



Protein Levels and Environmental Temperature Effects on Carcass Characteristics, Performance, and Nitrogen Excretion of Broiler Chickens from 7 to 21 Days of Age

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ABSTRACT

This trial was conducted to evaluate the utilization of low-protein diets formulated based on the ideal protein concept for broiler chickens from 7 to 21 days of age reared at different environmental temperatures. Nine hundred male Cobb-500® chickens were used. At day seven chicks were distributed according to a completely randomized design in a 3 x 3 factorial with four replications of 25 birds each. It was used three crude protein levels in the diet (21.5; 20.0 and 18.5%) and three environmental temperatures (low, thermoneutral and high). The performance, carcass characteristics (yield and chemical composition), and nitrogen ingestion and excretion were assessed. There was no significant interaction among the factors for the evaluated variables. Environmental temperatures affected differently chicken performance. High environmental temperature resulted in lower weight gain and higher wing fat percentage, whereas cold temperature resulted in higher feed conversion. On the other hand, low-protein diets decreased weight gain, breast yield, nitrogen excretion and influenced breast and wings chemical composition. Birds reared at high environmental temperature showed lower nitrogen intake and excretion. The results showed that the decrease in protein levels from 7 to 21 days of age contributed to lower nitrogen excretion in broiler chickens, but impaired performance and carcass characteristics independent of rearing temperature.

INTRODUCTION

The broiler chickens development and productivity are highly influenced by dietary protein levels (Bregendahl *et al.*, 2002) and environmental temperature (Ain Baziz *et al.*, 1996) in the initial rearing period.

Since proteins have several functions in the living organism, it is important to provide adequate protein levels in the diet to guarantee optimum animal performance. Current studies have evaluated practices that allow the dietary protein levels reduction, since this would not only reduce the nitrogen excretion into the environment (Aletor *et al.*, 2000; Bregendahl *et al.*, 2002), but would also reduce diet costs because protein is an expensive nutrient. Nevertheless, the broilers' performance results fed low-protein diets in the initial phase are not conclusive. Some studies have shown that protein reduction does not influence performance (Summers *et al.*, 1992; Deschepper & De Groote, 1995), whereas other studies have shown impaired bird performance (Hussein *et al.*, 2001; Bregendahl *et al.*, 2002).

Based on the ideal protein concept, it is possible to reduce crude protein levels in broiler diets and fulfill the amino acid requirements by supplementation with synthetic amino acids. Therefore, diet formulation based on the ideal protein concept permits to provide amino acids at



the exact amounts and proportions needed for both maintenance and maximum protein deposition.

Chick development, mainly during the first weeks of age, is crucial for future performance, since some physiological processes will greatly influence body weight and feed conversion in the adult bird, *e.g.*, cell hyperplasia and hypertrophy, maturation of the thermoregulatory system and cell differentiation in the gastrointestinal mucosa. It is well known that chick's thermoneutral temperature lies between 31 and 33° C in the first week of age. Therefore, higher temperatures than those may induce hyperthermia and dehydration, resulting in lower feed intake and delayed growth (Mickelberry *et al.*, 1966). On the other hand, temperatures much lower than thermoneutral temperatures might result in hypothermia and induce pulmonary hypertension syndrome (ascites) (Maxwell & Robertson, 1998).

Several practices might be adopted in order to minimize the impairment effects of high temperatures on the broiler chickens production characteristics. Some of these include management practices (feed restriction, rearing density), adequate housing and use of equipments (house height and localization, roof type, fans, fogging systems), genetic improvement (heat-resistant strains) and nutritional practices (levels of protein, energy and electrolyte in the diets). Among nutritional practices, the protein levels reduction is based on the fact that this nutrient has higher caloric increment than carbohydrates and lipids (Musharaf & Latshaw, 1999). Therefore, for many years it has been recommended to reduce dietary protein contents to decrease the amount of heat produced by broilers during heat stress (Waldroup, 1982). However, recent studies have shown that the utilization of diets with low crude protein levels resulted in poor performance of broilers reared under heat stress during the growing phase (Alleman & Leclerq, 1997; Faria Filho, 2003).

