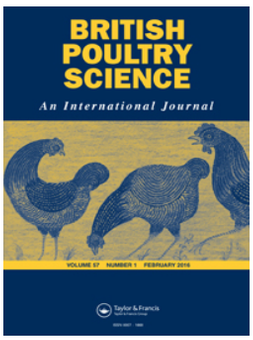

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Performance and carbon turnover in fast- and slow-growing broilers submitted to cyclic heat stress and fed on high-protein diets

D.M.B. CAMPOS, M. MACARI, M.F. FERNADEZ-ALARCON, W.C.L. NOGUEIRA, F.A. DE SOUZA, F.H. HADA, R. LUNEDO, AND J.C. DENADAI[1]

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Abstract 1. Two experiments were conducted to test the hypothesis that when using similar protein/amino acid diets and environment temperature conditions, the performance and carbon turnover in muscle and liver tissues, as measured by the incorporation of stable isotopes ($^{13}C/^{12}C$), must be different between fast-growing Cobb 500® and slow-growing Label Rouge broilers.

2. For both experiments (Cobb and Label Rouge), 21-d-old birds were distributed in a completely randomised, 3 × 3 factorial design; three environmental temperatures (cyclic heat stress *ad libitum*, 22°C *ad libitum*, and 22°C restricted) and three crude protein concentrations (189.1, 210 and 220 g/kg CP) were used.

3. The Cobb 500® had better performance with higher concentrations of crude protein. Cyclic heat stress (a temperature factor), negatively affected this genetic strain's performance. For the Label Rouge birds, the crude protein concentrations in the diet presented inconsistent results and cyclic heat stress did not affect the performance.

4. The carbon turnover rate was affected in the Cobb 500® strain, with a high protein content reducing carbon turnover in the evaluated tissues (liver and muscles). Feed intake had a greater impact on carbon turnover rates than cyclic heat stress. The Label Rouge birds were not affected by the evaluated factors, suggesting that genetic improvement has a leading role on tissue carbon turnover.

5. There is a genetic influence on carbon turnover in the liver and muscle tissues of broiler chickens. In addition, genetically fast-growing broilers are more susceptible to variations in diet composition and environmental temperature than less rapidly growing animals.

INTRODUCTION

Feed efficiency in broilers has increased markedly in recent decades. Havenstein *et al.* (2003*a*, 2003*b*) showed that 85–90% of this increased productivity is due to genetic improvement programmes. Performance improvement, especially in the development of muscle mass, is a function of advances in genetic potential. The greater protein mass in genetically improved chickens (broiler strains) is due to the balance between increased protein synthesis and degradation, i.e., turnover. These mechanisms of synthesis degradation are independent and have different influences and, with increased energy costs, they have become relevant efficiency factors (Doherty *et al.*, 2009).

Broiler rearing in hot environments is the subject of much scientific debate. Heat stress reduces feed intake (FI) and consequently, the animal's performance. Thus, changes in the nutritional composition of feed, such as increased crude protein/amino acids and changing of the diet particle sizes, have been suggested for maintaining productivity in heat stress conditions (Temim *et al.*, 2000*a*, 2000*b*; Gonzalez-Esquerra and Leeson, 2005; Faria Filho *et al.*, 2007).

Incorporation of carbon or nitrogen in tissues has been investigated through the use of stable isotopes. This natural isotopic abundance has

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been used by various authors to measure enrichment changes to the ratio of $^{13}C/^{12}C$ isotopes and infer turnover in different tissues (Martineau *et al.*, 1985; Martins *et al.*, 2012; Ducatti *et al.*, 2014). Furthermore, Tieszen *et al.* (1983) showed that the most metabolically active tissues, such as blood and the liver, rapidly alter isotopic enrichment, and consequently have a higher turnover than less metabolically active tissues, such as skeletal muscle and bone. A better understanding of how carbon incorporates in the different tissues of fast- and slow-growing broilers may aid in the development of better nutritional programmes for mitigating heat stress.

This study tested the hypothesis that when using similar protein/amino acid diets and environmental temperature conditions, the performance and carbon turnover of muscle and liver tissues, as measured by the incorporation of stable isotopes ($^{13}C/^{12}C$), must be different between Cobb 500® (a fast-growing, genetically improved strain) and Label Rouge (slower growing, not genetically improved) broiler strains.

MATERIALS AND METHODS

Birds and experimental design

The birds were raised in three climate-controlled chambers, each 6.0 m × 8.0 m, with concrete floors. The side and top walls consisted of insulating material with 4 exhaust fans. The chambers were equipped with cooling systems and infrared lamps controlled by thermostats. Lighting was 24L:0D (15 lx), with an average relative humidity of 51 ± 2%.

Two experiments, one for each genetic group, were performed. Experiment I used Cobb 500® broilers, a fast-growing genetic strain. This experiment was run from 21 to 45 d of age. Experiment II used Label Rouge naked-neck broilers, a slow-growing genetic group. Experiment II ran from 21 to 55 d, because of the longer maturity rate for these birds. The birds in both experiments received the same diet (Table 1) until 21 d of age and were grown at the temperature recommended for Cobb 500® broilers. At 21 d, the birds were weighed and experimental units were composed with chickens of the same average weight.

