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Crude Protein and Metabolizable Energy Levels for Layers Reared in Hot Climates

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ABSTRACT

The objective of the present study was to evaluate the effects of different dietary crude protein (CP) and metabolizable energy (ME) levels on the performance, internal and external egg quality, and cloacal temperature of commercial layers reared in hot climate. In this trial, 100 commercial Hy-Line W-36 layers between 20 and 32 weeks of age were distributed according to a completely randomized experimental design with a 2 x 2 factorial arrangement, consisting of two CP levels (15 e 18%) and two ME contents (2700 and 3100 kcal/kg) with five replicates per treatment and 10 birds per replicate. Performance (feed intake, egg production, egg mass, and feed conversion ratio), internal (Haugh units and albumen and yolk percentage) and external (eggshell % and thickness) egg quality parameters were evaluated. Cloacal temperature was measured in two birds per replicate at 8:00 h and 13:00 h. The diet containing 2700 kcal ME/kg promoted the best performance, whereas the worse performance observed in birds fed the diet with 3100 ME/kg was partially recovered when the diet contained 18% CP. Haugh units worsened as dietary CP level increased. The other external and internal egg quality parameters were not affected by dietary CP or ME levels. The cloacal temperature of birds fed 15% CP was lower in the morning and higher in the afternoon relative to those fed 18% CP, which temperature did not change during the day. It was concluded that dietary CP and ME levels influenced the performance and the body temperature of commercial layers.

INTRODUCTION

Birds are homoeothermic animals, that is, they need to maintain their body temperature within a narrow range around 41°C (Furlan & Macari, 2002), and therefore, rearing temperatures thermoneutral (Leeson *et al*., 2000). However, Brazil presents a predominantly tropical climate, with average temperatures usually above those recommended for commercial poultry production.

The main consequence of exposure to heat is that birds reduce their feed intake (Geraert *et al*., 1996) in order to diminish metabolic heat production in an attempt to maintain their body temperature (Koh & Macleod, 1999); however, major reduction in energy and nutrient intake may compromise productivity (Leeson *et al*., 2000).

The heat increment produced by protein metabolism is higher than that of carbohydrates, which in turn is higher than that of fat (Pond *et al*., 2005). This is the reason why it is recommended increasing dietary energy levels by the inclusion of oil and reducing protein level under heat stress situations (Leeson *et al*., 2000).

Metabolizable energy requirements of commercial layers depend on environmental temperature (Rostagno *et al*., 2005; Sakomura *et al*. 2005); it increases when the environment is cold or hot (Sakomura *et al*., 2005). Therefore, under heat stress situations, increasing energy levels in the diet of commercial layers by the inclusion of oil may compensate the low feed intake and supply the higher energy requirements. Nevertheless, Usayran *et al*. (2001) did not find any relationship between environmental temperature and dietary oil levels fed to commercial layers.

Because protein presents the higher heat increment among nutrients (Pond *et al*., 2005), it is usually recommended to reduce dietary protein levels when poultry are reared in hot environments. Nevertheless, recent studies with broilers have shown that it is better to increase dietary CP level to compensate the low protein intake resulting from exposure to heat (Faria Filho, 2006). No studies on this matter were found with commercial layers in literature.

The objective of the present study was to evaluate the effects of different dietary CP and ME levels on the performance, internal and external egg quality, and cloacal temperature of commercial layers reared in hot climate.

MATERIAL AND METHODS

This study was carried out at the poultry house of the Institute of Agrarian Sciences of the Federal University of Minas Gerais, Montes Claros, MG, Brazil.

In total, 200 commercial Hy-Line W-36 layers between 20 and 32 weeks of age were distributed according to a completely randomized experimental design with a 2 x 2 factorial arrangement, consisting of two CP levels (15 e 18%) and two ME contents (2700 and 3100 kcal/kg). with five replicates per treatment and 10 birds per replicate. Feed and water were supplied *ad libitum*, and a lighting program of 17 hours of light per day was adopted. Birds were submitted to conventional commercial management. The experimental diets are shown in Table 1.

Minimal, maximal and average environmental temperature and relative humidity during the experimental period are presented in Table 2.

The following performance parameters were evaluated when layers were 20 to 32 weeks old: feed intake (g/hen/day), egg production (%/hen/day), egg weight (g), egg mass (g/hen/day), and feed conversion ratio (g/g). Egg production was daily recorded, and the results are expressed as a percentage of the number of hens housed. Egg weight was determined by weighing all the eggs produced per experimental unit on the last experimental day, as calculating the average. Egg mass was calculated by multiplying egg production by egg weight. Feed conversion ratio was obtained by dividing feed intake by egg mass.

