Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Stradiotti, AC; Bendassolli, JA; Ducatti, C; Sartori, JR; Pelícia, VC; Araujo, PC; Maruno, MK; Pezzato, AC
Incorporation of Labeled Methionine as a Tissue Tracer in Broiler Chickens
Revista Brasileira de Ciência Avícola, vol. 18, núm. 4, octubre-diciembre, 2016, pp. 719-724
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Oct - Dec 2016 / v.18 / n.4 / 719-724
http://dx.doi.org/10.1590/1516-635x1804 719-724

Incorporation of Labeled Methionine as a Tissue Tracer in Broiler Chickens

■**Author(s)**

Stradiotti AC[I]
Bendassolli JA[II]
Ducatti C[III]
Sartori JR[IV]
Pelícia VC[I]
Araujo PC[I]
Maruno MK[I]
Pezzato AC[IV]

[I] Post-graduation students in Animal Sciences, FMVZ/UNESP, Botucatu, SP, Brazil
[II] Ass. Prof., PhD, Center of Nuclear Energy in Agriculture, CENA/USP, Piracicaba, SP, Brazil
[III] Ass. Prof., PhD, Department of Physics and Biophysics, IBB/UNESP, Botucatu, SP, Brazil
[IV] Ass. Prof., PhD, Department of Genetic Improvement and Animal Nutrition, FMVZ/UNESP, Botucatu, SP, Brazil

■**Mail Address**

Corresponding author e-mail address
Ana Cristina Stradiotti
Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina Veterinária e Zootecnia, Departamento de Melhoramento e Nutrição Animal. Distrito de Rubião Júnior, s/n. Caixa Postal 560. Botucatu, São Paulo, Brazil.
Zip code: 18.618-000.
Tel: +55-14-3880-2998
Email: ana.stradiotti@hotmail.com

■**Keywords**

Isotopic dilution, labeled isotopes, turnover.

Submitted: July/2016
Approved: September/2016

ABSTRACT

The objective of this study was to evaluate the process of L-methionine incorporation in the blood plasma, liver, breast muscle, and abdominal fat of 35- to 59-d-old broiler chickens using the carbon *stable isotope* (^{12}C and ^{13}C) technique for the estimation of methionine requirements. In this experiment, 51 male broiler chickens orally received a solution of L-[$^{13}C_1$] methionine (92 atm % ^{13}C) at 29 µmol/kg live weight/h for 6 h. Three birds were sacrificed for tissue collection at times 0 h (control), 0.5, 1, 2, 3, 4, 5, 6, 12, 24, 48, 72, 96, 120, 144, 168, and 336 h after the administration of the first dose. Tissue L-[$^{13}C_1$] methionine incorporation mass and percentage results were analyzed using Minitab 16 statistical software. Except for abdominal fat, tissue methionine levels gradually increased after the administration of the methionine solution. The calculated half-lives of methionine in the blood plasma, liver, and breast muscle were 2.52, 1.36, and 3.57 h, respectively, suggesting a greater rate of methionine incorporation in the liver, followed by blood plasma and breast muscle. The isotopic dilution showed that 2.81, 4.79, and 23.64% of the administered L-methionine were retained in the blood plasma, liver, and breast muscle, respectively. The methionine requirements of finisher broilers may be estimated using the carbon isotope technique, and approximately 3, 5, and 24% methionine is used for the synthesis of blood plasma, liver, and breast muscle, respectively, at the evaluated dose.

INTRODUCTION

Due to genetic improvement, modern broilers present greater potential for weight gain and premium cuts yield, particularly breast yield. Their management and feeding has become very complex, and synthetic essential amino acids are commonly added to the feed to supply their requirements.

Methionine, choline, betaine, and folic acid are methyl group donors, and therefore, essential for several metabolic routes. Labile methyl groups participate in the synthesis of amino acids, phospholipids, as well as of DNA and RNA (Saunderson & Mackinlay, 1990). Considering that poultry are not able to synthesize these substances, they must be supplemented in the diet. Methionine is the first limiting amino acid for feathering and body growth (Bertechini, 2006). Under field conditions, methionine is added to broiler diets to supply the specific requirements of this amino acid.

