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Effect of Protein, Carbohydrate, Lipid, and Selenium Levels on the Performance, Carcass Yield, and Blood Changes in Broilers

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ABSTRACT

The objective of this study was to evaluate the performance, carcass and parts yield, and blood changes in broilers fed different protein, carbohydrate, and lipid levels. Birds were fed a commercial diet until seven days of age. On day 8, birds were distributed according to a completely randomized experimental design in a 4 x 2 factorial arrangement (control diet, low protein diet, low carbohydrate diet or low lipid diet vs. supplementation of 0 or 0.3ppm organic selenium) with four replicates of 15 birds each. Broilers fed low protein presented lower body weight, feed intake, and worse feed conversion ratio on day 42, as well as lower carcass and breast yields, higher leg and abdominal fat yields, higher triglyceride and lower uric acid blood levels. Broilers fed the low carbohydrate diets presented low glucose levels on days 14 and 42.Creatine-kinase (CK) levels increased as birds aged. The livability of broilers fed the low protein diets improved and of those fed low carbohydrate diets worsened with dietary selenium addition on days 35 and 42. Selenium supplementation increased glucose levels in 42-d-old broilers. Changes in dietary protein caused more impact on broiler performance compared with carbohydrates and lipids. Changes in macronutrients caused metabolic changes in broilers. Selenium affected broiler livability as measured on days 35 and 42, and glucose blood levels.

INTRODUCTION

Nutrients are chemical compounds present in all foods, and are synthesized by the body. Essential nutrients are those that cannot be synthesized by the body, and therefore, must be supplied by the diet. These include amino acids derived from protein digestion, fatty acids from fats and oils, and vitamins and minerals.

All nutrients are divided in two well-defined classes: macronutrients and micronutrients. Those required in large quantities, such as protein, lipids, and carbohydrates, are classified as macronutrients, and their main functions are to supply energy and the compounds required for body growth, maintenance, and activity. On the other hand, micronutrients are required in small amounts, from milligrams to micrograms.

There are several studies reported in literature on feed formulation, and many focus on the influence of macronutrient ratios (protein, carbohydrate, lipid) on the live performance of broilers (Dairo *et al.*, 2010; Hosseini-Vashan *et al.*, 2010). The amount and ratio between metabolizable energy and protein levels in the diet have a strong influence on broiler performance. High energy to protein ratios increase fat deposition, but when dietary protein content is increased, performance is less efficient and there is higher nitrogen excretion (Malheiros *et al.*, 2004).

Despite the large number of studies on this subject, only a few aimed at identifying the physiological mechanisms responsible for the performance results. Many of these studies focused on energy and nitrogen metabolism, intermediate metabolism, and endocrine function (Malheiros *et al.*, 2003a; Swennen *et al.*, 2005), but few take into consideration the effect of changing the concentration of a specific macronutrient on the other nutrients, which makes it difficult to determine the individual effects of that nutrient (Buyse *et al.*, 2001). The studies with broilers often manipulate iso-energy diets replacing one macronutrient by another macronutrient, while the third macronutrient level is maintained (Collin *et al.*, 2003; Malheiros *et al.*, 2003a,b; Swennen *et al.*, 2004, 2005, 2010, 2011). This paired substitution is based on the assumption that the effect of the macronutrient on dietary energy content can be isolated, allowing a precise investigation on its effect on bird performance (Poppit *et al.*, 1998; Buyse *et al.*, 2001).

Selenium is a trace mineral that is essential for human and animal nutrition, as it is a component of at least 25 selenoproteins, which are involved in several physiological functions, such as growth, reproduction, and immunity (Surai, 2002). This trace mineral has a very versatile reduction-oxidation capacity, and it acts on the active center of the enzyme glutathione-peroxidase, which function is to inactive free radicals. It is also part of other enzymes, such as iodothyronine deiodinase, responsible for the conversion of thyroxine into its active form, triiodothyronine (McDonald *et al.*, 2002; Ortolani, 2002; Surai, 2006).

When an unbalance between the production of free radicals and the body's capacity to inactive them occurs, there is lipid peroxidation, which is a process that damages the lipids of the cell membrane, resulting in cell failure. In gastrointestinal cells, it impairs nutrient absorption, negatively affecting animal performance (Pappas *et al.*, 2008).