A total of 720 male chickens were used for each experiment. The birds were distributed in a completely randomised, 3 × 3 factorial design; three environmental temperatures (cyclic heat stress *ad libitum*, 22°C *ad libitum*, and 22°C restricted) and three crude protein concentrations (189.1, 210 and 220 g/kg CP). There were 9 treatments with 4 replicates of 20 birds each, totalling 36 experimental units in each experiment.

The birds in the cyclic heat stress treatment were subjected to an oscillating temperature scheme of 16 h at 22°C and 8 h at 32°C. For the 22°C restricted treatment; the birds received the same amount of feed as consumed by the birds under the cyclic heat stress *ad libitum* treatment on the previous day. The pair-feeding schedule was used to separate the effects of temperature and FI on the studied variables.

This study was conducted in accordance with the ethical principles for animal experimentation adopted by the Brazilian College of Experimentation (COBEA) and with approval of the local Committee for Ethical Animal Use (CEUA), São Paulo State University (UNESP), Jaboticabal, SP, Brazil.

Experimental diets

For the first 21 d in both experiments, the birds were fed on the same diet based on photosynthetic cycle C_3 plants. After 21 d, they were fed on diets based on photosynthetic cycle C_4 plants. This procedure standardised the isotopic values for the animals to differentiate it from the diet based on C_4 plants. The food ingredients derived from plants of the photosynthetic cycles C_3 and C_4 have different isotopic values, approximately 14‰.

During photosynthetic assimilation, plants with the photosynthetic cycle C_3 fix atmospheric CO_2 by the *Calvin–Benson* cycle and have $\delta^{13}C$ values between −22 and −34‰. Plants with the C_4 cycle fix CO_2 through the *Hatch–Slack* cycle and have values between −9 and −16‰. The negative sign indicates that the plants have lower concentrations of ^{13}C than the international standard. Therefore, C_3 and C_4 plants have different isotopic signatures ($^{13}C/^{12}C$) due to the fractionation that occurs during photosynthetic carbon fixation (Gannes *et al.*, 1998). This natural isotopic ratio difference permits the formulation of diets with different isotopic patterns to be used as natural tracers.

The control diet for both experiments was formulated with nutritional levels suggested by Rostagno (2011). Diets with high protein contents (210 and 220 g/kg CP) presented nutrient concentrations and metabolisable energy similar to the control diet (Table 1). However, in all experimental diets, the supplemented arginine is in excess of the amounts recommended. The arginine supplementation was designed to keep the lysine:arginine (LYS:ARG) relationship at 1.39.

Performance

The following performance indicators were obtained for the two periods from 21 to 45 d of age (Experiment I) and 21 to 55 d of age (Experiment II): FI, weight gain (WG), feed conversion (FC = FI/WG), protein intake (CPI) and protein feed conversion (PFC = CPI/WG). FI and FC

Table 1. *Diet composition (g/kg), calculated nutrient concentrations and values of the isotopic enrichment of the experimental diets*

		Grower (22–45 d)		
Ingredients g/kg	Starter (1–21 d)	189.1 CP	210 CP	220 CP
Maize meal	–	623.6	545.9	514.1
Rice, milled	556.6	–	–	–
Soybean meal, 450 g/kg CP	361.5	282.9	357.5	389.7
Soybean oil	39.8	45.4	58.4	63.2
Limestone	8.4	8.3	8.3	8.3
Dicalcium phosphate	18.0	12.2	11.6	11.3
NaCl	4.9	4.5	4.5	4.5
[1] Vitamin & mineral supplement	5.0	–	–	–
[2] Vitamin & mineral supplement	–	5.0	5.0	5.0
l-Lysine HCl	2.4	3.0	0.8	0.0
dl-methionine	1.8	1.6	1	0.7
l-threonine	1.4	1	–	–
L-arginine	–	4.0	1.9	1.2
Butylated hydroxytoluene (antioxidant)	0.1	0.1	0.1	0.1
Kaolin, inert	0.1	8.4	5	2
Experiment I $\delta^{13}C$ (‰), ($n = 5$)	–28.58	–19.22	–19.57	–22.61
Experiment II $\delta^{13}C$ (‰), ($n = 5$)	–28.45	–18.54	–21.10	–19.89
Calculated nutritional concentrations				
ME, MJ/kg	12.64	13.33	13.33	13.33
Crude protein, g/kg	216.0	189.1	210.0	220.0
Calcium, g/kg	8.7	7.0	7.0	7.0
Phosphorus available, g/kg	4.2	3.3	3.3	3.3
Sodium, g/kg	2.1	2.0	2.0	2.0
Lysine, g/kg	12.5	10.8	10.8	11.0
Methionine, g/kg	6.4	5.4	5.1	4.9
Methionine + cystine, g/kg	9.0	7.9	7.9	7.9
Threonine, g/kg	8.1	7.0	7.1	7.5
Tryptophan, g/kg	2.6	2.0	2.3	2.5
Arginine, g/kg	14.4	15.0	15.1	15.3
Arginine:lysine	1.15	1.39	1.40	1.39
Methionine:lysine	0.51	0.50	0.47	0.45

