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PERFORMANCE AND DUODENUM MORPHOMETRY OF BROILER CHICKENS SUBMITTED TO DIFFERENT METABOLIZABLE ENERGY LEVELS AND FEED PROGRAMS AT 42 TO 57 DAYS OF AGE

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ABSTRACT

This experiment was conducted to evaluate the use of different energy levels and amino acid recommendations on performance, carcass yield and intestinal morphometry of broilers from 42 to 57 days of age. We used 1,600 one-day old male broilers (Cobb 500) in a completely randomized design arranged in a 2x4 factorial scheme with 3,200 and 3,600 kcal ME/kg and four different feed programs. The metabolizable energy levels and the feed programs did not determine significant differences in carcass characteristics for choosing a level of energy or a feed program, thus the energy level and the recommendations of amino acids that determine the highest cost-benefit ratio should prevail. It was observed that 3,600 kcal ME/kg resulted in some improvement on performance and morphometry of the intestinal mucosa, also the fractioning of digestible amino acid requirements during two periods resulted in worse performance and intestinal villi height.

KEYWORDS: Digestible amino acids; metabolizable energy; nutritional requirements; small intestine.

DESEMPENHO E MORFOMETRIA DUODENAL DE FRANGOS DE CORTE SUBMETIDOS A DIFERENTES NÍVEIS DE ENERGIA E PROGRAMAS DE ALIMENTAÇÃO DE 42 A 57 DIAS DE IDADE

RESUMO

O experimento foi realizado com o objetivo de avaliar diferentes níveis de energia e programas de alimentação sobre o desempenho, as características de carcaça e a morfometria da mucosa do duodeno de frangos de corte de 42 a 57 dias de idade. Foram utilizados 1.600 pintos machos "Cobb 500", em um delineamento inteiramente ao acaso, em esquema fatorial 2X4, sendo dois níveis de energia (3.200 e 3.600 kcal EM/kg) e quatro programas de alimentação. Os níveis energéticos ou os diferentes programas de alimentação não determinaram diferenças expressivas nas características de carcaça que justifiquem a escolha de um nível de energia ou de um programa alimentar, devendo prevalecer o nível energético e as recomendações de aminoácidos que determinem o maior custo-benefício. Observou-se que o nível 3.600 kcal EM/kg proporcionou melhoria no desempenho das aves e na morfometria da mucosa intestinal e o fracionamento das exigências de aminoácidos digestíveis em dois períodos piorou os resultados de desempenho e de altura de vilosidade.

PALAVRAS-CHAVE: Aminoácidos digestíveis; energia metabolizável; exigências nutricionais; intestino delgado.

INTRODUCTION

The current high growth rate of broilers is in large part the result of genetic and production conditions improvement, such as nutrition and management (RAMOS et al., 2011). This high growth rate of broiler chickens (RAMOS et al., 2009) led to a greater appreciation of cut yields for subsequent commercialization, and these cuts can be directly influenced by diet and feeding programs (LIMA et al., 2008).

For a long time, the prevailing idea was that birds look for food to meet their energy requirements. However, many studies have demonstrated that food intake control is regulated by the energy density of the diet (NASCIMENTO et al., 2011).

The imbalance of dietary components, particularly amino acids, limits the growth of lean tissue and directs calories to adipocytes. The energy content also needs to be balanced with other nutrients, because, when unbalanced, the excess of energy will favor fat deposition in the carcass (LESSON & SUMMERS, 2001).

The fact that requirements have been largely determined on the basis of weight gain and feed conversion leads to a less adequate diet to maximize lean tissue growth. However, within the same protein level, supplementation of amino acids increases the protein content and reduces the fat content in broilers´ breast (SI et al., 2001).

Regarding possible changes in carcass composition due to nutrient levels, LIMA et al. (2008) concluded that the energy level of diets for broilers affects both the biological and economic performance.

The good performance of birds depends on adequate obtainment of energy and chemicals in the body. For this to occur, it is necessary for the digestive tract to present appropriate structural and functional characteristics from food intake to its absorption (CAMPOS et al., 2007). Mucosa is the functional element of the small intestine, and it may be characterized as a permeable layer to nutrients and a barrier against noxious compounds (OLIVEIRA et al., 2008).