Selenium deficiency is associated to the development of several diseases, such as exudative diathesis and nutritional encephalomalacia, liver and pancreas disorders, poor live performance, reproductive disorders, and economic losses related to morbidity and mortality (Leng *et al.*, 2003). Therefore, it would be interesting to evaluate the effect of dietary selenium supplementation when macronutrients are changes on broiler performance.

The objective of the present experiment was to evaluate the performance, carcass traits, and possible metabolic changes of broilers fed diets with different macronutrient levels and supplemented or not with selenium.

MATERIALS AND METHODS

A number of 480 one-d-old male Cobb-500® broilers were fed until seven days of age with a commercial diet containing the nutritional levels recommended by the strain manual. On day 8, birds were distributed according to a completely randomized experimental design in a 4 x 2 factorial arrangement (control diet, low protein diet, low carbohydrate diet or low lipid diet

Table 1 – Ingredients and calculated nutritional composition of the experimental diets, according to treatments.

	Experimental diets			
Ingredients	Control	Low protein	Low CHO	Low lipid
Corn	59.19	74.65	46.02	36.80
Soybean meal (45%)	32.00	13.74	34.40	30.61
Soybean oil	2.31	1.05	6.70	1.00
Gluten (60%)	1.00	1.00	1.00	5.00
Starch	1.00	1.00	1.00	21.87
Dicalcium phosphate	2.28	2.39	2.32	2.37
Limestone	1.06	1.09	1.02	1.00
Salt	0.35	0.35	0.35	0.35
Kaolin	----	2.30	6.20	----
BHT	0.01	0.01	0.01	0.01
Mineral and vitamin suppl.[1]	0.50	0.50	0.50	0.50
DL-methionine	0.24	0.43	0.25	0.22
L-lysine	0.05	0.80	0.12	0.24
L-threonine	0.01	0.29	0.11	0.03
L-tryptophan	----	0.04	----	----
L-arginine	----	0.36	----	----
Total	100	100	100	100
	Calculated composition			
Metabolizable energy (kcal/kg)	3000	3000	3000	3000
Crude protein (%)	20	13	20	20
Ether extract (%)	5.00	4.00	8.98	3.00
Ca (%)	1.00	1.00	1.00	1.00
Available P (%)	0.50	0.50	0.50	0.50
CHO+starch (%)	41.90	49.30	34.00	45.09
Ash (%)	6.34	5.61	6.32	6.11
Fiber (%)	3.10	2.19	3.03	2.67
Threonine (%)	0.80	0.80	0.80	0.80
Met+cys (%)	0.90	0.90	0.90	0.90
Tryptophan (%)	0.26	0.19	0.27	0.25
Lysine (%)	1.10	1.20	1.20	1.20
Arginine (%)	1.33	1.15	1.35	1.26

[1] Mineral and vitamin supplement (levels kg product) – Vitamin A 2,500,000 U.I., Vitamin D3 500,000 U.I., Vitamin B12 3,000 mcg, folic acid 150 mg, biotin 13 mg, niacin 8,750 mg, calcium pantothenate 2,800 mg, cobalt 25 mg, copper 1,500 mg, iron 12,500 mg, iodine 250 mg, manganese 16,250 mg, selenium 50mg, zinc 11,250 mg, choline chloride 50% 175,000 mg, DL-methionine 375,000 mg, growth promoter 20,000 mg, coccidiostat 20,000 mg, antioxidant 2,000mg, Vitamin E 3,700 mg, Vitamin K3 625 mg, Vitamin B1 375 mg, Vitamin B2 1,250 mg, Vitamin B6 375 mg.

vs. supplementation of 0 or 0.3ppm organic selenium) with four replicates of 15 birds each. The experimental diets are shown in Table 1. A split-plot design in a factorial arrangement, with time as subplot, was used for the analysis of metabolites.

Birds were housed in 3.75-m^2 pens, with wood-shavings litter and equipped with a tube feeder and a bell drinker, inside an experimental broiler house. A lighting program of 24 hours of light was adopted during the entire experiment. Water and feed were supplied *ad libitum*. Birds were vaccinated according to the program established for the region.

On days 14, 21, 28, 35, and 42, always at the same time, 5mL of blood were collected by puncture of the brachial of 10 birds per treatment using syringes with heparin. Blood samples were centrifuged at 3000rpm for 10 minutes at 4°C, and the sera were frozen until analysis.