[1]Guaranteed concentrations per kg of product: nicotinic acid 6930 mg; biotin 32 mg; manganese 15 252 mg; choline 120 mg; calcium pantothenate 1900 mg; retinyl acetate 420 mg; thiamine 356 mg; cyanocobalamin 2000 mcg; riboflavin 1920 mg; pyridoxine 693 mg; cholecalciferol 15 mg; α-tocopherol 5000 mg; menadione 196 mg; copper 25 000 mg; iodine 260 mg; selenium 54.6 mg; zinc 18 250 mg; antioxidant 100 mg; coccidiostat 25 000 mg; DL-methionine (minimum) 340 g; growth and feed efficiency promoter 10 000 mg.

[2]Guaranteed concentrations per kg of product: nicotinic acid 6930 mg; biotin 32 mg; choline 120 mg; manganese 15 252 mg; calcium pantothenate 1900 mg; retinyl acetate 420 mg; thiamine 356 mg; cyanocobalamin 2000 mcg; riboflavin 1920 mg; pyridoxine 693 mg; cholecalciferol 15 mg; α-tocopherol 5000 mg; menadione 196.5 mg; selenium 54.6 mg; copper 25 000 mg; iodine 260 mg; zinc 18 250 mg; antioxidant 100 mg; coccidiostat 22 000 mg; DL-methionine (minimum) 270 g; growth and feed efficiency promoter 10 000 mg.

used the mortality date to calculate the number of adjusted birds (Sakomura and Rostagno, 2007).

Incorporation of ^{13}C in muscle and liver tissues

For the Cobb 500® strain, 4 animals per treatment were killed on d 20, 21, 22, 23, 26, 30, 34, 40 and 45. Next, samples of the *pectoralis major* muscle, *sartorius* muscle and liver were taken. The same was done with 4 birds from the Label Rouge lineage on d 20, 21, 22, 23, 26, 30, 34, 40, 45, 50 and 55. The samples were immediately placed in Falcon tubes, identified and frozen at −20°C for isotopic analysis.

The isotopic analysis was carried out according to Zuanon *et al.* (2006). First, the samples were thawed and dried in a forced air oven (Marconi: Model MA 0351, Brazil) at 56°C for 48 h. Before grinding, only liver samples were defatted in ethylic ether in a Soxhlet apparatus for 4 h, because rich-lipid tissues are poor in ^{13}C as compared to low-lipid tissues (Tieszen *et al.*, 1983). Next, all tissues and experimental diets were ground in a Spex cryogenic mill (Model 6750 freezer/mill) at −196°C. All sample particles were ground finer than 60 µm. Determination of isotopic composition was performed on a Delta-V mass spectrometer (Thermo Scientific) coupled with an Elemental analyser Flash 2000 Organic EA (Thermo Scientific). The results were expressed in notation $\delta^{13}C$, in which the pattern adopted was the *Peedee Belemnite* (PDB), with the analysis error on the order of 0.2‰, according to the equation below.

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

$\delta^{13}C$ = enrichment of the ratio $^{13}C/^{12}C$ of the sample compared to standard PDB (‰);
R = isotope ratio ($^{13}C/^{12}C$) of the sample and standard, dimensionless.

The incorporation of ^{13}C into the tissues was measured using the Ducatti *et al.* (2002) model.

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

$\delta^{13}C_{(t)}$ = isotopic enrichment of carbon in the desired time;
$\delta^{13}C_{(i)}$ = isotopic enrichment in the initial tissue;
$\delta^{13}C_{(f)}$ = isotopic enrichment of carbon at the final;
k = is the carbon exchange constant (d^{-1});
t = unit of time (d).

The half-life ($t = T$) of ^{13}C in the tissues was measured using the following equation:

$$T = \ln 2/k \quad (3)$$

T = half-life time (d);
ln = Napierian logarithm;
the exchange time t (d).

$$t = \left(-\tfrac{1}{k}\right) \ln(1 - F) \quad (4)$$

$F = 0.95$ (95% of atoms exchanged).

Statistical analysis

For performance variables, data were subjected to analysis of variance using the General Linear Model procedure (GLM) of SAS® software (SAS Institute, 2002) and in cases of statistically significant differences, the means were compared by Tukey test at 5% probability. The incorporation of ^{13}C in bird tissues for the trial period was performed by exponential fit of the first order, as proposed by Ducatti *et al.* (2002), using Origin® 6.0 Professional (Microcal Software, 1999).

RESULTS

Performance

Fast-growing Cobb 500® broilers

FI and FC were affected by the diet's crude protein levels (CPL). Birds in the 220 CP treatment had lower FI and FC than animals in other treatments. The animals grown at 22°C *ad libitum* had higher FI than animals raised in 22°C restricted as well as those under cyclic heat stress. Animals raised in cyclic heat stress had lower FC than animals raised in 22°C *ad libitum* and 22°C restricted (Table 2).

