



Short Communication

Dietary crude protein reduction on growth and carcass performance of 22 to 42-day-old broilers reared under different temperatures¹

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ABSTRACT - This study was conducted to verify the effects of dietary crude protein reduction on growth and carcass performance of 22-42-day-old broilers reared under different temperatures. Treatments were set up in a five by two factorial arrangement, with five crude protein levels (220, 210, 200, 190 and 184 g/kg) and two temperatures (21.6 and 32.2 °C). Diets were isocaloric and essential amino acid-to-lysine ratio was maintained constant in all treatments. There was no interaction between crude protein reduction and environmental temperature for any of the parameters evaluated. Crude protein had no influence on feed intake. Nevertheless, weight gain and feed conversion ratio were linearly influenced by crude protein reduction. Worse performance was also observed in birds exposed to heat stress as compared with birds kept under thermoneutral temperature. There was no effect of crude protein reduction on breast and legs. Birds kept under heat stress had lower breast yield and higher leg yield as compared with broilers reared at 21.6 °C. Any crude protein reduction in the range of 220 to 184 g/kg for 22 to 42-day-old broilers has a negative effect on their performance. Heat stress worsens broiler performance and breast yield.

Key Words: breast yield, heat stress, temperature, thermoneutral temperature

Introduction

Performance and carcass yield of broilers are severely and negatively affected by high temperatures, which are common in tropical countries (Rostagno, 1995; Cheng et al., 1997; Lana et al., 2000; Furlan, 2006). This decrease in performance is caused by several physiological adjustments made by the bird to reduce body heat production. Feed intake, digestion and nutrient absorption generate a considerable amount of heat due to high metabolic activity of the organs involved in these processes, and if the bird cannot offset this heat by reducing feed intake, panting or increasing water intake, it may have its survival eventually compromised (Geraert et al., 1996; Baziz et al., 1997; Oliveira Neto et al., 2000).

As a function of the low feed intake observed in birds exposed to heat stress, some researchers have suggested increasing dietary protein content in order to compensate for the low amino acid intake (Temim et al., 1999; 2000; Gonzalez-Esquerre & Lesson, 2005). However, the metabolic heat generated by protein metabolism is relatively high

as compared with that of carbohydrates and fat (Musharaf & Latshaw, 1999). Moreover, diets with high crude protein content contain excessive levels of amino acids, which must be metabolized, producing more body heat, which has to be dissipated to the environment (Dionízio et al., 2005; Silva et al., 2006). Considering that high dietary crude protein levels increase metabolic heat and nitrogen excretion and ultimately impair poultry performance (Waldroup et al., 1976; Cheng et al., 1996; Cheng et al., 1997; Aletor et al., 2000) it has been suggested (Cheng et al., 1997) that dietary crude protein levels be reduced when broilers are reared under high environmental temperatures. Conversely, studies by Alleman & Leclercq (1997), Faria Filho et al. (2006) and Laganá et al. (2007) showed that reducing dietary crude protein in broilers kept under high temperatures impaired growth performance. All data aforementioned showed that crude protein reduction is still a subject not elucidated in the literature, especially considering different environmental temperatures.

Therefore, the objective of the present study was to evaluate the effect of dietary crude protein reduction on

growth and carcass performance of broilers reared in a thermoneutral or heat stress environment.

Material and Methods

All animal procedures were approved by the Committee of Animal Care and Use of Universidade Federal de Viçosa.

The experiment was carried out in climatic chambers at the Animal Bioclimatology unit of the Animal Science Department, Center for Agrarian Sciences, Universidade Federal de Viçosa, in Viçosa, MG, Brazil. Five hundred and sixty slow-feathering male broilers of the Cobb 500 strain, vaccinated against fowl pox and Marek's disease, were used for this study. During the starter phase (1 to 21 days of age), birds were reared in a conventional broiler house, and all were fed a common diet containing 3,000 kcal/kg metabolizable energy (ME) and 218 g/kg CP formulated to supply the nutritional requirements recommended by Rostagno et al. (2011).

At 22 days of age, 560 birds with 856 g average weight were distributed in a completely randomized

experimental design in a 5×2 factorial arrangement with 5 crude protein levels (220, 210, 200, 190 and 184 g/kg) and 2 environmental temperatures (21.6 and 32.2 °C). Each treatment was replicated eight times with seven birds per pen. The pen was considered the experimental unit. Lysine-to-crude protein ratios observed for all treatments were: 0.50, 0.52, 0.55, 0.58 and 0.60 g/kg.