The following blood metabolites were analyzed: glucose, creatine-kinase (CK), uric acid, and triglycerides, using commercial kits (LABTEST), according to the techniques described by Malheiros *et al.* (2003a). Samples were read in a spectrophotometer (LABQUEST, semi-automatic spectrophotometer), with the wavelength required for each test.

On day 42, 10 birds per treatment were randomly selected for carcass trait evaluation. Broilers were identified and fasted for six hours. Birds were then weighed, sacrificed by neck dislocation, bled, plucked, and eviscerated. Commercial parts were cut to determine carcass yield, breast yield, leg (drumstick+thigh) yield, and abdominal fat (fat located around the cloaca and gizzard) percentage.

Weight gain, feed intake, feed conversion ratio, live weight, and livability were evaluated for the periods of 7-28, 7-35, and 7-42 days of age.

SAS® statistical package (SAS, 2002) was used for data analyses. Data were evaluated for the assumptions of normality of the studentized residuals, homogeneity of variances, and matrix sphericity of the subplot data. The outliers identified were removed. Means were compared by the test of Tukey ($p < 0.05$).

The following parameters were transformed: uric acid and creatine-kinase to log(10), triglyceride to square root, and glucose as to its inverse (1/glucose). Tables and figures present the original, non-transformed, means.

Using the test of Mauchly, uric acid data did not comply with the assumption of matrix sphericity ($p = 0.0196$), and the procedure PROC MIXED of SAS® (SAS, 2002) showed that the Toeplitz covariance matrix provided the best fit.

RESULTS

Diets significantly ($p < 0.0001$) influenced body weight at the evaluated ages, as shown in Table 2. The birds fed the low-protein diets presented lower body weight compared with the other treatments in all studied periods. At 42 days of age, those fed the low-carbohydrate diets were heavier than those fed the low-lipid diet.

Table 2 – Weight gain, feed intake, feed conversion ratio, and livability of broilers during the evaluated periods

Factors	Evaluated parameters											
	Weight gain (g)			Feed intake (g)			FCR (g/g)			Livability (%)		
Feed (F)	7-28	7-35	7-42	7-28	7-35	7-42	7-28	7-35	7-42	7-28	7-35	7-42
Control	1373 a	1970 a	2639 ab	1893 a	2994 a	4278 a	1.56 a	1.65 a	1.72 a	100.0	99.2	99.2
Low protein	1116 b	1589 b	2093 c	1774 b	2766 b	3856 b	1.85 b	1.93 b	2.00 b	99.2	97.5	97.5
Low carbohydrate	1354 a	1958 a	2684 a	1840 ab	2982 a	4342 a	1.52 a	1.66 a	1.72 a	98.3	97.5	97.5
Low lipid	1330 a	1889 a	2537 b	1827 ab	2896 ab	4153 a	1.56 a	1.68 a	1.75 a	99.2	99.2	99.2
Selenium (Se)												
0.3 ppm	1307	1875	2512	1852	2936	4191	1.62	1.72	1.79	99.1	98.8	98.8
0 ppm	1280	1828	2465	1816	2883	4123	1.63	1.74	1.80	99.1	97.9	97.9
	Probability											
F	<0.0001	<0.0001	<0.0001	0.0047	0.0007	0.0001	<0.0001	<0.0001	<0.0001	0.5061	0.2869	0.2869
Se	0.0820	0.0749	0.1484	0.0936	0.1629	0.2709	0.1097	0.1346	0.3668	1.0000	0.3273	0.3273
F x Se	0.5684	0.8308	0.7701	0.5771	0.4758	0.3634	0.7869	0.9152	0.5389	0.0928	0.0026	0.0026
CV (%)	3.27	3.86	3.65	3.17	3.60	3.96	1.70	1.96	1.90	2.16	2.39	2.39

Means followed by different letters in the same column are different by the test of Tukey (5%).

Feed intake of the broilers fed low-protein was lower compared with those fed the control diet on day 28, the low-carbohydrate diet and control diet on day 35, and those fed all the other diets on day 42.

There was a significant ($p < 0.001$) effect of diet on feed conversion ratio at all ages (Table 2). The low-protein diet resulted in the worst feed conversion ratio, whereas the control, low-carbohydrate, and low-lipid diets were not different.