Studying the intestinal mucosa is fundamental because it is an important aspect of the physiology of the digestion, since it represents a large area of exposure to exogenous agents present in this region from the beginning of ingestion, digestion and absorption of nutrients (VIEIRA et al., 2006). The intestinal mucosa has continued growth and is affected by endogenous and exogenous factors, such as metabolic hormones, physical and chemical characteristics of nutrients and microorganisms in the intestines. The intestinal microbiota plays an important role in maintaining animal health (OLIVEIRA et al., 2008) and its composition can be both beneficial and harmful to the individual, depending on the nature and amount of microorganisms.

The number and size of villi in each segment of the small intestine give them certain characteristics, and in the presence of nutrients, the absorptive capacity of the segment is directly proportional to the number and size of villi and the surface area available for absorption (MACARI et al., 2002). The absorption of digestion products occurs entirely in the small intestine by two mechanisms: diffusion and active transportation. This absorption is facilitated, among other factors, by the placement of the mucosa in numerous projections called villi and invaginations of mucosa between the base of the villus, forming Lieberkhun crypts (BALOG NETO et al., 2008).

The objectives of this study were to evaluate the effects of different levels of energy and feeding programs on performance, carcass characteristics and intestinal morphology of broilers from 42 and 57 days of age.

MATERIAL AND METHODS

Initially, 1,600 male "Cobb 500" chicks, at one day of age, were housed in a conventional masonry shed with wood shavings bedding (approximately five centimeters high). In the periods of 1-21 days and 22-41 days of age, all birds received the same rations formulated to meet the nutritional requirements, according to ROSTAGNO et al. (2005). At the end of 41 days, all plots were weighted to ensure similar mean weight at the beginning of the experiment (42 days of age).

At 42 days of age, 1,600 male "Cobb 500" broilers were distributed in a completely randomized design in a 2 x 4 factorial arrangement, totaling eight treatments with five replicates of 40 birds. The factors analyzed were two metabolizable energy levels (EL) (3,200 and 3,600 kcal / kg) and four feeding programs (FP), comprising four recommendations of digestible amino acids, which varied according to the source of recommendation (POPE & EMMERT, 2001; ROSTAGNO et al., 2005) and the poultry breeding phase (42-57 days, 42-49 days, 50-57 days of age), represented in the scheme below:

- Feeding Program 1 (FP1): diet formulated according to the recommendations by ROSTAGNO et al. (2005), for lysine, methionine + cystine and threonine, provided from 42 to 57 days of age;
- Feeding Program 2 (FP2): diet formulated to contain lysine, methionine + cystine and threonine, according to the equation developed by POPE & EMMERT (2001), provided from 42 to 57 days of age;
- Feeding Program 3 (FP 3): diet formulated to contain lysine, methionine + cystine and threonine, according to the equation developed by POPE & EMMERT (2001), provided from 42 to 49 days of age, and PA4 diet, provided within the period of 50 to 57 days of age;
- Feeding Program 4 (FP 4): PA1 ration, provided from 42 to 49 days of age and a diet formulated to contain lysine, methionine + cystine and threonine, according to the equation developed by POPE & EMMERT (2001), provided from 50 to 57 days of age.

The regression equations of POPE & EMMERT (2001), developed to meet the requirements of male broilers at each feeding phase were as follows:

- Digestible Methionine + Cystine = 0.88 – 0.0063x
- Digestible lysine = 1.22 – 0.0095x
- Digestible Threonine = 0.8 – 0.0053x

x = average age within the desired period.

According to the application of the equations by POPE & EMMERT (2001), digestible methionine + digestible cystine levels were 0.57 (42 to 57 days), 0.59 (42-49 days) and 0.54 (50 to 57 days); digestible lysine levels were 0.75 (42 to 57 days), 0.78 (42 to 49 days) and 0.71 (50 to 57 days); and digestible threonine levels were 0.54 (42 to 57 days), 0.56 (42-49 days) and 0.51 (50 to 57 days). The experimental diets were formulated based on corn, soybean meal, soybean oil, dicalcium phosphate, limestone, vitamins and microminerals supplement (Table 1).

At the end of the experimental period, when the birds reached 57 days of age, data of weight gain, feed intake, feed conversion, caloric conversion (kcal consumed / kg weight gain) and intestinal morphology were evaluated.