On the last day of the experiment, internal egg quality was evaluated relative to Haugh units and yolk and albumen percentages. Haugh units (HU) were determined according to the equation of Nesheim *et al*. (1979): HU = 100 x log (h + 7.57 – 1.7 $p^{0.37}$), where h is albumen height (mm) and p is egg weight (g). Albumen height was measured at 1 cm of the yolk, using a 0.1-mm precision pachymeter. Eggs were weight in a 0.01-

Table 1 – Experimental diets.

Ingredients	Feeds			
	2700/15	2700/18	3100/15	3100/18
Corn	64.9	59.9	60.4	50.3
Soybean meal	18.5	26.0	19.5	27.8
Calcitic limestone	8.1	8.1	8.1	8.1
Soybean oil	0	0	6.0	7.8
Inert material (rice husks)	2.5	0	0	0
Supplement*	6.0	6.0	6.0	6.0
TOTAL	100	100	100	100
Energy and nutrients				
Metabolizable energy (kcal/kg)	2700	2700	3100	3100
Crude protein (%)	15	18	15	18
Calcium (%)	3.8	3.8	3.8	3.8

* Vitamin, mineral, and additive supplement – Levels per kg of feed: vitamin A 8818 IU; vitamin D3 2800 IU; vitamin E 5.01 mg; vitamin K 0.33 mg; thiamine 4.41 mg; riboflavin 1.14 mg; pyridoxine 5.01 mg; cyanocobalamin 12.42 mcg; folic acid 0.20 mg; pantothenic acid 7.01 mg; niacin 22.02 mg; choline 280.02 mg; antioxidant 324 mg; copper 16.2 mg; iodine 1.68 mg; selenium 0.29 mg; zinc 71.93 mg; iron 40.5 mg; cobalt 0.16 mg; manganese 84.24 mg; calcium 6 g; phosphorus 3.3 g; sodium 0.96 g; methionine 0.72 g; crude protein 17% of the supplement; metabolizable energy 1000 kcal/kg supplement.

Table 2 – Environmental temperature and air relative humidity during the experimental period.

Week of age	Environmental temperature (°C)			Air relative humidity (%)		
	Minimal	Maximal	Average	Minimal	Maximal	Average
20	22.1	32.6	27.4	43	90	67
21	21.4	30.3	25.9	40	92	66
22	21.9	30.3	26.1	38	88	63
23	19.0	29.5	24.3	50	87	69
24	19.6	31.3	25.4	48	92	70
25	20.7	32.0	26.4	49	85	67
26	20.0	29.0	24.5	40	88	64
27	18.9	29.8	24.4	48	85	67
28	20.7	30.6	25.7	45	84	65
29	22.0	30.6	26.3	48	88	68
30	20.0	29.3	24.7	59	92	76
31	21.9	30.6	26.3	62	86	74
32	21.0	32.3	26.7	54	90	72
Average	20.7	30.6	25.7	48	88	68

Table 3 – Effect of dietary metabolizable energy and crude protein levels on feed intake (FI; g/hen/day), egg production (PRO; %/hen/day), egg weight (EW; g), egg mass (EM; g/hen/day) and feed conversion ratio (FCR; g/g) of commercial layers reared in hot climate.

Factors	FI	PRO	EW	EM	FCR
Metabolizable energy (kcal/kg)					
2700	108.8	88.7	56.6	50.7	2.21 b
3100	90.4	79.3	55.8	44.2	2.07 a
Crude protein (%)					
15	96.8	81.1	54.9 b	44.8	2.23 b
18	102.4	86.9	57.6 a	50.1	2.04 a
Analysis of variance					
Metabolizable energy (ME)	0.000	0.000	0.119	0.000	0.010
Crude protein (CP)	0.021	0.001	0.000	0.000	0.002
ME x CP interaction	0.016	0.018	0.128	0.004	0.28
CV (%)	4.73	3.57	2.00	3.26	4.84

Means followed by different letter in the same column, within each factor, are different by the F test.

g precision scale. Yolk and albumen percentages; yolk and albumen were manually separated and weighed in a 0.01-g precision scale and were expressed as percentages relative to fresh egg weight.