The stable isotope technique has been increasingly applied in animal metabolism and feed digestibility studies. Studies have shown that the isotopic composition of animal tissues depends mainly on ingested feed, water, and inhaled gases, and their associated isotope effects are linked to metabolic processes (Kennedy & Krouse, 1990).

Animal nutrition studies have focused on protein balance, nutrient partition and tissue turnover, and the use of markers. Poultry nutrition studies using stable isotopes in diet as markers produce estimates the rate with which tissue stable isotopes are replaced by those present in the diet (Denadai *et al*., 2007; Gottmann *et al*., 2008; Pelícia *et al*., 2011; Araujo *et al*., 2011; Sernagiotto *et al*., 2013). Labeled compounds can also be used as biological markers (Stradiotti *et al*., 2013).

Therefore, the objective of the present study was to evaluate the incorporation of L-[$^{13}C_1$]methionine in the blood plasma, liver, breast muscle, and abdominal fat of broiler chickens from 35 to 49 days of age, using the carbon stable isotope technique, and therefore, to obtain a better understanding of the dynamics of methyl groups derived from labeled methionine in the nutrient metabolism of broilers.

MATERIALS AND METHODS

This study was carried out at the facilities of the Poultry Nutrition Laboratory of the School of Veterinary Medicine and Animal Science, State University of São Paulo (UNESP), Botucatu campus, Brazil.

At the start of the experiment, 270 one-day-old male Cobb chicks were allocated to nine floor pens (2.5-m^2 area), with 30 birds/pen, where they were reared until 49 days of age. Feed and water were offered *ad libitum* during the entire experimental period.

The chemical composition of the feedstuffs was analyzed at Bromatology Laboratory of UNESP, Botucatu campus, according to AOAC standards (2006) before diet formulation. The experimental feeds were formulated to supply the nutritional requirements of male broilers as recommended by Rostagno *et al*. (2005). The feeding program was divided into four phases: pre-starter (1-7 d), starter (8-21 d), grower I (22-35 d), and grower II (36-49 d). In order to maintain the dietary isotopic signal during the sampling period (35 to 49 days), the grower II diet was formulated to contain similar nutritional levels as those of the grower I diet, except for methionine (Table 1).

At 33 days of age, all birds were weighed, and 51 birds with 2,162.5 g ± 112.5 g average body weight were selected. On day 35, birds were orally fed a solution composed of enriched methionine (L-[$^{13}C_1$] methionine, with isotopic abundance of 92 atm % of ^{13}C; ISOTEC INC., Matheson, USA). The dose was 29 µmol (936,70 µg of ^{13}C) of L-[$^{13}C_1$] methionine/kg live weight/h for 6 h (adapted from Muramatsu *et al*., 1987). For that purpose, enriched methionine was diluted in saline solution at 0.9% in order to obtain 10.874 mg of enriched methionine per 0.6-mL dose.

One dose was administered every hour up to six hours after the first dose was given (time 0). At times 0 (control), 0.5, 1, 2, 3, 4, 5, 6, 12, 24, 48, 72, 96, 120, 144, 168, and 336 h after the administration of the first dose, three birds were electrically stunned and killed. Blood plasma, liver, breast muscle (*Pectoralis major*), and abdominal fat samples were collected to analyze the incorporation labeled methionine in these tissues.

Table 1 – Feedstuff composition and calculated nutritional values.