The objective of the present study was to evaluate the performance, carcass characteristics and nitrogen excretion of chicks from 7 to 21 days of age reared at low, thermoneutral or high temperatures and fed diets containing different protein levels formulated on ideal protein concept.

MATERIAL AND METHODS

Male Cobb-500® chicks were fed a control diet (Table 1) and reared at thermoneutral temperature (32 ± 2°C) and relative humidity of 55 ± 13% between

one and seven days of age (pre-experimental period). At seven days of age, 900 birds with body weight average of 162.1 ± 1.3g were distributed into nine treatments according to a completely randomized design in a 3 x 3 factorial arrangement, with four repetitions of 25 birds each. The studied factors were crude protein levels (21.5; 20.0 and 18.5%) and environmental temperature (low, thermoneutral and high). Water and diets were provided *ad libitum* and the birds were managed according to standard commercial practices.

Table 1 – Composition of experimental diets.

Ingredients	Crude protein	Ideal protein	
	21.5% (control)	20.0%	8.5%
Corn	54.72	60.45	66.76
Soybean meal	36.62	31.10	24.91
Soybean oil	4.76	3.70	2.53
Dicalcium phosphate	1.77	1.83	1.89
Calcitic limestone	1.08	1.08	1.08
Salt	0.45	0.30	0.13
Sodium bicarbonate	-	0.23	0.49
Potassium chloride 60%	-	0.16	0.33
Choline chloride 60%	0.10	0.10	0.10
Coxistac 12%	0.05	0.05	0.05
Zinc bacitracin 15%	0.03	0.03	0.03
Vitamin/mineral supplement ¹	0.10	0.10	0.10
L-lysine	-	0.18	0.39
DL-methionine	0.32	0.30	0.35
L-threonine	-	0.08	0.17
L-tryptophan	-	0.03	0.05
L-valine	-	0.13	0.23
L-arginine	-	0.07	0.23
L-isoleucine	-	0.08	0.18
Total	100.00	100.00	00.00
Energy and nutrients	Calculated composition		
Metabolizable energy (kcal/kg)	3,080	3,080	3,080
Crude protein (%)	21.5	20.0	18.5
Calcium (%)	0.97	0.97	0.97
Available phosphorus (%)	0.47	0.47	0.47
Potassium (%)	0.90	0.90	0.90
Sodium (%)	0.20	0.20	0.20
Chloride (%)	0.35	0.35	0.35
Electrolytic balance (meq/kg)	219	219	219
Digestible amino acids (%)			
Lysine	1.13	1.13	1.13
Methionine + cystine	0.87	0.81	0.81
Threonine	0.17	0.18	0.18
Tryptophan	0.76	0.76	0.76
Valine	1.27	1.19	1.19
Arginine	0.77	0.76	0.76
Isoleucine	0.83	0.87	0.87
Leucine	1.65	1.52	1.36
Histidine	0.52	0.47	0.42
Phenylalanine+tyrosine	1.51	1.37	1.21
Essential/Non-essential amino acids	49:51	50:50	52:48

1 - Levels per kg of diet: 1,500 IU Vitamin A; 500 IU Vitamin D3; 20 mg vitamin E; 0.5 mg vitamin K; 2 mg vitamin B1; 6.6 mg vitamin B2; 20 mcg vitamin B12; 0.1 mg folic acid; 10 mg pantothenic acid; 100 mg niacin; 125 mg antioxidant; 10 mg Cu; 50 mg Fe; 1.365 mg I; 88 mg Mn; 0.25 mg Se; 100 mg Zn.