Birds were housed in battery cages with 0.72 m² compartments, and the battery cages were kept in environmental chambers equipped with temperature and relative humidity controls to provide a thermoneutral environment (21 °C and 74% relative humidity) and a heat stress environment (32 °C and 66% relative humidity).

The environmental conditions inside the chambers were monitored and recorded twice daily (08h00 and 17h00) using dry bulb, wet bulb and black globe thermometers placed in the center of the room. Data were converted into WGTI (wet globe temperature index) for environmental characterization as described by Buffington et al. (1981). A continuous lighting program was used during the experimental period with 24 hours of artificial light, using 75 W fluorescent lamps.

Table 1 - Ingredient and calculated composition of the experimental diets (g/kg, as is)

Ingredient (g/kg)	Crude protein level (g/kg)				
	220	210	200	190	184
Corn (78 g/kg CP)	516.3	550.9	588.6	622.9	659.7
Soybean meal (460 g/kg CP)	388.5	358.0	324.9	294.1	260.5
Dicalcium phosphate	16.26	17.20	17.46	17.71	17.15
Limestone	9.83	9.23	9.23	9.23	9.80
Soybean oil	59.14	53.50	46.80	41.00	34.50
Salt	4.66	4.66	4.65	4.65	4.65
Premix ¹	3.50	3.50	3.50	3.50	3.50
DL-methionine (99%) ²	1.94	2.17	2.42	2.65	2.90
L-lysine HCL (78.5%)	---	0.91	1.90	2.82	3.82
L-threonine (98.5%)	---	0.05	0.48	0.90	1.38
L-valine (99%)	---	---	0.14	0.61	1.12
L-arginine (99%)	---	---	---	0.03	1.02
L-tryptophan (99%)	---	---	---	---	0.01
Calculated composition (g/kg)					
AMEn, kcal/kg	3,150	3,153	3,151	3,150	3,146
Crude protein	220	210	200	190	184
Digestible lysine	11.0 (100) ³	11.0 (100)	11.0 (100)	11.0 (100)	11.0 (100)
Digestible methionine + cystine	7.91 (72)	7.92 (72)	7.92 (72)	7.91 (72)	7.91 (72)
Digestible threonine	7.51 (68)	7.17 (65)	7.14 (65)	7.14 (65)	7.15 (65)
Digestible tryptophan	2.48 (23)	2.33 (21)	2.17 (20)	2.02 (18)	1.86 (17)
Digestible valine	9.30 (85)	8.84 (80)	8.47 (77)	8.47 (77)	8.46 (77)
Digestible isoleucine	9.69 (88)	9.15 (84)	8.56 (78)	8.01 (73)	7.41 (67)
Digestible arginine	14.1 (128)	13.3 (121)	12.4 (111)	11.5 (105)	11.5 (105)
Digestible glycine + serine	18.8 (171)	17.8 (163)	16.7 (152)	15.6 (142)	14.5 (132)
Available phosphorus	4.12	4.27	4.28	4.30	4.17
Calcium	8.89	8.83	8.83	8.83	8.84
Sodium	2.03	2.03	2.03	2.03	2.03

¹ Content/kg: vit. A - 4.286 kIU; vit. D3 - 428 kIU; vit. E - 4.29 IU; vit. B1 - 0.57 g; vit. B2 - 1.14 g; vit. B6 - 0.86 g; vit. B12 - 0.004 g; nicotinic acid - 7.14 g; pantothenic acid - 2.86 g; vit. K3 - 0.86 g; folic acid - 0.29 g; zinc bacitracin - 22.86 g; selenium - 71.4 mg; manganese - 22.86 g; iron - 12.86 g; zinc - 17.14 g; copper - 2.2910 g; iodine - 0.29 g; salinomycin - 18.86 g; avilamycin - 2.86 g; choline - 214 g; antioxidant BHT (butylated hydroxytoluene) - 22.86 g; excipient q.s. - 3,500 g.

² Crude protein values of all industrial amino acids were considered to estimate dietary crude protein.

³ Values between parentheses are amino acid-to-lysine ratios (ideal protein).