Table 1. Percentage composition and calculated nutrient levels of the experimental diets

Ingredients	Experimental diets							
	3,200 kcal ME/kg				3,600 kcal ME/kg			
	FP1	FP2	FP3	FP4	FP1	FP2	FP3	FP4
Corn grain	64.50	64.85	64.89	65.04	55.02	55.50	55.46	55.55
Soybean meal	27.35	27.30	27.30	27.25	29.13	29.00	29.00	29.00
Soybean oil	4.61	4.55	4.50	4.43	12.34	12.19	12.20	12.17
Dicalcium	1.43	1.43	1.43	1.44	1.45	1.45	1.45	1.45
Limestone	0.91	0.92	0.91	0.92	0.89	0.89	0.90	0.90
Supplement min + vit*	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
DL-Methionine 98%	0.16	0.055	0.075	0.024	0.17	0.066	0.086	0.035
L-Lysine HCl 78%	0.14	-	-	-	0.10	-	-	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values								
ME (kcal kg^{-1})	3,200	3,200	3,200	3,200	3,600	3,600	3,600	3,600
Crude protein	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Calcium (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Available phosphorus (%)	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Lysine (%)	0.94	0.86	0.86	0.86	0.94	0.86	0.86	0.86
Met.+ Cist. dig. (%)	0.67	0.57	0.59	0.54	0.67	0.57	0.59	0.54
Threonine (%)	0.61	0.60	0.61	0.61	0.61	0.60	0.61	0.61
Sodium (%)	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19

*Enriched per kilogram of diet: Vitamins: (A - 8,000 IU; E - 50 mg; D3 - 2,000 IU; K3 - 2.0 mg; B1 - 1.5 mg; B2 - 4.0 mg; B6 - 2.0 mg; B12 - 15.0 mcg); Pantothenic Acid - 12.0 mg; Folic Acid – 0.8 mg; Niacin - 30.0 mg; Biotin - 0.04 mg; Choline - 200 mg; Copper - 8.0 mg; Iodine - 1.0 mg; Selenium - 0.3 mg; Manganese - 70.0 mg; Zinc - 80.0 mg; Iron - 50.0 mg; Antioxidant BHT - 50.0 mg.

For the evaluation of carcass traits, four birds from each plot were taken randomly, identified, weighed and separated into individual boxes, where they remained fasting for a period of about six hours, receiving only water. Then, they were transferred to the slaughterhouse where they were weighed again, and slaughtered.

After evisceration, the chickens went through two bath systems in cold water called chiller and pre-chiller, which prepare the carcass for cooling or freezing. During this process, the carcass absorbs the water, which freezes along with the product if an adequate dripping is not carried out. After a short period of dripping (remaining hence a larger amount of water in the carcass), the carcasses were weighed again without feet, head and neck, and the cuts and respective yield evaluations were performed: breast, thigh + drumstick and abdominal fat.

On the 57th day of the experiment, a random sample of two birds per repetition was taken, weighed and slaughtered by decapitation for blood flow. Afterwards, the collection of a middle portion of the duodenum of about four centimeters was carried out and fixed by immersion in Bouin solution for 24 hours. The tissue fragments were used for histological examination with inclusion of the material in Paraplast. After microtomy, 15 longitudinal and semi-serial cuts of seven micrometers thick were obtained, stained by Periodic Acid-Schiff (PAS) and hematoxylin and observed by light microscopy.

Readings of 30 measurements of villus height and crypt depth of the intestines were made (micrometer) for each treatment, by means of an image analyzer from Kontron Elektronik (Video Plan) coupled to a binocular microscope. Measurements of villus height were taken from the basal region of the intestinal mucosa, coincident with the upper portion of the crypts until its apex. The crypts were measured from its base to the crypt:villus transition region. After the results were obtained, they were statistically analyzed.

Statistical analyzes were performed using the program ESTAT (1994). In case of statistical significance, means were compared by Tukey test at 5% probability.

RESULTS AND DISCUSSION

Table 2 presents the averages of performance data for the period from 42 to 57 days of age. There was no interaction (P> 0.05) between the energy levels and feeding programs for feed intake, weight gain and feed conversion. A significant effect (P <0.05) of the energy levels was observed only on feed conversion, with the best results found in the level of 3,600 kcal ME / kg.

Table 2. Feed intake (FI), weight gain (WG), feed conversion (FC) and caloric conversion (CC) of birds according to the energy levels of the ration (EL) and feeding programs (FP) and the unfolding of interaction EL x FP for CC, from 42-57 days of age