	Phases			
Ingredients (%)	Pre-starter	Starter	Grower I	Grower II
Corn	56.220	59.320	62.140	62.130
Soybean meal	37.240	34.430	30.840	30.860
Soybean oil	2.070	2.300	3.260	3.270
Dicalcium phosphate	1.950	1.800	1.670	1.670
Limestone	0.940	0.900	0.840	0.840
DL-Methionine	0.217	0.155	0.155	0.134
L-Lysine HCl	0.335	0.185	0.203	0.203
L-Threonine	0.135	0.050	0.050	0.050
Choline chloride	0.060	0.050	0.050	0.050
Anticoccidial agent[1]	0.025	0.025	0.025	0.025
Sodium chloride	0.515	0.495	0.470	0.470
Vit/min supplement[2]	0.300	0.300	-	-
Vit/min supplement[3]	-	-	0.300	0.300
Nutritional Levels				
Metabolizable energy, kcal/kg	2,950	3,000	3,100	3,100
Crude protein, %	22.040	20.790	19.410	19.410
Calcium, %	0.940	0.880	0.820	0.820
Available phosphorus, %	0.470	0.440	0.410	0.410
Methionine, %	0.519	0.447	0.429	0.407
Methionine+Cystine, %	0.940	0.810	0.770	0.770
Lysine, %	1.330	1.150	1.070	1.070
Threonine, %	0.870	0.750	0.700	0.700
K, %	0.590	0.590	0.590	0.590
Na, %	0.220	0.210	0.210	0.210
Cl, %	0.200	0.190	0.180	0.180
Mean Isotopic Values[4]				
$\delta^{13}C$, ‰	-18,8	-18,4	-18,1	-18,1

[1]Monenpac mc 400®: Sodium monensin (40%).

[2]Starter vitamin/mineral supplement TORTUGA® (per kg feed): manganese 559.86 mg, iron 434.37 mg, zinc 433.56 mg, copper 85.65 mg, iodine 5.61 mg, selenium 3.39 mg, vitamin A 106,560 IU, vitamin D3 25,537.5 IU, vitamin E 148.77 mg, vitamin K3 18 mg, vitamin B1 20.1 mg, vitamin B2 45 mg, vitamin B6 24.9 mg, vitamin B12 120 mcg, niacin 300 mg, folic acid 117.45 mg, pantothenic acid 7.5 mg, biotin 0.99 mg, antioxidant 42 mg.

[3]Finisher vitamin/mineral supplement TORTUGA® (per kg feed): manganese 559.85 mg, iron 434.37 mg, zinc 433.56 mg, copper 85.65 mg, iodine 5.61 mg, selenium 3.39 mg, vitamin A 88,054.5 IU, vitamin D3 21,264.23 IU, vitamin E 124.17 mg, vitamin K3 15 mg, vitamin B1 16.74 mg, vitamin B2 37,47 mg, vitamin B6 20,73 mg, vitamin B12 99.9 mcg, niacin 249.75 mg, folic acid 97.74 mg, pantothenic acid 6.27 mg, biotin 0.84 mg, antioxidant 42 mg.

[4]Isotopic mean values expressed as $\delta^{13}C$, ‰ relative to the *PeeDee Belemnite* (PDB) standard.

Due to the high velocity of carbon isotope dilution in tissues with fast metabolic rates, such as blood, blood plasma and liver, sampling times were concentrated around the first hours of the experimental period.

Breast muscle, liver, and abdominal fat samples were dried on a forced-ventilation oven at 56°C for 72 h. Both experimental diets and dried tissue samples were ground in a *cryogenic mill* to obtain a homogeneous material with fine particle size (smaller than 60 µm) (Ducatti, 2007). After grinding, fat was extracted from liver samples for 4 hours in ethyl ether using a Soxhlet extractor.

Isotopic analysis was carried out at the facilities of the Center of Stable Isotopes of the Biosciences Institute, UNESP, Botucatu campus, Brazil.

For the determination of isotopic composition, samples were weighed in tin capsules and submitted to an isotope ratio mass spectrometer (Delta S-Finnigan Mat, Bremen, Germany) coupled to an element analyzer (EA 1108 – CHN – Fisons Instruments, Rodano, Italy). Samples were quantitatively burnt to obtain CO_2.

Minitab 16 Statistical Software (2010) was used to calculate the incorporation speed of L-[$^{13}C_1$] methionine represented by carbon substitution speed, applying an exponential time function in first-order exponential equations. Tissue incorporation percentage of labeled methionine was calculated using the isotopic dilution method.