Eggshell quality was determined on the last experimental day in terms of eggshell thickness (mm) and percentage (% relative to fresh egg weight). Eggshells were first dried for 72 hours at room temperature, and then their thickness was measured using a 0.01-mm precision pachymeter. Eggshell weight was determined in a 0.01-g precision scale.

Cloacal temperature was measured in two hens per replicate by inserting a 0.1°C precision digital thermometer in the cloaca. This procedure was repeated for three consecutive days in the morning (starting at 8:00) and in the afternoon (starting at 13:00). During the morning, average environmental temperature was 21.8°C and average air relative humidity was 90%, whereas in the afternoon, average environmental temperature was 31.4°C and average air relative humidity was 48%.

Cloacal temperature data were analyzed according to a completely randomized experimental design with a 2 x 2 x 2 factorial arrangement, which factor were dietary CP levels (15 or 18%), dietary ME levels (2700 or 3100 kcal/kg), and period (morning or afternoon), with two replicates of five hens each.

Data were checked for the presence of outliers, normality of studentized residuals (Cramer–Von–Misses test), and variance homogeneity (Brown Forsite test). When the assumptions of the model were accepted, data were submitted to analysis of variance and results were considered significant at $p<0.05$. The analyses were performed using SAS® (Statistical Analysis System), according to the procedures described by Littell *et al*. (2002).

RESULTS AND DISCUSSION

The recorded maximal and minimal temperatures were 30.6 and 20.7°C, respectively (Table 2). It must be mentioned that the maximal temperature is above that considered optimal. During the afternoon of all experimental days, maximal temperature was higher than the thermoneutral temperature, and hens' behavior indicated they were suffering heat stress. Hens panted, moved very little and remained sitting, with the wings spread away from the body, which, according to Furlan & Macari (2002), is a typical behavior of heat-stressed birds. On the other hand, the average and minimal temperatures recorded during the experiment were within the thermoneutral range for commercial layers, which, according to Leeson *et al*. (2000) is between 19 and 27°C.

There was no significant interaction between CP and ME for egg weight and feed conversion ratio (Table 3). The diets containing 18% CP increased egg weight, as expected, because protein is an important component of eggs (Leeson *et al*., 2000), and higher CP contents determine higher CP deposition in the egg. These results agree with the findings of Pinto *et al*. (2002) with Japanese quails, who observed a positive linear

Table 4 - Interaction between dietary metabolizable energy and crude protein for feed intake, egg mass, and egg production of commercial layers reared in hot climate.

Crude protein (%)	Metabolizable energy (kcal/kg)	
	2700	3100
Feed intake (g/hen/day)		
15	108.94 aA	84.69 bB
18	108.64 aA	96.16 ab
Egg production (%)		
15	87.64 Aa	74.51 bB
18	89.80 aA	84.08 ab
Egg mass (g/hen/day)		
15	49.33 bA	40.35 bB
18	52.05 Aa	48.07 ab

Means followed by different small letters in the same row or capital letter in the same column are different by the F test (95%).

effect of CP (16, 18, 20, 22, and 24%) on egg weight. ME levels did not influence egg weight, as previously reported by Costa *et al*. (2009).

Relative to feed conversion ratio, the highest CP and ME levels generated the best results. This may have been a consequence of the reduction in feed intake (Table 4). In addition, egg mass was higher when the diet contained 18% CP compared with 15%, independently of dietary ME content (Table 4). The lower feed intake, together with the higher egg mass, promoted better feed conversion ratio.

Feed intake, egg production, and egg mass were affected by the interaction between ME and CP levels (Table 4). The hens fed 3100 kcal ME/kg presented lower feed intake, egg production, and egg mass relative to those fed 2700 kcal ME/kg; however, this effect was less pronounced in the birds fed 18% CP.

The hens fed the diet with high ME content presented lower feed intake, in agreement with Moraes *et al*. (1991) and Harms *et al*. (2000). This response may be explained by the fact that birds regulate their intake according to dietary energy level (Bertechini, 2006). However, when the diet contained 18% CP and 3100 kcal ME/kg, the reduction in feed intake was not pronounced, probably because, due to the higher dietary protein content, energy requirement increased. Another explanation for the lower feed intake of the hens fed 3100 kcal ME/kg was the high oil content of the feeds. Under these situations, feed passage from the gizzard to the duodenum is slower because the fat digestion in the duodenum is slow (Mateos & Sell, 1981; Mateos *et al*., 1982; Andreotti *et al*., 2004) because fats need to be emulsified, thereby reducing the appetite.