The crude protein levels (Micro-Kjeldahl) and amino acid (chromatography) in the corn and soybean meal that were used in the diets were previously evaluated according to AOAC (1984). Low-protein diets were formulated based on the ideal protein concept, so that the requirements of digestible amino acids were expressed as a percentage of digestible lysine (Baker & Han, 1994), as follows: methionine+cystine 72%, threonine 67%, tryptophan 16%, valine 77%, arginine 105% and isoleucine 67%. The leucine, histidine and phenylalanine+tyrosine levels were closer to the ideal proportion proposed by Baker & Han (1994), *i.e.*, 109, 32 and 105% of lysine, respectively, with the decreasing levels of crude protein (Table 1). The diets contained similar levels of nutrients, except for protein and amino acid levels. Diet samples were also collected for crude protein and amino acid analyses (AOAC, 1984). The actual protein and amino acid values were similar to the calculated values.

During the experimental period (7 to 21 days), the birds were distributed into three environmentally controlled rooms with low, thermoneutral and high temperatures (Table 2). Heating was provided by infrared lamps and cooling was provided by cooling unities. The temperature was controlled by thermostats.

Table 2 – Environmental temperature and relative humidity measured from 7 to 21 days of age.

Temperature	Environmental temperature (°C)	Relative humidity (%)
Low		
07 to 14 days	21.1±1.37 ¹	60.8±10.9
15 to 21	18.3±1.2	72.1±9.1
Thermoneutral		
07 to 14 days	29.0±0.9	54.2±9.9
15 to 21	26.3±1.5	72.6±10.9
High		
07 to 14 days	33.6±0.9	50.8±8.4
15 to 21	33.3±0.7	57.4±11.3

¹ Mean±standard deviation.

Production characteristics (feed intake, weight gain and feed conversion) were evaluated from 7 to 21 days of age. Carcass yield, abdominal fat percentage and breast, thighs+drumsticks and wings yields were evaluated at 21 days in two birds per replication. Commercial cuts were made only by one person that had been previously trained. Carcass yield and percentage of abdominal fat were expressed as a function of the live weight and the other characteristics were expressed as a function of carcass weight with feet and head. Dry matter, ether extract, crude protein

and ashes were evaluated in the breast, thighs+drumsticks and wings according to the recommendations of AOAC (1984).

Nitrogen ingestion was calculated multiplying feed intake by dietary nitrogen levels. Nitrogen retention was evaluated in a reference slaughter of 20 birds at 7 days and two birds per experimental unity at 21 days of age. Whole birds were ground (with feathers, blood and viscera), and nitrogen levels were determined. Nitrogen retention was calculated as the difference between nitrogen levels at 21 and 7 days of age. Nitrogen excretion was estimated as ingested nitrogen minus retained nitrogen in the organism. The nitrogen levels in the carcasses and diets were assessed using the Micro-Kjeldahl method (AOAC, 1984).

The statistical package SAS[®] was used to assess the normal distribution of the studentized errors (Cramer-Von Mises test) and the homogeneity of variance (Brown-Forsythe test). After it has been assured that all presuppositions of the model had been attended, data were submitted to analysis of variance using the General Linear Models (GLM) procedure of SAS[®] (SAS Institute, 2002). Statistically different means ($p < 0.05$) were compared using the Tukey's test at 5% of probability.

RESULTS AND DISCUSSION

Feed intake, weight gain and feed conversion results are shown in Table 3. There were no significant interactions between dietary crude protein levels and environmental temperature for these characteristics.

Table 3 – Means of feed intake, weight gain and feed conversion of broiler chickens between 7 and 21 days of age.

Factors	Characteristics		
	Feed intake (g)	Weight gain (g)	Feed conversion (g/g)
Environmental temperature			
Low	1006±24.5 a ¹	667±20.2 b	1.51±0.06 c
Thermoneutral	991±21.6 a	720±21.4 a	1.38±0.04 a
High	788±26.9 b	540±30.8 c	1.46±0.06 b
Crude protein (%)			
21.5 (control)	929±97.0	663±74.3 a	1.40±0.07 a
20.0	934±116.5	641±86.9 b	1.46±0.08 b
18.5	923±104.7	623±81.1 c	1.48±0.06 b
CV (%)	2.54	2.78	2.87

¹ - Means±standard deviation followed by different letters in the same column and within each factor are different by Tukey's test (5%).