Results were expressed in $\delta^{13}C$ against the Peedee Belemnite (PDB) standard, with an analytical error of 0.2‰ (equation 1):

$$\delta^{13}C_{(sample,\ standard)} = [(R_{sample}/R_{standard})-1] \times 10^3 \quad (1)$$

Where: $\delta^{13}C$ = relative enrichment of the $^{13}C/^{12}C$ ratio of the sample relative to the PDB standard. Dimensionless.; R = isotopic ratio ($^{13}C/^{12}C$) of the sample and the standard. Dimensionless.

An exponential time function (equation 2) (Ducatti *et al*., 2002), obtained using the method of exponential equations of the first order, was used to quantitatively measure the velocity of tissue carbon substitution by orally administered carbon after a given period of time:

$$\delta^{13}C(t) = \delta^{13}C(f) + \left[\delta^{13}C(i) - \delta^{13}C(f)\right] e^{-kt} \quad (2)$$

Where: $\delta^{13}C$ (t) = tissue isotropic enrichment at any time (t). Dimensionless.; $\delta^{13}C$ (f) = isotopic enrichment of tissue at equilibrium or final condition. Dimensionless.; $\delta^{13}C$ (i) = isotopic enrichment of tissue at starter condition. Dimensionless.; k = turnover constant in units of time^{-1}; t = time (in hours) elapsed since the administration of solutions containing L-[$^{13}C_1$] methionine.

The half-life ($T_{50\%}$) of ^{13}C in the tissues when 50% of the ^{13}C atoms were substituted was measured by (equation 3):

$$T_{50\%} = \ln 2/k \quad (3)$$

Where: T = half-life, time (hours); ln = Napierian logarithm; k = tissue turnover rate constant, time^{-1}, providing an estimate of the velocity of stable isotope exchange in the tissue (Ducatti *et al*., 2002; Ducatti, 2007).

Dry tissue mass and residual carbon percentage of each tissue were obtained for the calculation of tissue isotopic dilution.

The isotopic dilution method is based on the isotopic balance between isotopes of an element in a sample before and after the addition of a material enriched with one of the isotopes. Equations (4 and 5), adapted by Trivelin *et al*. (1994) and Sant Ana Filho (2011), show the isotopic mass balance under the conditions of the current experiment using L-[$^{13}C_1$] methionine:

$$f_{met} \times Ab_{met} + f_{nat} \times Ab_{nat} = (f_{met} + f_{nat}) \times Ab_p \quad (4)$$

$$f_{met} + f_{nat} = 1 \quad (5)$$

Where: f_{met} and f_{nat} = carbon fractions in L-[$^{13}C_1$] methionine and natural sources respectively; Ab_{met}, Ab_{nat} and Ab_p = abundance (% of ^{13}C atoms) in the amino acid L[$^{13}C_1$]methionine, natural sources and tissues (breast muscle, liver, abdominal fat, or blood plasma), respectively.

The mass balance allows to differentiate the contributions of carbon from L-[$^{13}C_1$]methionine and from the natural source for isotope ^{13}C content in the final products. The ^{13}C incorporation rate in the tissues was calculated relative to the dose supplied.

The natural abundance (Ab_{nat}) of isotope ^{13}C was obtained for each fraction or product (blood plasma, liver, and breast muscle) evaluated in the present study.

The following equation is derived from equations 4 and 5:

$$f_{met} = \frac{(Ab_p - Ab_{nat})}{(Ab_{met} - Ab_{nat})} \quad (6)$$

In terms of percentage, the previous equation (6) may be written as:

$$\%\ CPP_{met} = \frac{(Ab_p - Ab_{nat})}{(Ab_{met} - Ab_{nat})} \times 100 \quad (7)$$

Where: % CPP_{met} = percentage of carbon in the product from L-[$^{13}C_1$]methionine.

The amount of carbon in each fraction can be obtained based on the mass of the evaluated product (M_p) (equation 8):

$$CPP_{met} = [(Ab_p - Ab_{nat}) / (Ab_{met} - Ab_{nat})] \times (\%Ct_p) \times (M_p) \quad (8)$$

Where CPP_{met} = carbon in the product derived from L-[$^{13}C_1$]methionine (µg); M_p = total product mass (µg); % Ct_p = percentage of total carbon in the product.