There was no effect of selenium on body weight, feed intake, or feed conversion ratio. However, there was a significant interaction ($p = 0.0026$) between diet and selenium supplementation when broilers were 35 and 42 days old. Relative to the factor diet, the low-protein diet promoted significantly higher livability when it was supplemented with selenium (Se), whereas the low-carbohydrate diet resulted in higher livability when it was not supplemented with Se. Considering the factor Se supplementation, the low-carbohydrate diet with no Se supplementation resulted in higher livability when compared with the low-protein diets, and an opposite effect was obtained when it was supplemented with Se.

Because no birds died in the period of 35-42 days of age, there no changes in livability values when compared with the period of 7-35 (Table 2, Figure 1). Therefore, the table of the details of effects of the interaction on this parameter is not presented.

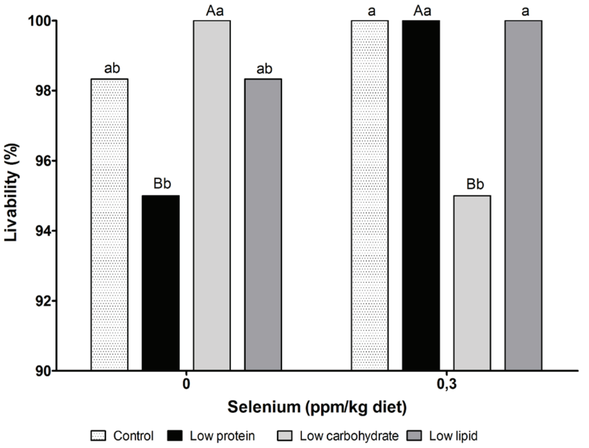

Figure 1 – Details of the interaction between feed and selenium of 35-d-old broilers. Means followed by the same uppercase letters for feed and lowercase letter for Se levels are not different.

Parts yield and abdominal fat were significantly influenced by feed (Table 3). The birds fed the low-lipid diets obtained better carcass yield results compared with those supplied with the low-protein diet. Low-protein diets resulted in lower breast yield, and higher leg yield and abdominal fat percentage.

Table 3 – Carcass, breast, leg, and abdominal fat (AF) yields of 42-d-old broilers.

Factors	Analyzed parameters[1]			
	Carcass	Breast	Leg	AF
Feed (F)		%		
Control	71.59 ab	34.61 a	30.24 b	1.36 b
Low protein	69.90 b	31.24 b	31.95 a	2.17 a
Low carbohydrate	70.76 ab	34.65 a	30.12 b	1.08 b
Low lipid	72.02 a	35.30 a	30.04 b	1.28 b
Selenium (Se)				
0.3 ppm	71.11	33.69	30.69	1.56
0 ppm	71.02	34.21	30.48	1.39
	Probability			
F	0.0291	< 0.0001	0.0009	< 0.0001
Se	0.8661	0.2242	0.5622	0.0560
F x Se	0.0857	0.0853	0.2618	0.7976
CV (%)	3.14	5.24	5.01	25.78

Means followed by different letters in the same column are different by the test of Tukey (5%).[1] Carcass and abdominal fat yields are expressed relative to live weight; parts yields are expressed relative to carcass weight.

The details of the interaction between feed and age for glucose are shown in Figure 2-A. Within the age factor, 14-d-old broilers fed the control and the low-protein diet presented higher blood glucose concentration compared with those fed the low-carbohydrate diet. On day 42, the glucose levels of birds fed the low-protein diet were higher relative to those fed the low-carbohydrate diets. At the other evaluated ages, there were no significant differences among the feeds. Considering feeds, the control diet promoted higher glucose levels in 14-d-old broilers, which were significantly different from those obtained on day 28.

The details of the effect of interaction between selenium and age on blood glucose levels (Figure 2-B) show that Se addition significantly increased glucose levels in 42-d-old birds. Considering the age factor, the lowest glucose level was obtained in 28-d-old birds fed Se-supplemented diets, and in 42-d-old birds when Se was not added to the diets.

The low-protein diets resulted in lowest triglyceride levels (Table 4). In addition, triglyceride blood levels decreased as birds aged.

The details of the interaction between feed and age for glucose are shown in Figure 2-A. Within the age factor, 14-d-old broilers fed the control and the low-protein diet presented higher blood glucose concentration compared with those fed the low-carbohydrate diet. On day 42, the glucose levels of

birds fed the low-protein diet were higher relative to those fed the low-carbohydrate diets. At the other evaluated ages, there were no significant differences among the feeds. Considering feeds, the control diet promoted higher glucose levels in 14-d-old broilers, which were significantly different from those obtained on day 28.