The main effects (Table 3) indicated that chicks reared at high environmental temperature showed lower feed intake in comparison to the birds subjected



to low or thermoneutral temperatures. Since energy requirements for maintenance decrease with increasing environmental temperature, heat stress adaptation involves the feed intake reduction as an attempt to reduce the production of endogenous heat (Daghir, 1995). On one hand, feed intake of birds reared at low environmental temperature was not different from birds at thermoneutral temperature, which might be due to bird behavior in order to conserve heat. At low environmental temperatures, the birds spend most of the time in groups as a means to decrease heat dissipation, and therefore the time spent eating or drinking water is reduced (Furlan & Macari, 2002).

The present results showed that environmental temperature affected body weight gain. Birds reared at thermoneutral temperature showed higher weight gain than birds submitted to low or high environmental temperatures, and the worst results were seen in the birds kept under high temperatures (Table 3). It was also seen that the birds reared at low environmental temperature showed higher feed conversion, whereas birds raised at thermoneutral temperature showed better feed conversion. Intermediate feed conversion was seen in the high temperature-reared birds. Such results indicate that the energy spent to produce heat in the low environmental temperature was greater than the energy spent to dissipate heat in the high temperature. Indeed, high environmental temperatures are essential for chicks in the initial phase. Besides the fact that their thermoregulatory system is not completely developed yet, their area/volume ratio is greater; therefore, more heat is dissipated (Furlan & Macari, 2002).

Crude protein levels had no effect on feed intake; nevertheless, body weight gain and feed conversion were impaired with decreasing protein levels in the diet. Similar results have been observed by Hussein *et al.* (2001) and Bregendahl *et al.* (2002), *i.e.*, that reported decreased performance of birds fed low-protein diets. Other studies have also reported that protein levels had no effects on feed intake (Ferguson *et al.*, 1998; Araújo, 2001). Gonzales (2002) stated that the diet intake control is affected not only by the protein content, but also by protein quality, *i.e.*, amino acid balance. In the present study, low-protein diets showed no amino acid deficiency, which contributed to the similar results of feed intake.

The reasons for the performance decrease in broilers fed low-protein diets have been studied by Rérat *et al.* (1992) cited by Bregendahl *et al.* (2002). It has been suggested that lower concentrations of free amino

acids are found in the portal blood if diets are supplemented with synthetic amino acids in comparison to amino acids from intact protein (corn and soybean meal). This indicates that free amino acids are preferably metabolized within enterocytes, decreasing their bio-availability if compared to the amino acids provided as peptides (intact protein). Pinchasov *et al.* (1990) and Rutz (2002) reported that peptides from the digestion of intact proteins are absorbed more rapidly by enterocytes than the free amino acids of diets supplemented with synthetic amino acids. The lower absorption rate of synthetic amino acids results in lower availability of essential amino acids for protein synthesis in the tissues (Pinchasov *et al.*, 1990). Besides, Batal & Parsons (2002) have shown that purified diets containing 14.4% of crude protein and ideal profile of amino acids resulted in lower villi height and width and smaller crypt depth in comparison to a corn and soybean-based diet with 23% of protein. The present findings also indicated that birds fed low protein levels had worse performance. The diets with 21.5; 20.0 and 18.5% of protein had 99.1; 96.4 and 91.9% of intact protein (corn +soybean meal), respectively. The remaining nitrogen was provided by synthetic amino acids. Such findings demonstrate the importance of intact protein on the initial performance of broiler chickens.

There was no significant interaction between rearing temperature and dietary protein levels on the carcass yields and commercial cuts, and on abdominal fat percentage (Table 4). Nevertheless, at high-temperature birds showed better carcass yield in relation to birds reared at low and thermoneutral temperatures, which were not different. Such results are related to lower development rate of the viscera and slower feathering in high temperature birds, allowing a proportional increase in carcass yield (Ain Baziz *et al.*, 1996). Although the birds submitted to high environmental temperature have shown the best carcass yield, this fact was not reflected in the absolute body weight gain (Table 3).