For WG, the CPL did not have a significant effect, but temperature did. Birds kept in the 22°C *ad libitum* environment showed higher results than 22°C restricted and cyclic heat stress (Table 2).

Protein intake was affected by the evaluated factors. For CPL, the lowest consumption was observed for the 189.1 CP treatment, the other two treatments did not differ. For temperature, the 22°C treatment *ad libitum* had the highest protein intake, followed by restricted 22°C and cyclic stress (Table 2).

Finally, PFC was affected by both the ration's CPL and the experimental temperatures. Under the CPL factor, the 220 CP had the best conversion rate; the other treatments did not differ. For the temperature factors, the best PFC was observed for cyclic stress, followed by 22°C *ad libitum* and 22°C restricted (Table 2).

Slow-growing label rouge broilers

FI was affected by temperature and CPL (Table 3). Birds fed on 189.1 CP had higher FI under the 22°C *ad libitum* treatment. However, for the other protein concentrations, the temperature scheme did not affect FI ($P > 0.05$). For 22°C *ad libitum*, animals fed on 210 CP showed lower FI when compared to 189.1 CP and 220 CP.

WG was affected by both CPL and temperature. Treatments with 220 CP and 210 CP were different ($P < 0.05$); however, the 189.1 CP treatment did not differ from the others. For temperature factors, the 22°C restricted treatment had the smallest WG ($P < 0.05$), and the cyclic heat stress treatment did not differ from 22°C *ad libitum* (Table 2).

Both temperature and CPL had a significant effect on FC (Table 2). Animals under treatment 210 CP showed the best FC rates, followed by treatments 189.1 CP and 220 CP. For temperature factors, the best FC was found under cyclic heat stress, and treatments 22°C restricted and 22°C *ad libitum* did not differ significantly ($P > 0.05$).

Protein intake was also affected by both factors (CPL and temperature). Increased dietary protein content resulted in a gradual increase in protein intake. As for the temperature factor, the 22°C treatment *ad libitum* had the highest protein intake (Table 2).

The PFC was worse with the increased protein diet ($P < 0.05$). However, for the temperatures studied, the cyclic stress presented the best conversion. The 22°C restricted and 22°C *ad libitum* treatments did not differ (Table 2).

Incorporation of ^{13}C

Fast-growing Cobb 500® broilers

The change in $\delta^{13}C$ values, enrichment equations ($\delta^{13}C$) over time (t) in d and determination coefficient for the liver, *pectoralis major* and *sartorius* muscles are shown in Figure 1 and is further illustrated in Figure 3. Table 4 contains the half-life values (d) from 21 d of age (date of the change in diet), and Table 5 contains the values of the exchange time (d) for 95% of the carbon atoms for the different tissues.

The liver showed the lowest half-life value (Table 4). The half-life values for the *pectoralis major* and *sartorius* muscle were similar for the different factors studied. For liver tissue and muscles, the birds of treatment 189.1 CP showed the lowest half-life in the temperature treatment 22°C *ad libitum* (0.8, 3.6 and 3.0), followed by cyclic heat stress (1.2, 4.7 and 3.6) and 22°C restricted (1.5, 4.9 and 4.2).

For the different tissues and temperature schemes, the treatment with 220 CP had the largest half-life values (Table 4).

The exchange time for 95% of the carbon atoms in the liver for treatment 22°C *ad libitum* with 220 CP was 23.7 d. For the *pectoralis major*, birds of 22°C *ad libitum* with 220 CP and cyclic stress with 220 CP had values of 26.3 and 28.6 d, respectively. As for the *sartorius* muscle, birds raised in 22°C *ad libitum* with 220 CP exchanged 95% of the carbon atoms in 27.9 d. It should be noted that the Cobb 500® experiment period was 24 d (from d 21 to 45), and therefore, the exchange time for 95% of carbon atoms was

Table 2. *Feed intake (FI), weight gain (WG), feed conversion (FC), protein intake (CPI) and crude protein feed conversion (CPFC) of Cobb broilers from 21 to 45 d old and Label Rouge broilers from 21 to 55 d old (mean ± SEM)*