RESULTS AND DISCUSSION

The carbon percentages determined in the breast muscle, liver, abdominal fat, and blood plasma were 41.56, 41.21, 69.44, and 2.71%, respectively. Dry mass weight, using as reference a 2.5-kg broiler, of the breast muscle, liver, and abdominal fat were determined as 133.22, 9.37, and 37.93 g, respectively. Considering that the blood plasma accounts for 5% of the live weight of a broiler (Macari & Luquetti, 2002), a 2.5-kg broiler has 125 g of blood plasma.

Applying those results in equation 8, the following amounts of carbon were determined in the breast muscle, liver, abdominal fat, and blood plasma: 55,365; 9,372; 37,926; and 2,667 mg of carbon, respectively.

Based on the average isotopic values (δ ^{13}C, ‰) of each tissue, obtained by mass spectrometry, Table 2 shows the relationship between isotopic enrichment ($\delta^{13}C$, ‰) of the blood plasma, breast muscle, liver, and abdominal fat and time.

These results shows the (^{13}C) tissue enrichment 336 few hours after the administration of the last dose of the solution containing L-[$^{13}C_1$]methionine to the birds. The isotope data presented in Table 2 shows a gradual change of δ ^{13}C values with time. The peak of blood plasma and liver enrichment was observed 12 hours after of the first administration of the solution containing L-[$^{13}C_1$] methionine. However, in the breast muscle, maximum ^{13}C enrichment was obtained 72 hours after of administration of the first dose, indicating lower carbon isotope exchange compared with the blood plasma and the liver. No changes in abdominal fat isotope signals were detected, indicating that ^{13}C from methionine was not incorporated in this tissue.

After the time of tissue enrichment peak, and depending on the continuity of tissue metabolic synthesis and turnover, a dilution of the isotopic signal was observed, i.e., the ^{13}C concentration from L-[$^{13}C_1$] methionine was reduced. This is completely normal given the discontinuation of the administration of enriched amino acid.

The results of tissue enrichment as a function of time are shown in Figures 1, 2, and 3 for the blood plasma, liver, and breast muscle, respectively. These graphs were built according to the following equations: $\delta^{13}C = -13.01-6.51e^{-0.5094t}$ ($R^2 = 0.998$) with a carbon half-life of 2.52 hours for blood plasma (Figure 1); $\delta^{13}C = -13.01-6.51e^{-0.5094t}$ ($R^2 = 0.937$) with a carbon half-life of 1.36 hours for the liver (Figure 2); and $\delta^{13}C = -17.62-1.86e^{-0.1942t}$ ($R^2 = 0.965$) with a carbon half-life of 3.57 hours for the breast muscle (Figure 3).

Table 2 – Average $\delta^{13}C$ values (mean ± standard deviation) expressed as ‰ of blood plasma (BP), liver (LI), breast muscle (PM), and abdominal fat (AF) of 35- to 49-d-old broilers orally fed a solution containing L-[$^{13}C_1$]methionine, as a function of time.