The details of the interaction between feed and age for creatine-kinase (CK) levels are shown in Figure 2-D. Considering the factor age, CK levels increased with bird age, independently of treatment. Relative to age, 21-d-old broilers fed the low-lipid diet presented lower CK blood levels compared with those fed the low-carbohydrate and the control diets.

Table 4 – Blood parameters (glucose, triglycerides, uric acid, and creatine-kinase (CK)) of 14-, 21-, 28-, 35-, and 42-d-old broilers

Factors	Evaluated parameters (mg/dL)			
	Glucose	Triglyceride	Uric acid	CK
Feed (F)				
Control	240.41	94.56 b	7.35	5128
Low protein	247.67	127.65 a	5.44	3283
Low carbohydrate	237.10	92.14 b	7.83	4425
Low lipid	239.86	90.77 b	7.51	4473
Selenium (Se)				
0.3 ppm	242.22	97.96	7.01	4301
0 ppm	240.30	104.60	7.06	4353
Age (A)				
14	246.00	114.42 ab	7.89	559
21	248.38	115.37 a	7.26	1747
28	230.84	97.63 bc	7.68	3827
35	242.06	95.63 c	6.09	5623
42	239.02	83.35 d	6.23	9880
	Probability			
F	0.0047	< 0.0001	< 0.0001	0.0505
Se	0.3638	0.1290	0.7012	0.5668
A	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F x Se	0.7283	0.3126	0.7275	0.0966
F x A	0.0005	0.0600	< 0.0001	< 0.0001
Se x A	0.0451	0.7567	0.6407	0.6311
F x Se x A	0.0801	0.5108	0.0250	0.0132
CV plot (%)	6.24	15.41	7.42	6.52
CV subplot (%)	6.43	14.84	9.58	5.67

Means followed by different letters in the same column are different by the test of Tukey (5%).

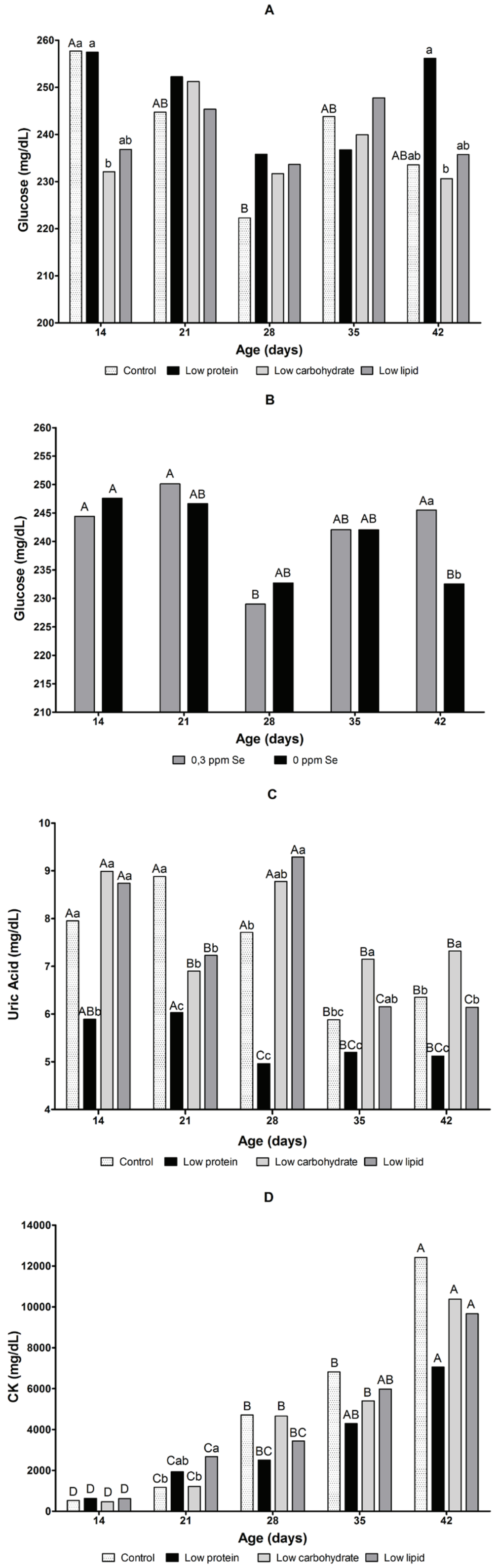

Figure 2 – Means of the details of the interaction between feed and age for glucose blood level (A), between selenium and age for blood glucose level (B), between feed and age for uric acid level (C), and between feed and age for CK levels (D).Means followed by the same uppercase letters for feed and lowercase letter for age are not different (A, C, D).Means followed by the same uppercase letters for feed and lowercase letter for Se levels are not different (B).