AMEn - apparent metabolizable energy, corrected by nitrogen balance; CP - crude protein.

The basal diet (Table 1) was corn- and soybean meal-based with DL-methionine, minerals and vitamins to supply the nutritional requirements of birds as recommended by Rostagno et al. (2011). Protein levels of the experimental diets were obtained by adjusting corn and soybean meal contents. Digestible lysine and ideal amino acid ratios according to the ideal protein profile suggested by Rostagno et al. (2011) were maintained by supplementing DL-methionine, L-lysine HCl, L-threonine, L-valine, L-arginine HCl and L-tryptophan as needed. Animals had free access to water and feed. Water was changed three times daily.

Birds were weighed on d 22 and 42 to determine weight gain (WG). Feed intake (FI) was calculated as the difference between the total amounts of feed supplied minus feed residue in the feeders on d 42. Feed conversion ratio (FCR) was calculated based on feed intake and weight gain, and was adjusted for mortality. On d 42, three birds per pen with body weight closest to the average of the pen were randomly selected for slaughter. They were fasted for 12 hours and then sacrificed for evaluation of breast, thigh and drumstick weights in order to calculate yields, which was done based on carcass weight.

Feed chemical analyses were carried out according to the methods described by Silva (1990) at the Animal Nutrition Laboratory of the Department of Animal Science of Universidade Federal de Viçosa. All statistic analysis were performed using statistical package SAS (Statistical Analysis System, version 7.0). Feed intake, WG, FCR and carcass yield evaluation between environment temperatures were done by analysis of variance, and means were compared by the Student-Newman-Keuls test ($\alpha = 0.05$). The analysis of crude protein level for every temperature was conducted independently, given that there was no interaction, and regression analysis was used to reach the optimum level of crude protein for each parameter evaluated.

Results and Discussion

According to the Cobb management guide (2008), the optimal temperature range to raise broilers between 22 and 42 days of age is between 17.5 and 26 °C. Higher temperatures could cause heat stress in broilers, which might generate the stimulation of thermoregulation mechanisms that nearly always impair broiler performance, at least partially. It has been shown that black globe humidity indexes (BGHI) equal or higher than 83 correspond to heat stress in 22 to 42-day-old broilers (Valerio et al., 2003). Therefore, thermoneutral and heat stress environments were well defined in the present study (Table 2). There

was no interaction between crude protein (CP) reduction and environmental temperatures ($P > 0.10$), and hence these parameters were individually evaluated. The only exception was leg yield, which presented crude protein and temperature interaction, where no influence of crude protein was observed on the legs of broilers reared under heat stress conditions, but on the other hand, the legs of those reared at thermoneutral temperature were quadratically affected by crude protein (Table 3).

Dietary crude protein reduction from 220 to 184 g/kg, which caused lysine-to-crude protein ratio to range from 0.50 to 0.60 g/kg, did not influence feed intake (FI) significantly ($P > 0.10$). The lack of effect of dietary CP reduction on FI indicates that high or low-protein diets, in the range studied, promoted similar intake in 22 to 42-day-old broilers receiving balanced essential amino acid content. Cheng et al. (1997) and Faria Filho (2006) also did not observe any influence of CP reduction on the FI of broilers subjected to heat stress (32 °C). Considering the high heat increment generated by excess protein (Musharaf & Latshaw, 1999; Aftab et al., 2006) and the fact that metabolic heat production is one of the factors that cause feed intake reduction in birds maintained in hot temperatures (Curtis, 1983; Rostagno, 1995; Mendes et al., 1997; Oliveira Neto et al., 2000; Siqueira et al., 2007), the results of the present study did not confirm the beneficial effect of CP reduction on FI of broilers exposed to heat stress, since no increase was observed on FI neither at 21.6 nor 32.2 °C (Table 4).

Table 2 - Temperature, relative humidity (RH) and black globe humidity index (BGHI)

Environment	Temperature (°C) ¹	RH (%)	BGHI ²
Thermoneutral	21.6±0.7	74.0±4.0	70.0±0.9
Heat stress	32.2±0.6	66.0±3.2	83.0±0.9

¹ As described by Buffington et al. (1981).

² Temperature was measured three times daily, at 08h00, 12h00 and 18h00.