Sampling time (h)	BP		LI		PM		AF	
0	-19.3	±0.1	-18.7	±0.4	-19.5	±0.2	-21.5	±0.5
0.5	-18.5	±0.3	-17.6	±0.3	-19.3	±0.2	-21.3	±0.3
1	-17.9	±0.2	-17.4	±0.2	-19.0	±0.2	-22.2	±0.3
2	-18.1	±0.6	-16.8	±0.5	-19.2	±0.2	-21.9	±0.4
3	-15.9	±0.4	-15.4	±0.5	-18.6	±0.1	-21.6	±0.2
4	-15.0	±0.3	-14.1	±0.2	-18.4	±0.2	-21.5	±0.1
5	-14.8	±0.9	-13.8	±0.1	-18.4	±0.4	-21.6	±0.3
6	-13.4	±0.4	-13.4	±0.3	-18.1	±0.4	-21.7	±0.4
12	-13.3	±0.1	-13.3	±0.1	-17.9	±0.5	-22.0	±0.1
24	-15.2	±0.5	-15.0	±0.5	-17.8	±0.4	-22.3	±0.2
48	-16.2	±0.3	-16.0	±0.5	-17.8	±0.2	-21.5	±0.5
72	-17.1	±0.2	-16.7	±0.2	-17.7	±0.2	-21.8	±0.1
96	-17.7	±0.4	-17.4	±0.8	-18.0	±0.2	-22.5	±0.3
120	-18.0	±0.4	-17.4	±0.2	-18.0	±0.3	-21.9	±0.4
144	-18.1	±0.4	-17.5	±0.2	-18.1	±0.4	-21.7	±0.1
168	-18.1	±0.2	-17.1	±0.1	-18.0	±0.2	-21.7	±0.1
336	-18.8	±0.2	-18.5	±0.3	-18.5	±0.6	-22.7	±0.4

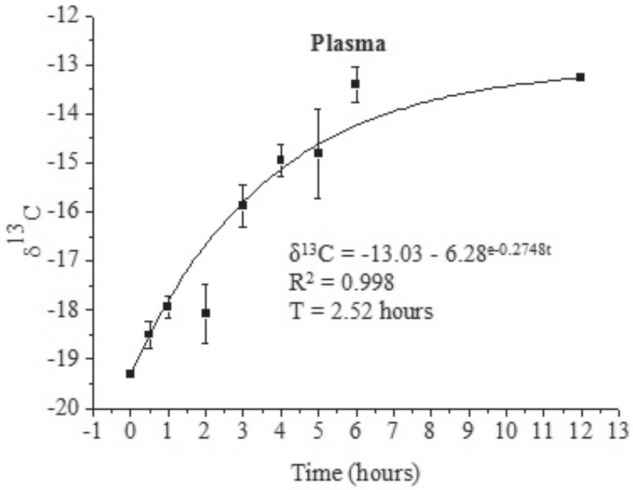

Figure 1 – Exponential model of the period of isotopic ^{13}C enrichment of the blood plasma of 35- to 49-d-old broilers orally receiving a solution containing L-[$^{13}C_1$] methionine.

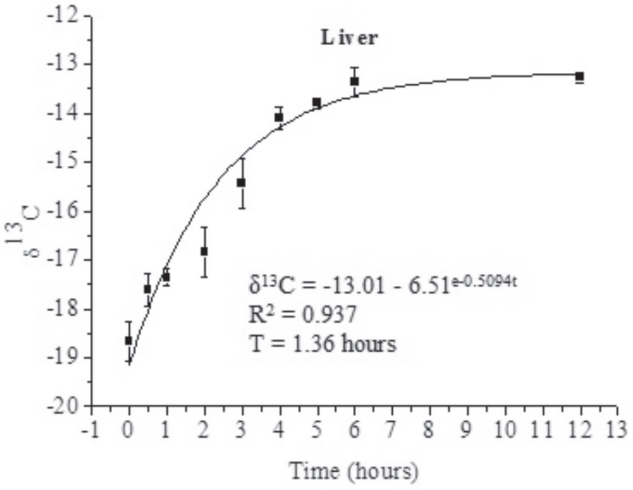

Figure 2 – Exponential model of the period of isotopic ^{13}C enrichment of the liver of 35- to 49-d-old broilers orally receiving a solution containing L-[$^{13}C_1$] methionine.