DISCUSSION

Considering the periods evaluated in this study, in general, the broilers fed low-protein diets presented worse performance compared to those fed the control, low-carbohydrate, and low-lipid diets. These results are consistent with those of Malheiros *et al.*(2003a), who carried out an experiment with diets similar to those evaluated in the present experiment, and concluded that broilers fed low-protein diet presented worse FCR compared with those supplied with low-lipid and low-carbohydrate diets.

Also, Ghazanfari *et al.* (2010), tested feeds with different crude protein levels (22.3, 19.3, and 16.3%) and observed that low protein levels impaired feed conversion ratio and live weight of broilers during the periods of 0-21 and 22-32 days of age. Consistent live weight results were also reported by Swennen *et al.*(2004 and 2011) and Jlali *et al.* (2012).

Therefore, the performance results obtained in the present study agree with Malheiros *et al.* (2003a), who asserted that dietary protein content has a stronger effect on broiler growth compared with the macronutrients carbohydrates and lipids.

At 42 days of age, the broilers fed the low lipid diets presented lower body weight than those fed the low-carbohydrate diets. A possible explanation may be their lower feed intake: despite not being statistically different, the low-lipid diets resulted in 4.4% lower feed intake relative to the low-carbohydrate diets, which corresponds to 189g lower feed intake per bird.

This lower feed intake may have been caused by the feedstuffs used for food manufacturing, as the low-lipid diets contained high starch levels to compensate the metabolizable energy derived from oil in order to maintain equal dietary energy level. This may have caused worse diet palatability, slightly influencing feed intake during the evaluated period. However, this hypothesis needs to be confirmed by experiments investigating this effect on feed intake.

The broilers fed the low-protein diets presented numerically lower feed intake at 28 days of age. On day 35, this diet resulted in lower feed intake compared with the control and the low-carbohydrate diets, and despite of statistical significance, the feed intake of birds fed the low-protein diets was 4.5% lower than those fed the low-lipid diet. This effect was clear during the period of 7 to 42 days of age, when feed intake of the broilers supplied with the low-protein diet was lower than those submitted to the other treatments.

Collin *et al.* (2003) studied the effect of dietary protein, carbohydrate, and lipid levels on the performance of 7- to 42 -d-old broilers and observed lower feed intake in broiler fed low-protein diets (12. 5% CP) compared with those supplied with low-lipid and low-carbohydrate diets (19.6% and 19.7% CP, respectively). On the other hand, Malheiros *et al.* (2003a) did not find any changes in feed intake when feeding broilers with iso-energy diets containing low protein (15.8% CP), carbohydrate, or lipid (19.6% and 19.5% CP, respectively) levels.

Swennen *et al.* (2007) reported that broilers fed low protein adjust their feed intake according to protein dietary level, i.e., broilers fed diets with significantly reduced protein levels reduce their feed intake. However, when protein reduction is small, broilers may increase their feed intake or consume the same amount of feed as broilers fed higher protein levels. This may explain why Malheiros *et al.* (2003a) did not find significant differences in feed intake, because the reduction in protein levels applied by these authors (3.8% and 3.7% PB relative to the low-lipid and low-carbohydrate diets, respectively) was not as severe as those used by Collin *et al.* (2003) (7.1% and 7.2% reduction to the low-carbohydrate and low-lipid diets, respectively) and in the present study (7% relative to the low-lipid, low-carbohydrate, and control diets).

Deficiency of some essential amino acids in the feed (glycine+serine, valine, isoleucine, phenylalanine+tyrosine), which were not supplemented, and may consequently caused dietary amino acid imbalance. According to Gonzales (2008), the aminostatic theory of feed intake control states that intake is mediated not only by the dietary level of crude protein, but also by the quality and balance among amino acids.

Selenium supplementation did not statistically affect broiler performance in the present study, which is partially in agreement with the findings of Payne & Southern (2005), who did not find significant differences in the performance of broilers supplemented with selenium yeast or sodium selenite at 0.3 ppm. Consistent results were observed by Yoon *et al.* (2007) and Cai *et al.* (2012).