Table 3 - Regression analysis and coefficient of determination (R^2) of significant parameters

Parameter	Equation	R^2
Linear		
Feed intake ¹	3141 + 37.98 FI	0.12
Lysine intake ¹	34.56 + 0.42 LI	0.11
Feed conversion ¹	1.60 + 0.037 FCR	0.48
Legs ¹	0.267 + 0.0021 Legs	0.11
Quadratic		
Lysine/weight gain	16.98 + 0.93 Lys/WG - 0.086 Lys/WG ²	0.52
Legs ²	0.298 - 0.0187 Legs + 0.0037 Legs ²	0.32

¹ Thermoneutral temperature.

² Heat stress.

FI - feed intake; LI - lysine intake; FCR - feed conversion ratio; Lys - lysine; WG - weight gain.

Regarding the different temperatures studied, FI was reduced by 22% ($P < 0.01$) under heat stress conditions, as compared with broilers maintained at 21.6 °C. It is known that broilers exposed to heat stress reduce FI through physiological mechanisms to ensure its survival. Many metabolic changes such as plasma thyroid (Oliveira Neto et al., 2000) and corticosterone (Yunianto et al., 1997) concentrations; liver and intestine relative weight (Geraert et al., 1996; Oliveira Neto et al., 2000); blood flow on the body surface and core contribute to FI changes; all of them occur to reduce body heat production.

Broiler WG was linearly influenced by dietary crude protein level, decreasing from 1542 g at 220 g/kg to 1455g at 184 g/kg of CP, which meant 5.6% (87 g) of WG reduction. Results showed that lowering CP from 220 (1532 g) to 210 g/kg (1525 g) had little effect on WG. Nevertheless, when CP was reduced to less than 210 g/kg WG reduced more. This can probably be explained by lysine-to-CP ratio (Lys/CP). Changing Lys/CP from 0.50 (220 g/kg CP) to 0.52 (210 g/kg CP) did not influence WG much. However, any higher CP reduction, such as to 200 g/kg increased Lys/CP (0.55), which might not bear the same muscle accretion. This may be related to non-essential amino acid-to-lysine ratio, such as glycine (Dean et al., 2006).

Broilers exposed to heat stress had 43% of WG reduction ($P < 0.001$) as compared with those maintained at 21.6 °C (1906 vs 1088 g). As FI was reduced 22% and higher reduction was observed for WG (43%) in broiler kept at 32.2 than those ones at 21.6 °C, it was clear that feed conversion ratio (FCR) underwent enormous influence from temperatures utilized. Low thyroid and high corticosterone plasmatic levels influence metabolic rate of broilers under heat stress conditions. For example, high corticosterone levels stimulate much higher muscle breakdown, which contributed to the lower WG observed in birds kept

in heat stress than those in a thermoneutral environment (Geraert et al., 1996; Yunianto et al., 1997).

Feed conversion and also lysine utilization efficiency-to-weight gain (Lys/WG) ratios were linearly influenced ($P < 0.002$) by dietary crude protein levels. The highest protein level fed (220 g/kg CP) resulted in a FCR of 1.93 and Lys/WG of 21.2, which was 7% better than that observed at the lowest CP levels evaluated for these parameters. This demonstrates that any reduction of crude protein, even considering ideal protein, made it much less efficient for the bird to convert feed or lysine into muscle mass. As previously mentioned, non-essential amino acids might be involved in this (Dean et al., 2006).

Considering the evaluated temperatures, data showed worse FCR (1.71 vs 2.31) and also worse Lys/WG ratio (18.8 vs 25.4), of 35%, for broilers kept at 32.2 °C than at 21.6 °C. This meant worse feed or lysine utilization efficiency to produce broiler muscles. Physiological changes in broilers exposed to heat stress explain why these birds became less metabolically efficient to growth (Yunianto et al., 1997; Oliveira Neto et al., 2000). As part of feed and lysine are not used to produce meat, nutrients were probably transformed into subcutaneous and visceral fat, and hence why birds under heat stress are fatter than those reared under thermoneutral temperatures (Baziz et al., 1997).

Breast and leg yields were not affected ($P > 0.10$) by dietary CP levels (Table 5). This showed that even though broilers presented higher WG when their diets were supplemented with high CP levels, no change was verified in body composition from dietary CP concentration. The absence of effect of dietary protein level on breast yield is consistent with the results obtained by Alleman & Leclercq (1997) and Sabino et al. (2004). Leg yield results obtained by Kerr & Kidd (1999) and Laganá et al. (2007) also did not show any influence of different CP levels on leg yield.

