentiate barn-raised vs. free-range chickens

The main feature of barn-raised corn-fed *Caipirinha*, and free-range *Caipirinha* chickens was their higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, in relation to the barn-raised corn-soybean-fed *Caipirinha* chickens (Fig. 1). Although the isotopic equilibrium was not yet reached, the increase observed in stable isotopic values of barn-raised corn-fed

Caipirinha chickens could be interpreted as a consequence of a change in the diet after 28 days (Fig. 1). The free-range *Caipirinha* chickens also received milled corn and their $\delta^{15}\text{N}$ increase with age was significantly higher than barn-raised corn-fed *Caipirinha* chickens (Fig. 1). At 120 days, the $\delta^{15}\text{N}$ values of free-range *Caipirinha* chickens were approximately 1‰ higher than the corn-fed chickens. At the isotopic equilibrium, the $\delta^{15}\text{N}_n$ of the free-range chickens would be equal to 4.6‰, which would be 0.4‰ higher than the $\delta^{15}\text{N}_n$ of the corn-fed chickens (Table 3).

Therefore, based on the above information, it is reasonable to speculate that milled corn alone would not be enough to justify the increase in the $\delta^{15}\text{N}$ values observed in free-range chickens. The same ^{15}N enrichment found here was found in free-range eggs relative to barn-laid eggs (Rogers, 2009; Rossmann, 2001). However, it is important to speculate about the causes for such differential increase in the $\delta^{15}\text{N}$ values between free-range and barn-raised corn-fed chickens.

One possibility is that the fractionation tissue-diet was different between chickens under different diets, and also that this fractionation varied in time, as shown in our study. It has been shown that diets with different compositions may cause differences in tissue-diet fractionation (McCutchan, Lewis, Kendall, & McGrath, 2003; Pearson, Levey, Greenberg, & del Rio, 2003; Vanderklift & Ponsard, 2003). This could be true in our case because the turnover time of free-range chickens was faster than the corn-fed chickens (Table 3), suggesting that free-range chickens were growing faster than the corn-fed chickens, as already discussed. This possible difference in the nutritional composition of the diets and the possible difference in chicken growth may lead to a different fractionation between tissue-diet that in turn would lead to different $\delta^{15}\text{N}$ values between home-grown and corn-fed *Caipirinha* chickens (McCutchan et al., 2003; Pearson et al., 2003; Vanderklift & Ponsard, 2003).

Another possibility is that the grass itself, and many soil invertebrates, such as earthworms and insects, can be an important additional protein source for free-range chickens (Fanatico, 2006; Ipek et al., 2009; Mattocks, 2002). This hypothesis is corroborated by recent studies that showed a small increase in animal protein in chicken diets led to a detectable increase in $\delta^{15}\text{N}$ values (Carrizo et al., 2006; Denadai et al., 2008; Mori et al., 2007; Rogers, 2009). Chicken broilers with 8% animal protein in their diet showed a 1‰ higher $\delta^{15}\text{N}$ ratio than broilers under a strictly grain-based diet (Carrizo et al., 2006).

This increase could be interpreted as the N-15 enrichment that normally occurs along the food chain (Minagawa & Wada, 1984). We hypothesised that barn-raised corn-soybean-fed *Caipirinha* chickens at age 28-day old began to tap animal protein sources when they became free-range chickens and had access to soil areas. Secondly, this increase could also be related to the fact that the $\delta^{15}\text{N}$ values of earthworms, insects and grasses could be higher than the $\delta^{15}\text{N}$ of grains composing the grain-based diets (Rogers, 2009). For instance, the $\delta^{15}\text{N}$ values of grass samples collected in the pasture area used in our feeding trials and of the soil organic matter of the same area had the highest $\delta^{15}\text{N}$ values among several diets (Table 2). Therefore, earthworms and insects feeding in these pasture areas would also have an elevated $\delta^{15}\text{N}$ value (Rogers, 2009). Ferreira (2008) found $\delta^{15}\text{N}$ values varying from 4‰ to 10‰ for terrestrial insects in a pasture located in the same region of our study. Additionally, Schmidt, Curry, Dyckmans, Rota, and Scrimgeour (2004) found that soil-feeding species of soil invertebrates had significantly higher $\delta^{15}\text{N}$ ratios than the soils from which they were feeding.

Based on the above results we are tempted to propose that at least for Brazilian conditions where most grasses are of C₄ type, carbon and nitrogen stable isotopes can be used as an initial screening device to authenticate claims that poultry had access

to forage areas and are really free-range chickens. This would require that these chickens would have high $\delta^{13}\text{C}$ combined with high $\delta^{15}\text{N}$ values. However, it is also important to consider that a confounding factor of this technique would be poultry fed with diets containing a high proportion of corn (C₄) and a low proportion of soybean (C₃) in order to increase $\delta^{13}\text{C}$ values, combined with any type of animal protein added to the diet, such as bone meal, fish meal, feather meal, etc., in order to increase the $\delta^{15}\text{N}$ values. On the other hand, it would be most unlikely that chickens with low $\delta^{15}\text{N}$ values would come from a free-range system.

We have to be cautious in recommending carbon and nitrogen stable isotope composition as a means of certifying free-range chickens, since certain combinations of ingredients in a diet could also lead to similar stable isotope composition found in free-range chickens. It would be useful to test whether the same tool could be applied in other countries around the world, especially in temperate regions, where most of the grasses used to feed free-range chickens are of the C₃ type.

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