The dietary supplementation of 0.3 ppm organic selenium improved broiler livability on days 35 and 42 (Figure 1). According to Boiago (2006), this effect may be related to a stronger immune system, enhancing leukocytosis and therefore, humoral and cell-mediated responses against pathogens (Macpherson, 1994).

Relative to carcass and parts yields (Table 3), broilers fed the low-protein diets presented lower breast yield compared with those fed the low-lipid diets. Testing different dietary crude protein levels (22.3, 19.3, and 16.3%), Ghazanfari *et al.* (2010) observed that reduced protein levels resulted in lower carcass yield in 49-d-old broilers.

Swennen *et al.* (2011) and Jlali *et al.* (2012) obtained lower breast yield when dietary crude protein level was reduced, in agreement with the results shown in Table 3. In the present study, this result is probably due to the deficiency of some amino acids (glycine+serine, valine, isoleucine, phenylalanine+tyrosine) that were not supplemented in the low-protein diets, causing amino acid imbalance, as mentioned above. According to Fisher (1994), broiler breast muscle is negatively affected when essential amino acids are deficient.

Silva *et al.* (2003) tested four protein and three energy levels, with energy/protein ratios of 188, 168, 148, and 128, and reported a linear reduction in leg (drumstick+thigh) weight when protein levels were reduced. Ghazanfari *et al.* (2010) also observed lower leg yield when feeding broilers with a diet containing 16.3% CP compared with a diet with 22.3% CP.

Although the results indicate a significant increase in the leg yield of broilers fed the low-protein diet, this is not necessarily an advantage, because in the present study, leg yield is expressed relative to live weight and, as the broilers fed this diet presented lower body weight, this results was expected.

The broilers fed the low-protein diet had higher abdominal fat percentage. This is in agreement with the findings of Collin *et al.* (2003), who evaluated different macronutrient ratios and also obtained a significant increase in abdominal fat in broilers fed low-protein diets. Consistent results were obtained by Swennen *et al.* (2005), Swennen *et al.* (2011), and Jlali *et al.* (2012).

The increase in abdominal fat percentage may be due to the effect of low-protein diets on feed intake, which increases when there are marginal CP reductions, and diminishes when protein reduction is severe (Collin *et al.*, 2003; Swennen *et al.*, 2005). On the other hand, energy intake increases, and the energy excess is directed to heat production, fat deposition or both (Malheiros *et al.* 2003a, Swennen *et al.* 2007). Therefore, diets with high energy/protein ratios make broilers consume energy in excess of their requirements, because according to the aminostatic theory of intake regulation (Gonzales, 2008), broilers eat to supply their protein/amino acid requirements.

Dietary Se supplementation did not affect carcass or breast yield, which is in agreement with the findings of Payne & Southern (2005), who did not find any differences in these parameters when supplementing broiler diets with Se yeast or sodium selenite at 0.3 ppm. Consistent results were obtained by Boiago (2006) and Medeiros *et al.* (2012).

Considering the effect of the interaction between feed and age on glucose blood levels, as shown in Figure 2-A, it was observed that diets did not influence this parameter on days 21, 28, and 35. This is in agreement with Malheiros *et al.* (2003a), who tested iso-energy diets and using paired replacement of macronutrients similarly to the presented study and also reported that blood glucose levels were not changed by dietary macronutrient content. Consistent results were described by Collin *et al.* (2003) and Swennen *et al.* (2005, 2006). The observed absence of influence of diet composition on blood glucose levels is probably due to the strict regulation of carbohydrate metabolism in broilers. Blood glucose levels tend to remains constant, even when broilers are submitted to fasting (Swennen *et al.*, 2007).

The study of the interaction between Se and age (Figure 2-B) show that, at 42 days of age, broilers supplemented with Se presented higher blood glucose levels than those fed diets with no Se addition. To our knowledge, there are no studies that directly investigates the relation between dietary Se addition and blood glucose levels in broilers. However, in humans, Yang *et al.* (2010) reported a positive association between Se concentration and glucose in elderly patients; however, the authors say that literature data are still inconsistent and further research on this matter is warrented.

The broilers fed the low-protein diet presented high blood triglyceride levels (Table 4), which was also reported by Malheiros *et al.* (2003a) and Swennen *et al.* (2005, 2006, 2009).