	CPL			Temperature			*P*-value			
Variable	189.1	210	220	Cyclic heat stress	22°C Restricted	22°C *ad libitum*	CPL	Temperature	CPL*Temperature	CV
Experiment I. Cobb 500®										
FI (kg)	3.86 ± 0.06^{a}	3.84 ± 0.05^{a}	3.74 ± 0.04^{b}	3.62 ± 0.0^{c}	3.86 ± 0.02^{b}	3.96 ± 0.04^{a}	0.0001	0.0128	0.1438	2.52
WG (kg)	2.46 ± 0.04	2.46 ± 0.03	2.47 ± 0.02	2.43 ± 0.03^{b}	2.42 ± 0.03^{b}	2.54 ± 0.03^{a}	0.9929	0.0114	0.3269	4.13
FC (kg/kg)	1.57 ± 0.03^{b}	1.56 ± 0.01^{b}	1.52 ± 0.02^{a}	1.49 ± 0.01^{a}	1.59 ± 0.02^{b}	1.56 ± 0.01^{b}	0.0092	0.0001	0.3211	2.54
CPI (kg)	0.73 ± 0.01^{b}	0.81 ± 0.01^{a}	0.82 ± 0.01^{a}	0.75 ± 0.01^{c}	0.79 ± 0.01^{b}	0.82 ± 0.01^{a}	0.0001	0.0001	0.2666	2.51
CPFC (kg/kg)	0.33 ± 0.005^{b}	0.32 ± 0.003^{b}	0.29 ± 0.004^{a}	0.30 ± 0.005^{a}	0.33 ± 0.006^{c}	0.32 ± 0.006^{b}	0.0001	0.0001	0.2713	2.56
Experiment II. Label Rouge										
FI (kg)	4.09 ± 0.05	4.00 ± 0.02	4.08 ± 0.04	4.00 ± 0.03	4.03 ± 0.01	4.15 ± 0.05	0.046	0.0011	0.0365	2.33
WG (kg)	1.96 ± 0.02^{b}	1.98 ± 0.02^{a}	1.90 ± 0.02^{b}	1.98 ± 0.02^{a}	1.89 ± 0.02^{b}	1.96 ± 0.02^{a}	0.0106	0.0018	0.5982	3.17
FC (kg/kg)	2.09 ± 0.02^{b}	2.02 ± 0.02^{a}	2.15 ± 0.02^{c}	2.02 ± 0.02^{a}	2.13 ± 0.02^{b}	2.12 ± 0.02^{b}	0.0001	0.0001	0.3031	2.77
CPI (kg)	0.77 ± 0.009^{c}	0.84 ± 0.004^{b}	0.90 ± 0.08^{a}	0.82 ± 0.018^{b}	0.83 ± 0.017^{b}	0.86 ± 0.014^{a}	0.0001	0.0022	0.0867	2.4
CPFC (kg/kg)	0.39 ± 0.004^{a}	0.42 ± 0.005^{b}	0.47 ± 0.004^{c}	0.42 ± 0.011^{a}	0.44 ± 0.011^{b}	0.44 ± 0.009^{b}	0.0001	0.0001	0.3789	2.81

Means in a row and within a factor with no common superscript letter differ significantly by Tukey test ($P > 0.05$).

Table 3. *Feed intake (kg) as affected by temperature and feeding different concentrations of dietary crude protein to Label Rouge birds from 21 to 55 d of age*

	Crude protein concentrations		
Temperature	189.1 CP	210 CP	220 CP
Cyclic heat stress	3.98 ± 0.034^{aB}	3.99 ± 0.051^{aA}	4.04 ± 0.079^{aA}
22°C Restricted	4.01 ± 0.001^{aB}	3.99 ± 0.003^{aA}	4.07 ± 0.002^{aA}
22°C *ad libitum*	4.29 ± 0.06^{aA}	4.02 ± 0.023^{bA}	4.15 ± 0.078^{aA}

Means in a column with no common uppercase superscript letter or in a row with no common lowercase superscript letter differ significantly by Tukey test ($P < 0.05$).

generally greater than the experimental period. This could have caused errors in the calculation of half-life values.

Slow-growing label rouge broilers

The change in $\delta^{13}C$ values, enrichment equations ($\delta^{13}C$) over time (t) in d, and determination coefficient for liver, *pectoralis major* and *sartorius* muscles are shown in Figure 2 and further illustrated in Figure 3. Half-life values for the liver (Table 4) were lower than those for the *pectoralis major* and *sartorius* muscles for both environmental temperatures and diet protein concentrations. This was similar to the data for the Cobb 500® birds. Also for the liver, the 210 CP treatment in all temperatures showed higher half-life values when compared to other protein concentrations (Table 5). For the muscle tissue, the *pectoralis major* showed slightly higher half-life values than those of the *sartorius* (Table 4).

Unlike the genetically improved birds, the amount of time required to exchange 95% of the carbon atoms for all tissues evaluated was less than the experimental time period (Table 5).

DISCUSSION

The dietary CPL, as well as the feeding schemes in different environmental temperatures, affected performance and carbon incorporation into the liver and muscle tissue of the two genetic groups.

With regard to environmental stress, the genetically improved, fast-growing chickens (Cobb) had lower FI under cyclic heat stress and worse WG and lower crude protein consumption