The reduction in protein levels had no effects on the carcass and wings yields, however, reduces breast yield and increases thigh+drumstick yield. Summers *et al.* (1988) reported no decrease in breast meat yield at 21 days of age in birds fed low-protein diets. On the other hand, birds fed low-protein diets in the initial phase had lower breast yield at 42, 49 and 56 days of age (Salmon *et al.*, 1983). The control diet resulted in lower percentage of abdominal fat in comparison to the diet with 18.5% CP, corroborating results reported



Table 4 – Means of carcass yield, breast, thighs+drumsticks (Legs) and wings yields, and percentage of abdominal fat (Fat) of broiler chickens at 21 days of age.

Factors	Characteristics (%) ¹				
	Carcass	Breast	Legs	Wings	Fat
Environmental temperature					
Low	81.80±0.92 b ¹	25.15±1.16 a	26.11±0.80	10.65±0.46 b	1.09±0.35 b
Thermoneutral	81.25±0.88 b	24.10±1.10 b	26.48±0.73	10.96±0.54 ab	1.46±0.24 a
High	83.71±1.05 a	23.98±1.09 b	26.60±0.49	11.25±0.49 a	1.54±0.33 a
Crude protein (%)					
21.5 (control)	82.17±1.15	25.01±0.97 a	26.05±0.51 b	11.08±0.49	1.17±0.31 b
20.0	82.41±1.59	24.65±1.09 a	26.23±0.71 b	10.95±0.52	1.40±0.43 ab
18.5	82.18±1.57	23.57±1.13 b	26.92±0.58 a	10.82±0.63	1.52±0.26 a
CV (%)	1.18	3.98	2.24	4.42	20.55

1 - Means ± standard deviation followed by different letters in the same column and within each factor are different by Tukey's test (5%).

by Kerr & Kidd (1999) and Aletor *et al.* (2000) in market-age broiler chickens.

Breast and wing yields were significantly affected by environmental temperature (Table 4). There was a reduction in breast yield and an increase in wing yield in birds kept at high environmental temperature compared to low temperature birds. The energetic characteristics of muscle fibers are related to the differential effect of heat on the breast meat and wing meat. Ain Baziz *et al.* (1996) observed that broilers chronically exposed to heat showed proportional reduction in breast meat, whereas the leg meat was slightly reduced. Howliger & Rose (1989) also reported a reduction in breast muscle, but no changes in red meat (thighs), which has mostly red fibers and oxidative enzymes. The reduction in muscle growth observed during heat exposure suggests changes in the growth of different fiber types, through changes in the metabolism and in the rates of protein synthesis and degradation.

Some researches have shown that body composition, particularly carcass fat, might be influenced by environmental factors such as temperature. Kubena *et al.* (1974) reported that the

increase in environmental temperature resulted in higher percentage of abdominal fat. According to Howliger & Rose (1987) there were increases of 0.81% and 1.6% in abdominal fat and total carcass fat of broilers, respectively, for each degree increased in the environmental temperature. The present findings also showed higher abdominal fat percentages in the birds reared at thermoneutral and high temperatures when compared to the low temperature. According to Ain Baziz *et al.* (1996) the higher fat deposition in broilers reared at high environmental temperature is due an increase in lipoprotein lipase enzyme activity in the birds adipose tissue. This enzyme is responsible for fatty acids release from lipoproteins and incorporation in adipocytes.

The values in Table 5 show that there was no interaction between environmental temperature and crude protein levels on the breast and thighs+drumsticks chemical composition. Environmental temperature had no effects on the chemical composition of breast and thighs+drumsticks, although the increase in temperature resulted in decreasing protein levels and increasing fat contents in the wings. Crude protein levels in the diet had no effect on the

Table 5 – Means of dry matter (MS, %), crude protein (%MS), ether extract (%MS) and ash (%MS) of the breast, thighs+drumsticks (Legs) and wings of broilers at 21 days of age.