Treatments	FI (g)	WG (g)	FC	CC (kcal/g)	
		Energy levels			
3,200	3,000	1,088	2.76 b	8.828	
3,600	2,891	1,118	2.59 a	9.107	
Feeding Program					
				3200 kcal	3600 kcal
PA1	2,577 c	1,127	2.29 a	7.715 Ca	6.938 Ba
PA2	2,529 c	1,059	2.40 a	8.221 BCa	8.073 Ba
PA3	3,219 b	1,109	2.90 b	9.145 ABb	10.625 Aa
PA4	3,456 a	1,117	3.09 b	10.228 Aa	10.789 Aa
F Values					
EL	3.6479^{NS}	1.6407^{NS}	6.3799^{*}	1.5265^{NS}	
FP	65.9570**	1.7039^{NS}	33.6509^{**}	43.0051^{**}	
EL x FP	1.1542^{NS}	0.6883^{NS}	1.8672^{NS}	4.6038^{*}	
CV(%)	5.48	5.91	7.07	7.12	

Means in the columns followed by capital letters and in the lines followed by different small letters are significant by Tukey test (P <0.05). ** P <0.01, * P <0.05 NS = non-significant.

No significant effects (P> 0.05) of energy levels were observed on feed intake and weight gain in this study. LIMA et al. (2008) observed in a study with different energy levels (2,900; 3,000 and 3,100 kcal / kg), lysine and methionine + cystine, that the lowest feed intake was obtained with the lowest energy level (P <0.05). It is known that increased intake may be associated with a higher level of oil in medium and high energy rations, which increases the palatability, favoring ingestion and the increase of food intake. CELLA et al. (2009), studying different levels of lysine (1.14; 1.18; 1.22 and 1.26% digestible lysine), found that there was a quadratic effect (P <0.05) on the weight gain of the birds, being the best digestible lysine level found at 1.18%.

BARBOSA et al. (2008), working with different energy levels and room temperatures, found similar responses to those obtained in this study for gain weight, and this variable was not influenced by the energy levels of the ration.

Feeding programs had a significant effect (P <0.01) on feed intake and feed conversion, with the lowest intake and better feed conversion values provided by the programs FP1 and FP2.

Regarding caloric conversion, there was a significant interaction (P <0.05) among treatments (Table 2). Within the level of 3,200 kcal / kg diet, we found that the best caloric conversion was obtained with the feeding program FP1 and the worst one with programs FP3 and FP4. At the level of 3,600 kcal / kg diet, no difference was found between the programs FP1 and FP2 and between the programs FP3 and FP4. However, FP1 and FP2 were different from FP3 and FP4, and the best results were found for programs FP1 and FP2.

Therefore, programs FP1 and FP2, by adopting the amino acids recommendations by ROSTAGNO et al. (2005) or by applying the equations by POPE & EMMERT (2001) to determine the levels of amino acids, considering a single amino acid level for the period from 42 to 57 days, provided similar performance compared to each other, and better in comparison to the other programs.

Table 3 presents data from carcass breast and thigh + drumstick and abdominal fat.

TABLE 3. Carcass yield (CY), breast yield (BY), thigh + drumstick yield (TY+DY) and abdominal fat (AF) of broilers slaughtered at 57 days of age according to the energy levels of the diet (EL) and feeding programs (FP)

Treatments	CY*[1] (g)	BY (g)	TY+DY (g)	AF (%)
		Energy level (EL)		
3,200	81.28	34.10	35.70	2.00
3,600	81.21	34.50	35.25	2.14
Feeding programs (FP)				
FP1	81.81	34.79	35.41	2.16
FP2	81.68	33.91	35.52	2.25
FP3	81.36	34.27	35.54	2.15
FP4	81.14	34.23	35.43	1.73
F values[1]				
EL	0.0093^{NS}	0.8070^{NS}	1.2802^{NS}	0.4199^{NS}
FP	0.2665^{NS}	0.6377^{NS}	0.0260^{NS}	1.1722^{NS}
EL x FP	1.4585^{NS}	2.7194^{NS}	1.6640^{NS}	0.5362^{NS}
CV(%)	2.78	3.69	3.25	29.44

**P< 0.01; *P < 0.05; NS = non-significant;
[1]Eviscerated carcasses, without the feet, head and neck, weighed right after chiller.

No significant interaction among the factors studied nor isolated effect of these factors on characteristics evaluated was verified (P> 0.05). These results are in accordance with the ones found by LIMA et al. (2008), in which the carcasses of males showed no interactions (P> 0.05) between energy and amino acids levels (lysine and methionine + cystine) of the experimental diets. SUMMERS et al. (1988), working with levels of 0.73, 0.93 and 1.03% lysine, also found no significant differences in carcass yield in broilers fed with different levels of lysine and methionine + cystine in the final phase. SILVIA FILHA et al. (2004), studying different energy levels in the ration, found higher carcass yield (81.28%) for the level of 3,150 kcal / kg.