In broilers, triglycerides are the product of *de novo* lipogenesis in the liver, and therefore, this metabolite is increased broilers fed low-protein diets particularly due to higher liver lipogenesis (Swennen *et al.*, 2007). This is associated with an increase in the activity of liver lipogenic enzymes, also *in vitro*, which may explain the higher abdominal fat percentage in the broilers fed the low-protein diet (Rosebrough *et al.*, 2004 e Swennen *et al.*, 2007).

The metabolic parameter triglyceride level was significantly affected by bird age ($p < 0.0001$): it was high on days 14 and 21, and gradually decreased, presenting its lower level on day 42. Consistent findings were obtained by Malheiros *et al.* (2003a) and Swennen *et al.* (2010).

The interaction between feed and age shows that uric acid blood levels (Figure 2-C) was lower in broilers fed the low-protein diet. At 35 days of age, these levels were 11.6% lower than in those in the control treatment, despite not being statistically different. Uric acid levels gradually decreased between days 14 and 42, independently of feed, and these results are consistent with the finding of Malheiros (2003a, b).

Differently from carbohydrates and lipids, proteins contain nitrogen atoms in their amino acids, in addition of carbon, oxygen, and hydrogen; when nitrogen is removed, it is called amino group ($NH4^+$) that when in excess, is converted into uric acid and excreted (Rutz, 2008). Therefore, in the present study, the lower concentration of uric acid found in broilers fed the low-protein diet suggest lower amino acid breakdown as a consequence of low protein supply, compared with low-carbohydrate and low lipid diets (Collin *et al.*, 2003), and therefore can be used as a biomarker of amino acid breakdown (Swennen *et al.*, 2007).

The reduced uric acid levels obtained in the present experiment and in the studies of Rosebrought *et al.* (1996), Collin *et al.* (2003), and Swennen *et al.* (2005, 2006) support the assertion of Jackson *et al.* (2005, 2006) that protein utilization is inversely related to protein intake.

The study of the effect of the interaction between feed and age on creatine kinase (CK) presented in Figure 2-D shows that low-lipid diet presented statistically higher levels of this metabolites compared with the control and the low-carbohydrate diets. This is not in agreement with the findings of Malheiros *et al.* (2004), who performed iso-energy replacements between fats and carbohydrates in diets and did not find significant differences in CK levels between broilers fed low-carbohydrate or low-lipid levels; however, their levels were statistically lower than those obtained with the control diet.

For the other evaluated ages, CK levels were not influenced by diet composition, in agreement with the study of Malheiros *et al.* (2003a), who also highlighted the wide variation among individuals, as found in the present study. Malheiros *et al.* (2003b) attributed this variation in CK levels, including in birds submitted to the same treatment, to genetics.

Blood CK concentration increased with age, particularly after three weeks of age, s previously reported by Malheiros *et al.* (2003a, b). The blood activity of CK is considered a myopathy (muscle damage) marker when there is cell membrane damage (sarcolemma) and permeability changes (Sandercock *et al.*, 2001). In the study of Silva *et al.* (2007) to establish references for blood parameters of HYBRO-PG broilers, CK activity was higher in older birds possibly due to muscle development.

Sandercock *et al.* (2001) also observed an increase in CK blood activity in larger and older broilers, and therefore this increase may be dependent on age and size. Those authors also considered that this may be a consequence of genetic selection for higher growth rate and muscle accretion, which may have cause deleterious effect on the structure, metabolism, and functional parameters of the skeletal muscles, resulting in spontaneous myopathy. Reinforcing this hypothesis, it was observed in the present experiment that birds fed the low-protein diets presented the lowest CK levels at 28, 35, and 42 days of age relative to the other diets, which is possibly related to the lower weight gain of these birds during these periods. Consequently, their size was smaller, indicating the dependence between size and CK, as mentioned above.

The dietary addition of 0.3 ppm selenium, except for the effect of the interaction between Se and age on glucose, had no influence on the other evaluated blood parameters (triglycerides, uric acid, CK) of birds fed diets containing different macronutrient levels.

CONCLUSIONS

Changes in dietary protein content had a stronger influence on broilers performance compared with changes in carbohydrate and lipid levels. Low-protein diets increased abdominal fat percentage and reduced carcass and breast carcass yields.

The changes in macronutrient levels caused metabolic alterations in broilers, and dietary protein level had a strong impact on blood triglyceride and uric acid levels.

The dietary addition of 0.3 ppm selenium affected the livability of 35- and 42-d-old broilers fed low-protein diets, and increased blood glucose levels at 42 days of age.

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