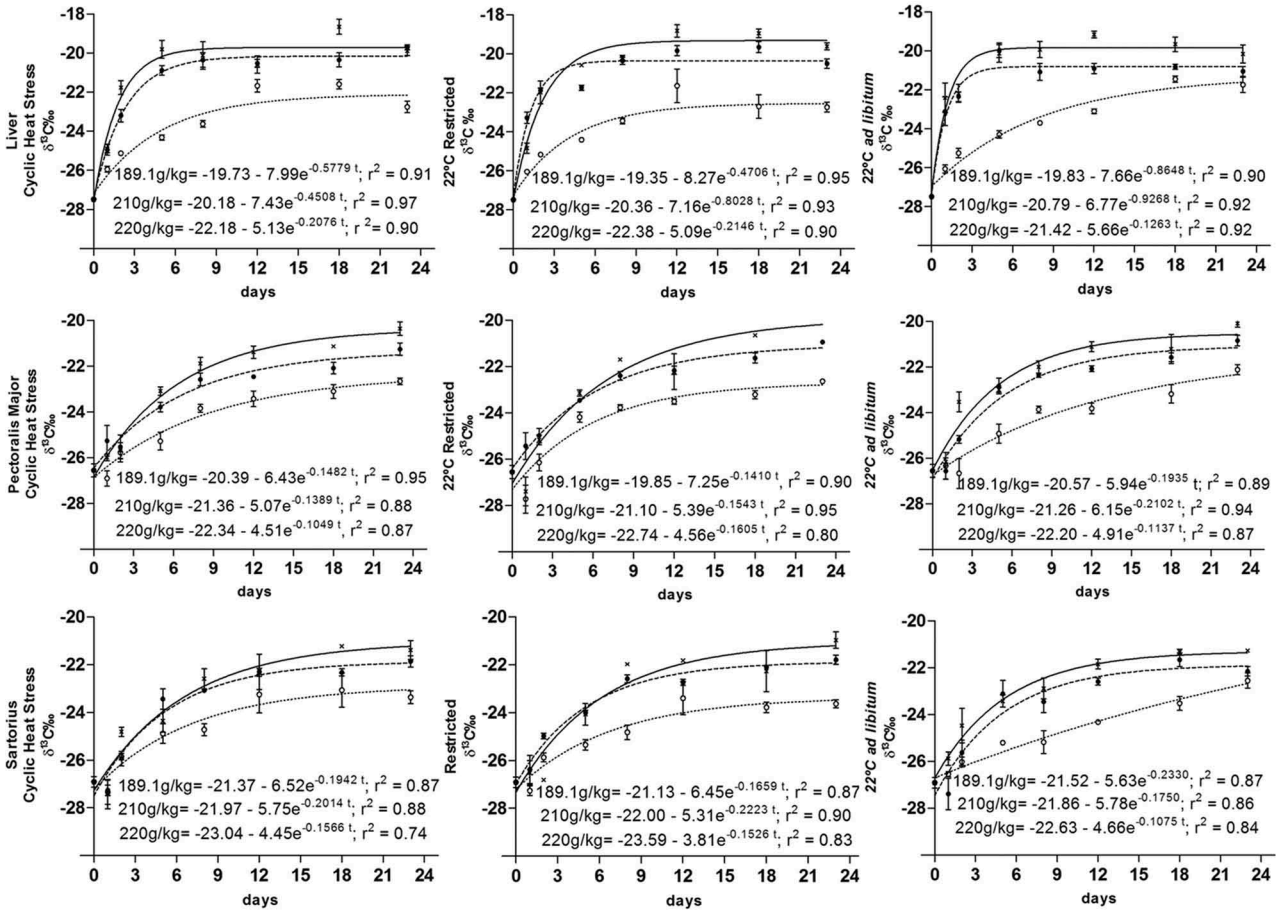

Figure 1. *Stable carbon-isotope exponential models of liver, pectoralis major and sartorius muscles of Cobb broilers submitted to experimental treatments from 21 to 45 d of age, enrichment equations ($\delta^{13}C$) over time (t) in d and determination coefficients. Data are means ± SEM and sample sizes are n = 4 for each point.*

Table 4. *Half-life values (d) for liver tissue, pectoralis major and sartorius muscles for Cobb broilers 21 to 45 d of age and Label Rouge broilers 21 to 55 d of age*

			CPL					
			189.1 CP		210 CP		220 CP	
Tissue		Temperature	Cobb 500®	Label Rouge	Cobb 500®	Label Rouge	Cobb 500®	Label Rouge
Liver		22°C *ad libitum*	0.8	1.9	0.7	2.8	5.5	2.2
		22°C Restricted	1.5	2.1	0.9	2.4	3.2	2.3
		Cyclic heat stress	1.2	1.9	1.5	2.4	3.3	1.6
Muscle	*Pectoralis major*	22°C *ad libitum*	3.6	7.7	3.3	6.9	6.1	6.1
		22°C Restricted	4.9	6.4	4.5	6.4	4.3	6.7
		Cyclic heat stress	4.7	6.4	5.0	7.2	6.6	6.6
	Sartorius	22°C *ad libitum*	3.0	5.0	4.0	6.7	6.4	4.2
		22°C Restricted	4.2	5.1	3.1	4.7	4.5	5.2
		Cyclic heat stress	3.6	5.0	3.4	6.1	4.4	6.0

Table 5. *Length of time (d) to replace 95% of the carbon atoms in the liver, pectoralis major and the sartorius muscles, for Cobb broilers from 21 to 45 d of age and Label Rouge from 21 to 55 d of age*