Regarding abdominal fat, no significant effects were observed (P> 0.05) among the energy levels studied or feeding programs, contrary to the findings by BARBOSA et al. (2008), in which the percentage of breast and abdominal fat of birds was affected (P <0.01) by dietary energy levels (2,800; 2,900; 3,000; 3,100 and 3,200 kcal / kg).

Table 4 presents the results of the duodenum morphometry of broilers slaughtered at 57 days of age and the significant interactions among energy levels and feeding programs for villus height (P <0.05). The level of 3,200 kcal / kg and the program FP1 presented lower villus height. This result agrees with those found by PELICANO et al. (2003), who observed lower villus height for 3,200 kcal / k g and 0.935% dietary lysine. Likewise, ANDRADE et al. (2004), studying moisture corn silage and its association with additives in the diets, verified that the lower villus height was found at level 3,202 kcal / kg for the control treatment. However, these results differ from those found by BALOG NETO et al. (2008), who found no effect of energy on villus height.

Nevertheless, the amino acid recommendation did not affect villus height (P> 0.05) at the level of 3600 kcal / kg.

Regarding the energy level, the level 3,600 kcal/kg provided greater intestinal villus height, except for program FP1. The characteristics of the intestinal mucosa, such as villus height among other factors, are affected by the physical and chemical characteristics of nutrients, which can be evidenced by the higher energy level.

TABLE 4. Villus height (VH), crypt depth (CD) and VH/CD relation of the duodenum of broilers slaughtered at 57 days of age according to the energy levels of the diet (EL) and feeding programs (FP), and the EL × FP interaction for VH

Treatments	VH (µm)		CD (µm)	HV/PC
	Energy level (EL)			
3,200	1246.30		403.60	3254.40
3,600	1317.30		411.40	3404.40
	Feeding programs (FP)			
	3,200 kcal	3,600 kcal		
FP1	1.1210 Ab	1.4553 Aa	471.10 A	2884,30 B
FP2	1.3818 Aa	1.2675 Aa	346.60 B	3935,10 A
FP3	1.2060 Aa	1.2663 Aa	400.80 AB	3176,60 AB
FP4	1.2765 Aa	1.2803 Aa	411.50 AB	3321,80 AB
	F values			
F EL	1.8790 ^{NS}		0.1219 ^{NS}	0.4234 ^{NS}
F FP	0.4935 ^{NS}		5.2863 **	3.6908 *
F ELXFP	3.3626 *		1.3042 ^{NS}	1.5946 ^{NS}
CV(%)	11.43		15.41	19.58

Means in the column followed by capital letters and in the line followed by different small letters are significant by Tukey test (P <0.05). ** P <0.01, * P <0.05 NS = non-significant.

There was no significant effect (P> 0.05) of the tested energy levels on the parameters of crypt depth (PC) and villus height / crypt depth ratio (VH / CD). These results agree with the findings by BALOG NETO et al (2008) and ANDRADE et al. (2004), who did not find any effect of the diet energy on crypt depth and villous / depth ratio; however, crypt depth tended to be numerically higher according to diet with higher energy level.

Regarding feeding programs, the best values of crypt depth (CD) were obtained with the program FP1, although it did not differ from programs FP3 and FP4. These results disagree with the findings by PELICANO et al (2003), who verified lower values than those of the current study for crypt depth for all treatments. The intestinal epithelium of birds is constantly renewed through mechanisms of development and repair of the intestinal mucosa (*turnover*), which can be changed under the action of pathogens or nutritional agents, becoming hyperplastic, with deeper crypt depth (CAMPOS et al., 2007).

For the villus height / crypt depth ratio (VH / CD), the best values were obtained for the program FP2, and it did not differ from programs FP3 and FP4.

The development of intestinal mucosa consists of increasing villous height and density, which corresponds to increase epithelial cell, giving better digestion and intestinal absorption (CAMPOS et al. 2007), as it was evidenced at higher energy level, which provided greater villus height and increased animals performance.

CONCLUSIONS

The higher energy level and the feeding programs FP1 and FP2 provided some improvement in bird performance, whereas the partition of digestible amino acid requirements in two periods, as in the programs FP3 and FP4 led to worse outcomes.

The energy levels or the different feeding programs do not determine significant differences in carcass quality to justify the choice of a level or a feeding program; therefore, the energy level and the recommendations of amino acids that determine the best benefit-cost ratio must prevail.

The highest energy level (3,600 kcal / kg), except for the feeding program 1 (FP1), provided better results for villous height.

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