Table 4 - Effect of dietary crude protein reduction on broiler performance, considering different environmental temperatures

Item	T (°C)	Dietary CP level (g/kg)					CV (%)	Mean	P-value		
		220	210	200	190	184			CP	T	CP×T
LI	21.6	35.2	35.3	35.2	36.8	36.6	4.66	35.8a	0.037	0.001	0.502
	32.2	27.8	28.9	28.0	27.9	26.8	12.78	27.9b	0.495		
Lys/WG	21.6	17.8	18.5	19.0	19.3	19.5	3.19	18.8b	0.001	0.001	0.733
	32.2	25.6	25.4	25.6	25.9	26.1	12.79	25.7a	0.563		
FI	21.6	3204	3207	3199	3346	3325	4.66	3256a	0.037	0.001	0.502
	32.2	2525	2631	2547	2540	2435	7.10	2536b	0.454		
WG	21.6	1978	1909	1852	1910	1879	4.91	1906a	0.084	0.001	0.231
	32.2	1085	1139	1093	1077	1025	4.87	1084b	0.090		
FCR	21.6	1.62	1.68	1.73	1.75	1.77	3.27	1.71b	0.001	0.001	0.732
	32.2	2.33	2.31	2.33	2.36	2.38	7.66	2.34a	0.635		

Mean values within a column with no common letters are significantly different ($P < 0.001$) as a result of a least significance difference comparison.

Eight replicates were used per treatment, with 10 birds in each.

CP - crude protein; CV - coefficient of variation; FCR - feed conversion ratio; FI - feed intake; LI - lysine intake (g); Lys/WG - lysine (g) to form 1 kg of weight gain; P-value of CP - value considering linear regression; T - temperature; WG - weight gain.

Table 5 - Effect of dietary crude protein (g/kg) reduction on breast and leg yields (g/kg) of broilers reared under different temperatures

Item	T (°C)	Dietary CP level (g/kg)					CV (%)	Mean	P-value		
		220	210	200	190	184			CP	T	CP×T
Breast	21.6	328	324	335	331	330	3.32	330a	0.266	0.001	0.857
	32.2	300	302	308	304	300	3.43	303b	0.121		
Legs	21.6	268	260	259	258	258	3.26	261b	0.035	0.001	0.002
	32.2	281	277	280	276	289	4.35	281a	0.001		

Mean values within a column with no common letters are significantly different ($P < 0.001$) as a result of a least significance difference comparison. CP - crude protein; CV - coefficient of variation; T - temperature.

Regarding temperatures studied, the results obtained in the present study confirm data found by Oliveira Neto et al. (2000) and Baziz et al. (1997), who also showed worse breast yield in broiler groups reared under heat stress as compared with broilers maintained at thermoneutral temperatures. Broilers under 21.6 °C presented 346.5 g/kg of breast yield, while those under 32.2 °C had breast yield of 303.0 g/kg, which meant 14% of difference in this carcass parameter. On the other hand, leg yield had opposite behavior, showing its value 8.5% higher at heat stress, in relation to thermoneutral temperature (282.8 vs 260.6%). This might be explained by the type of muscle fiber found in these different tissues (breast and legs). Breast muscle cells use glucose as main energy source, and have few mitochondria. According to Baziz et al. (1997), this fiber type produces more heat and is less efficient in producing energy like ATP, while leg muscles consist of fibers that contain many mitochondria and are more efficient to produce energy (ATP) using fat, for instance. Only a few studies have shown the effect of temperature on broiler leg yield (Oliveira Neto et al., 2000; Lu et al., 2007), where higher leg yield was verified in birds exposed to heat stress as compared with those maintained in a thermal comfort environment.

Conclusions

Dietary crude protein reduction from 220 to 184 g/kg impairs performance of 22 to 42-day-old broilers. Crude protein at 220 g/kg is the best level, which allows birds to achieve maximum response. This crude protein level represents a lysine/crude protein ratio of 0.50 g/kg and 3.49 g/kg of digestible lysine per Mcal of metabolizable energy. Heat stress has a negative influence on broiler performance and breast yield, so in order to reach maximum genetic response, broilers should be reared close to the thermoneutral zone for each age.

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