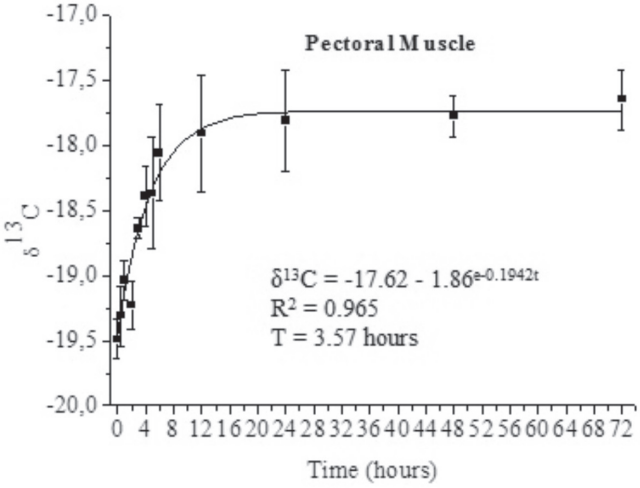

Figure 3 - Exponential model of the period of isotopic ^{13}C enrichment of the breast muscle plasma of 35- to 49-d-old broilers orally receiving a solution containing L-[$^{13}C_1$] methionine.

The percentage of carbon-13 mass derived from L-[$^{13}C_1$] methionine (Table 3) was calculated, and represent the amount of methionine incorporated in each tissue at each time analyzed.

At the time of enrichment peak, 2.81, 4.79, and 23.64% of the dose of L-[$^{13}C_1$] methionine administered were retained in the blood plasma, liver and breast muscle, respectively.

These results indicate that, out of all the enriched methionine retained in the broiler body, about 23.64% is used for breast muscle synthesis, demonstrating the importance of methionine for the formation of this tissue. No incorporation of ^{13}C was detected in abdominal fat, which was expected because the main

Table 3 – Mass (µg) and percentage (%) of ^{13}C incorporated into the blood plasma (BP), liver (LI), and breast muscle (PM) of 35- to 49-d-old broilers as a function of the dose of a solution containing L-[$^{13}C_1$]methionine and time.

Sampling time (h)	Dose* (µg ^{13}C)	^{13}C recovery (µg)			^{13}C incorporation (%)		
		BP	LI	PM	BP	LI	PM
0.5	936.70	22.82	46.41	35.04	2.44	4.95	3.74
1	936.70	38.21	53.90	266.15	4.08	5.75	28.41
2	1873.40	37.15	83.40	209.77	1.98	4.45	11.20
3	2810.10	79.57	132.82	611.50	2.83	4.73	21.76
4	3746.80	110.43	210.95	779.29	2.95	5.63	20.80
5	4683.50	106.35	261.58	914.61	2.27	5.59	19.53
6	5620.20	157.04	262.05	1166.51	2.79	4.66	20.76
12	5620.20	158.62	270.64	1241.58	2.81	4.79	21.97
24	5620.20	98.85	176.38	1205.93	1.75	3.12	21.34
48	5620.20	78.91	152.25	1288.50	1.40	2.69	22.80
72	5620.20	59.01	115.99	1336.09	1.04	2.05	23.64
96	5620.20	50.93	71.98	1196.88	0.90	1.27	21.18
120	5620.20	40.88	70.44	1124.73	0.72	1.25	19.90
144	5620.20	39.05	74.78	1070.51	0.69	1.32	18.95
168	5620.20	39.93	94.92	1080.92	0.71	1.68	19.13
336	5620.20	27.51	30.66	791.75	0.49	0.54	14.01

*Actual amount of ^{13}C in each dose of the orally-administered L-[$^{13}C_1$] methionine solution.

utilization of the ingested amino acids is protein tissue synthesis.

The present study showed that the process of L-methionine incorporation in the tissues of finisher broilers can be determined by the technique of carbon stable isotopes. The velocity and percentage of ^{13}C incorporation are different among tissues. The liver and blood plasma incorporate ^{13}C faster, but at a lower percentage compared with the breast muscle.

Further studies with marked methionine and amino acids are warranted to estimate their incorporation percentages in different broiler tissues and during different rearing phases to evaluate the importance of each amino acid for tissue synthesis, thereby supporting broiler nutrition research.

ACKNOWLEDGMENTS

We would like to thank Fundação de Amparo a Pesquisa do Estado de São Paulo – FAPESP (Process n. 07/57776-0), for grant given to the first author.

ETHICS AND BIOSECURITY COMMITTEE

All the procedures in this study followed the guidelines of the Ethics and Research Committee of the School of Veterinary Medicine and Animal Science, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu campus, SP, Brazil (Protocol n. 139/2007).