The obtained lower egg production when high-energy diets were fed is consistent with the finding of Rosa *et al*. (1996), who observed higher egg production when layers were fed 2800 kcal ME/kg compared with those fed 3100 kcal ME/kg. This may have been due to the lower feed intake of the hen fed the diet with 3100 kcal ME/kg and 15% CP, and the resulting lower energy intake reduced egg production.

This may also explain the lower egg mass observed in those birds. This effect was not as pronounced in the layers fed 18% CP, who presented higher egg weight compared with those fed 15% CP. Rabello *et al*. (2007) observed that low dietary protein level reduced albumen percentage, which may reduce egg weight, and consequently, egg mass.

There was no significant interaction of the tested factors for the evaluated internal and external egg quality parameters (Table 5). The eggs of layers fed 18% CP presented lower Haugh unit values than those fed 15% CP. This may be a result of the higher egg weight obtained for the birds fed 18% CP, which is used to calculate Haugh units. Dietary ME levels did not influence Haugh units. Yolk, albumen, and eggshell percentages and eggshell thickness were not affected by dietary ME or CP levels. Pavan *et al*. (2005) also did not observe any effect of CP levels (18, 20, or 22%) on the egg quality of layers.

Table 5 – Effect of dietary metabolizable energy and crude protein levels on yolk percentage (YO, %), albumen percentage (AL; %), eggshell percentage (ES; %), Haugh units (HU), and eggshell thickness (ET, mm) of commercial layers reared in hot climate.

Factors	YO)	AL	ES	HU	ET
Metabolizable energy (kcal/kg)					
2700	26.0	57.72	15.5	86.9	0.32
3100	26.3	57.10	16.4	85.6	0.32
Crude protein (%)					
15	26.0	57.21	16.1	87.5 a	0.32
18	26.4	57.61	15.8	85.1 b	0.32
Analysis of variance					
Metabolizable energy (ME)	0.204	0.130	0.227	0.199	0.193
Crude protein (CP)	0.188	0.316	0.310	0.025	0.859
ME x CP interaction	0.569	0.540	0.115	0.074	0.723
CV (%)	2.47	1.41	4.36	2.47	1.63

Means followed by different letters in the same column, within each factor, are different by the F test (95%).

There was a significant interaction between CP levels and period of the day on cloacal temperature (Tables 6 and 7). The layers fed 18% CP presented higher cloacal temperature than those fed 15% CP only in the morning. Independently of dietary CP level, cloacal temperature was higher in the afternoon. ME levels did not affect cloacal temperature, as observed by Ost and Peixoto (2000). CP levels did not interfere on cloacal temperatures during the hot period of the day (afternoon); however, when at thermal comfort temperatures (morning), hens fed 15% CP presented lower body temperature than those fed 18% CP. An explanation for this increase in temperature may be the high heat increment of protein. During the afternoon, the average environmental temperature of 30.6°C may have reduced feed intake, and consequently, protein intake.

Table 6 - Effect of dietary metabolizable energy and crude protein levels on the cloacal temperature in the morning and in the afternoon of commercial layers reared in hot climate.

Factors	Cloacal temperature (°C)
Metabolizable energy (kcal/kg)	
2700	41.16
3100	41.11
Crude protein (%)	
15	41.12
18	41.15
Period of the day	
Morning (21.8°C)	41.02 a
Afternoon (31.4°C)	41.26 b
Analysis of variance	
Metabolizable energy (ME)	0.0952
Crude protein (CP)	0.2817
Period of the day (PD)	< 0.0001
ME x CP interaction	0.3620
ME x PD interaction	0.8176
CP x PD interaction	0.0619
ME x CP x PD interaction	0.9572
CV (%)	0.40

Means followed by different letters in the same column, within each factor, are different by the F test (95%).

Table 7 – Effect of the interaction between period of the day and dietary crude protein level on cloacal temperature (°C) of commercial layers reared in hot climate.

Period of the day	Crude protein (%)	
	15	18
Morning (21.8°C)	40.97 bB	41.06 ab
Afternoon (31.4°C)	41.25 Aa	41.27 aA

Means followed by different small letters in the same row or capital letter in the same column are different by the F test (95%).

CONCLUSION

It was concluded that dietary CP and ME levels influenced the performance and the body temperature of commercial layers.

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