Characteristics (%)	Environmental temperature (°C)			Crude protein (%)			CV (%)	
	Low	Neutral	High	21.5	20.0	18.5		
Breast	Dry matter	28.7±0.18 ¹	28.7±0.16	28.7±0.14	28.6±0.08 b	28.6±0.07 b	29.7±0.08 a	1.26
	Crude protein	64.7±0.93	64.6±0.76	64.5±0.94	64.8±0.90	64.8±0.56	64.2±1.00	1.40
	Ether extract	24.7±0.77	24.8±0.82	24.7±0.97	24.2±0.23 b	24.1±0.23 b	25.8±0.41 a	1.27
	Ash	10.5±0.80	10.6±1.06	10.8±1.22	10.9±0.85 ab	11.1±0.69a	9.9±1.13 b	9.01
Legs	Dry matter	30.3±0.17	30.4±0.20	30.7±0.21	30.4±0.22	30.4±0.20	30.7±0.18	1.44
	Crude protein	50.8±1.56	50.4±1.22	48.8±1.20	50.9±1.12	50.7±1.07	48.4±1.21	1.54
	Ether extract	31.7±1.68	35.8±1.46	38.1±1.48	34.3±2.90	34.2±2.87	37.1±2.75	1.82
	Ash	17.5±0.90	13.8±0.97	13.1±0.65	14.9±2.24	15.1±2.06	14.4±2.17	5.95
Wings	Dry matter	32.8±0.12	32.8±0.08	32.6±0.14	32.7±0.05	32.8±0.04	32.9±1.00	1.16
	Crude protein	50.9±1.37 a	48.6±1.91 b	49.0±1.83 b	50.7±1.03 a	48.9±2.37 b	47.9±1.88 b	2.21
	Ether extract	35.0±0.43 c	37.7±2.88 b	38.4±2.30 a	34.9±1.06 c	37.7±2.13 b	38.6±2.73 a	1.78
	Ash	14.2±1.51	14.7±1.74	12.5±0.93	14.4±1.79	13.4±1.64	13.5±1.54	9.5

1 - Means±standard deviation followed by different letters in the same row and within each factor are different by the Tukey's test (p<0.05).



chemical composition of thighs+drumsticks. Nevertheless, the fat and ash deposition were higher and lower, respectively, in the breast of broilers fed low-protein diets. Low-protein diets have also increased fat deposition and decreased protein deposition in the wings.

The environmental temperatures and dietary crude protein levels effects on the ingestion and excretion of nitrogen in chicks from 7 to 21 days of age are shown in Table 6. There was no significant interaction between the factors for these variables. Although feed intake has not been influenced by crude protein levels in the diet (Table 3), nitrogen ingestion decreased with the reduction in dietary protein levels. In regard to environmental temperature, nitrogen ingestion decreased with increasing environmental temperature, as a result of lower feed intake (Table 3).

Table 6 – Means of nitrogen ingestion and excretion of broiler chickens from 7 to 21 days of age.

Factors	Nitrogen (g/bird)	
	Ingestion	Excretion
Environmental temperature		
Low	35.7±2.37 a ¹	14.1±1.93 a
Thermoneutral	35.1±1.82 a	11.5±0.81 b
High	27.9±2.07 b	11.3±1.62 b
Crude protein (%)		
21.5 (control)	35.1±3.66 a	13.8±1.78 a
20.0	33.1±4.12 b	12.2±1.69 b
18.5	30.6±3.47 c	10.8±1.33 c
CV (%)	2.55	6.90

1 - Means±standard deviation followed by different letters in the same column and within each factor are different by the Tukey's test (5%).

Nitrogen excretion gradually decreased with reducing levels of crude protein in the diet. Diets with 20 and 18.5% of protein decreased nitrogen excretion in 11.6 and 21.7%, respectively, in comparison to the control diet. Such results are in accordance with previously results of Blair *et al.* (1999) that concluded that nitrogen excretion might be reduced between 10 and 27% with decreasing diet protein levels. Although low-protein diets in the present study have caused lower nitrogen excretion, their use is not justified, since broiler performance has been impaired (Table 3).

CONCLUSIONS

The dietary protein levels reduction from 7 to 21 days of age contributed to lower nitrogen excretion, however, impaired performance, yield and commercial cuts composition in broiler chickens, independent of the rearing temperature.

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