REFERENCES

Araujo PC, Sartori JR, Cruz VC, Pezzato AC, Ducatti C, Stradiotti AC, et al. Rastreabilidade de farinha de vísceras de aves por isótopos estáveis em penas de frangos de corte. Pesquisa Agropecuária Brasileira 2011;46(5):538-545.

AOAC International. Official methods of analysis. 17th ed. Gaithersburg: Association of Official Analytical Chemists; 2006.

Bertechini AG. Nutrição de monogástricos. Lavras: UFLA; 2006.

Denadai JC, Ducatti C, Pezzato AC, Carrijo AS, Caldara FR, Oliveira RP. Studies on carbono-13 turnover in eggs and blood of commercial layers. Brazilian Journal of Poultry Science 2007;8:251-256.

Ducatti C. Isótopos estáveis ambientais [apostila]. Botucatu (SP): Instituto de Biociências, Universidade Estadual Paulista; 2007. p. 184

Ducatti C, Carrijo AS, Pezzato AC, Mancera PFA. Modelo teórico e experimental da reciclagem do carbono-13 em tecidos de mamíferos e aves. Scientia Agrícola 2002;59:29-33.

Gottmann R, Pezzato AC, Ducatti C, Denadai JC, Mituo MAO, Móri C, et al. Rastreabilidade de subprodutos de origem animal em dietas com levedura e trigo para frangos. Pesquisa Agropecuária Brasileira 2008;43(12):1641-1647.

Kennedy BV, Krouse HR. Isotope fractionation by plants and animals: implications for nutrition research. Canadian Journal of Physiology and Pharmacology 1990;68:960-972.

Macari M, Luquetti BC. Fisiologia Cardiovascular. In: Macari M, Furlan RL, Gonzales E. Fisiologia aviária aplicada a frangos de corte. Jaboticabal: Funep; 2002. p.279-297.

MINITAB 16 Statistical software [computer software]. Belo Horizonte: Minitab; 2010.

Muramatsu T, Hiramoto K, Tasaki I, Okumura JI. Effect of protein starvation on protein turnover in liver, oviduct and whole body of laying hens. Comparative Biochemistry and Physiology 1987;87(2):227-232.

Pelícia VC, Zavarize KC, Ducatti C, Stradiotti AC, Pezzato AC, Araujo PC, Mituo MAO, Madeira LA, Sartori JR. Nucleotídeos na dieta de frangos de corte e seus efeitos sobre a taxa de turnover da mucosa intestinal antes e após lesões causadas por coccidiose. Ciência Rural 2011;41(9):1652-1659.

Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Ferreira AS, Oliveira RF, et al. Tabelas brasileiras para aves e suínos: Composição de alimentos e exigências nutricionais. Viçosa: UFV; 2005.

Sant Ana Filho CR. Síntese de uréia enriquecida com o isótopo ^{13}C e/ou ^{15}N [tese]. Piracicaba (SP): Universidade de São Paulo; 2011.

Saunderson CL, Mackinlay J. Changes in body weight, composition and hepatic enzyme activities in response to dietary methionine, betaine and choline levels in growing chicks. British Journal of Nutrition 1990;63:339-349.

Sernagiotto ER, Ducatti C, Sartori JR, Stradiotti AC, Maruno MK, Araujo PC, et al. The use of carbon and nitrogen stable isotopes for the detection of poultry offal meal in meat-type quail feeds. Brazilian Journal of Poultry Science 2013;15:65-70.

Stradiotti AC, Ducatti C, Bendassolli JA, Sartori JR, Pelícia VC, Araujo PC, et al. Methionine incorporation into the blood plasma of broiler chickens at the last week of age. Poultry Science Association Annual Meeting Proceedings 2013. Poultry Science 2013; 92(E-Suppl. 1):112.

Trivelin PCO, Lara Cabezas WAR, Victória RL, Reichardt K. Evaluation of a ^{15}N plot design for estimating plant recovery of fertilizer nitrogen applied to sugar cane. Scientia Agricola 1994;51:226-234.