			CPL					
			189.1 CP		210 CP		220 CP	
Tissue		Temperature	Cobb 500®	Label Rouge	Cobb 500®	Label Rouge	Cobb 500®	Label Rouge
Liver		22°C *ad libitum*	3.5	8.2	3.2	12.0	23.7*	9.7
		22°C Restricted	6.4	9.1	3.7	10.3	14.0	9.9
		Cyclic heat stress	5.2	8.3	6.6	10.3	14.4	6.7
Muscle	*Pectoralis major*	22°C *ad libitum*	15.5	33.4	14.3	29.8	26.3*	26.2
		22°C Restricted	21.2	27.6	19.4	27.9	18.7	28.9
		Cyclic heat stress	20.2	27.8	21.6	31.1	28.6*	28.6
	Sartorius	22°C *ad libitum*	12.9	21.6	17.1	28.9	27.9*	18.2
		22°C Restricted	18.1	22.2	13.5	20.4	19.6	22.5
		Cyclic heat stress	15.4	21.7	14.9	26.3	19.1	26.0

*Change time (d) in excess of the experimental period.

when compared to the animals grown under 22°C and fed *ad libitum*. This performance reduction was also found by Zhang *et al.* (2012) in Arbor Acres broilers (also fast growing), between 4 and 6 weeks of age and subjected to a constant heat stress of 34°C, cyclic stress (6 h of daily stress at 36°C), or thermoneutral temperature (23°C). The cyclic and constant heat stress reduced WG 8.1 and 18.2%, respectively, when compared to the thermoneutral temperature. In this study, there was a WG reduction of 4.33% in Cobb for cyclic heat stress treatments and 22°C restricted (isolating the temperature effect) when compared to the animals in thermoneutral conditions. These findings suggest that for genetically improved strains, the duration of the heat stress is an important factor for body weight reduction, as the longer the period of stress is, the greater the body weight loss.

The cyclic heat stress did not affect the performance of the slow-growing Label Rouge birds. These findings show higher heat resistance for these animals and corroborate data from other authors (Rosa *et al.*, 2007; When *et al.*, 2007).

When the amount of CPL was increased from 189.1 to 220 CP, a reduction in FI was observed in the Cobb line. This was accompanied, however, by increased protein intake, FC and PFC.

Increasing CPL has been suggested for heat-stressed broilers, because it can compensate for the reduced intake of amino acids important for growth that results from the decreased feed consumption of heat stressed birds (Gonzalez-Esquerra and Leeson, 2005). The present findings show that diets with higher CPL did enable the animals to have a higher intake of amino acids, with better FC and better protein conversion. Thus, despite lower food intake and no difference in WG, our data suggest that metabolic efficiency was higher in these animals. Other research with high CPL and heat stress conditions has also showed improved performance for fast-growing broiler lines (Temim *et al.*, 1999, 2000*a*, 2000*b*; Faria Filho *et al.*, 2007).

For isotopic analysis, the half-life values (T) of ^{13}C depend on, in part, tissue synthesis or degradation, and even re-synthesis of certain molecules. The average composition of the skeletal muscle and liver are 18.5 and 18.7% of protein, 3 and

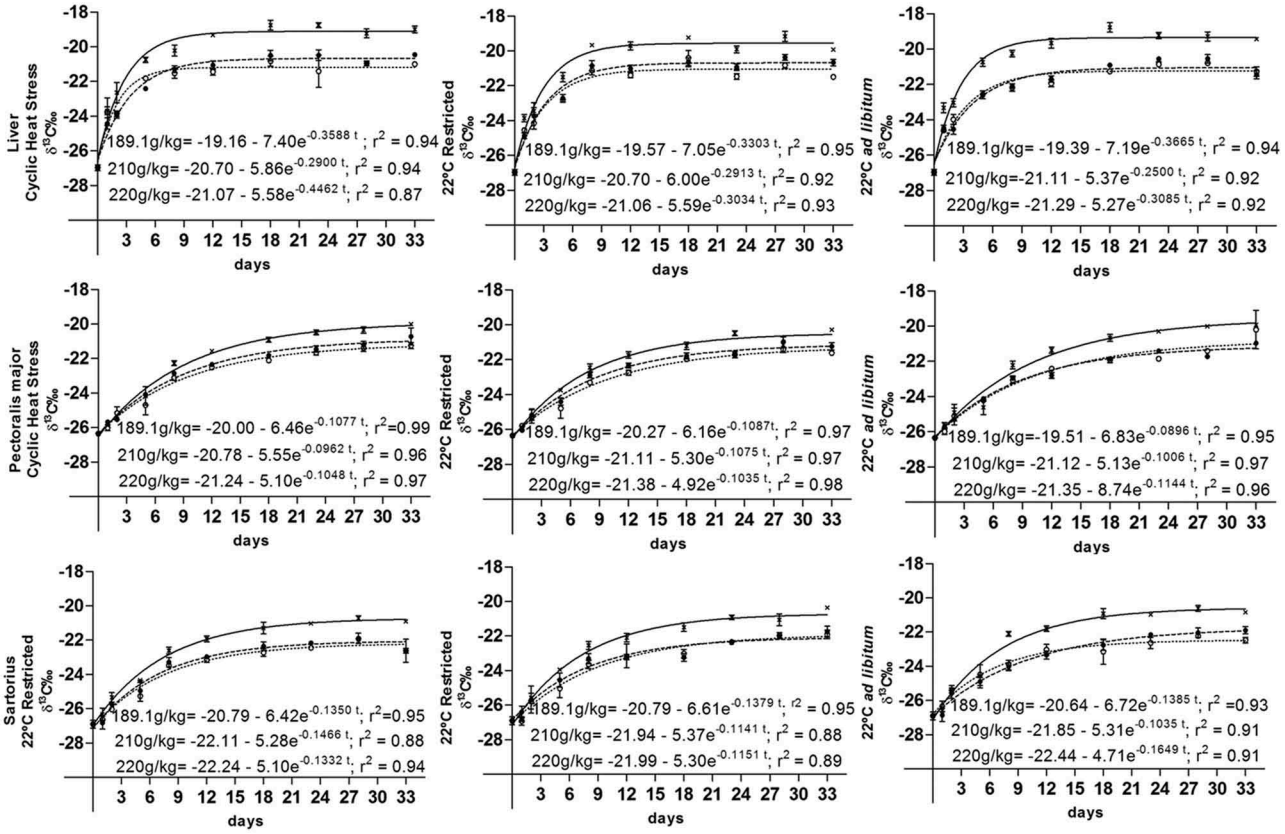

Figure 2. *Stable carbon-isotope exponential models of liver, pectoralis major and sartorius muscles of Label Rouge birds submitted to experimental treatments from 21 to 55 d of age, enrichment equations ($\delta^{13}C$) over time (t) in d and determination coefficients. Data are means ± SEM and sample sizes are n = 4 for each point.*

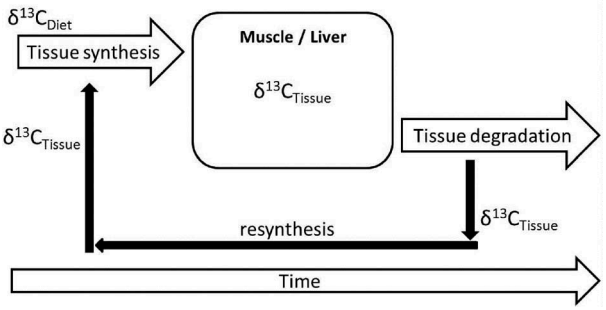

Figure 3. *Illustration of isotopic enrichment factors of ^{13}C over time in evaluated tissues.*

15.4% fat and 0.8 and 8.2% carbohydrate, respectively. Thus, it could be assumed that changes in the ^{13}C incorporation rate reflect the changes in carbon turnover of the evaluated tissues (Cruz *et al.*, 2005; Martins *et al.*, 2012).

The half-life values for ^{13}C in the liver of the improved Cobb strain were 0.8 *vs* 1.5 d for broilers fed on 189.1 CP raised at 22°C *ad libitum* and 22°C restricted, respectively (FI effect). For the temperature effect, the half-life of the liver's carbon showed little difference in chickens raised in cyclic heat stress *vs* 22°C *ad libitum*; 1.2 *vs* 1.5 d, respectively. Thus, these findings reveal that temperature and FI determine different patterns of carbon turnover in the liver tissue of genetically improved chickens.

This same pattern was observed for the *pectoralis major* and *sartorius* muscles. However, for the non-genetically improved Label Rouge birds, this pattern was not observed. This suggests that genetic improvement has a leading role on tissue carbon turnover, probably due to the metabolic activity of these tissues (Tieszen *et al.*, 1983), and FI has a greater impact on turnover rate than cyclic heat stress, but only in improved lines.

Studies on increasing CPL in hot-climate broiler diets suggest that this can improve performance; however, nothing has been described about carbon turnover in broilers fed on diets from C_3 and C_4 plants and the use of the stable carbon isotope technique. Our data show that when increasing the diet's CPL (220 CP), the carbon half-life in both liver and muscle is increased (Cobb strain only), on average 0.8–5.5 d for the liver tissue, 3.6–6.1 d for the *pectoralis major* muscle, and 3.0–6.4 d for *sartorius* muscle, for birds reared under 22°C and fed *ad libitum*. These data show that the carbon turnover in muscle and liver tissue of genetically improved poultry is reduced, suggesting a reduced metabolic activity.

It has been suggested that the better performance of genetically improved broilers fed on high-protein diets and raised at high environmental temperatures is due to proteolysis reduction (Temim *et al.*, 2000*b*). Our data revealed that carbon turnover was reduced when dietary protein was increased; giving evidence that tissues development is not only based on proteolysis, but also on synthesis and re-synthesis; however, based on the data of this study, it is not possible to quantify how much the carbon turnover in tissue is influenced by re-synthesis. Thus, further studies are needed to elucidate these issues.

In conclusion, the results support genetic influence on carbon turnover in the liver and muscle tissues of broiler chickens. Also, genetically improved broilers are more susceptible to variation in diet composition and environmental temperature than genetically non-improved animals.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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