

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Protein Levels for Heat-Exposed Broilers: Performance, Nutrients Digestibility, and Energy and Protein Metabolism

Daniel Emygdio de Faria Filho^{1*}, Daniel Mendes Borges Campos², Karoll Andrea Alfonso-Torres², Bruno Serpa Vieira², Paulo Sérgio Rosa³, Aiani Maria Vaz², Marcos Macari² and Renato Luis Furlan²

¹Universidade Federal de Minas Gerais - Núcleo de Ciências Agrárias - Montes Claros, MG, Brazil

²Universidade Estadual Paulista - Faculdade de Ciências Agrárias e Veterinárias - Jaboticabal, SP, Brazil

³Embrapa Suínos e Aves and Universidade do Contestado - Concórdia, SC, Brazil

Abstract: Heat stress causes significant economic losses on broilers production due to poorer performance and carcass quality. Considering that protein has the highest heat increment among nutrients, it has been suggested that protein levels should be reduced in diets for heat-exposed broilers. Nevertheless, there are no conclusive results on the benefits of such practice, and further studies should be performed to elucidate some reported discrepancies. Thus, a trial was carried out to evaluate the effects of dietary protein levels (17, 20 and 23%) and environmental temperature (22 and 32°C) on the performance, nutrients digestibility, and energy and protein metabolism of broiler chickens from 21 to 42 days of age. Nutrients digestibility was determined by total excreta collection, and energy and protein metabolism was evaluated by comparative slaughter method. It was concluded that (1) heat exposure impairs broilers performance and increases nitrogen excretion, but do not change nutrients digestibility; (2) high-protein diets are technically feasible and promotes lower heat production for broilers reared under thermoneutral or hot environments, however, high-protein diets increases nitrogen excretion.

Key words: Comparative slaughter, heat stress, ideal protein, nitrogen excretion, total excreta collection

Introduction

Heat stress causes significant economic losses on broilers production due to poorer performance, lower breast meat yield, and greater carcass fat deposition (Ain Baziz *et al.*, 1996). Among the proposed nutritional practices to alleviate heat stress effects, it has been suggested the decrease of crude protein levels (Hruby *et al.*, 1995; Cheng *et al.*, 1999), since it has highest heat increment among nutrients (Musharaf and Latshaw, 1999). Nevertheless, Alleman and Leclercq (1997) and Faria Filho (2003) have reported that low-protein diets impair broilers performance in hot environments. Thus, the increase in crude protein and/or amino acid levels for heat-exposed broilers to help to withstand the poor feed intake has shown some positive results (Temim *et al.*, 1999; Temim *et al.*, 2000a; Gonzalez-Esquerria and Lesson, 2005) or no effects (Zarate *et al.*, 2003a,b). In this sense, studies on nutrients digestibility, and energy and protein metabolism are needed to better understand the results involving protein levels and heat exposure.

In regard to energy metabolism, low-protein diets were shown to increase heat production in broilers (Buyse *et al.*, 1992; Nieto *et al.*, 1997; Swennen *et al.*, 2004), whereas other studies have reported no influence (Macleod, 1990, 1992, 1997). Temim *et al.* (2000b) have shown broilers reared under 32°C and fed high-protein diet did not modify protein synthesis. Since heat production is highly associated to protein accretion

(Macleod, 1997), such results indicate that high-protein diets have not increased heat production, nevertheless, this statement should still be confirmed.

Changes in nutrients digestibility might also occur in heat-exposed broilers, but results are widely inconsistent. Geraert *et al.* (1992) have described that dietary metabolizable energy are not changed in heat-exposed broilers, whereas Keshavarz and Fuller (1980) reported higher contents and Yamazaki and Zi-Yi (1982) found lower metabolizable energy levels. In regard to the coefficient of digestibility, heat-related broilers showed lower digestibility of dietary amino acids, although such effect occurred mainly in females (Wallis and Banalve, 1984). Bonnet *et al.* (1997) reported that heat exposure decreases protein and fat digestibility and the reduction explains partially the poorer performance of heat-exposed broilers.

When defining protein levels for broilers diet, it must be considered not only technical and economical aspects, but also the impact of such diets on nitrogen excretion. From total nitrogen intake, 51.1% is retained on broilers carcass, 30.6% remains in the litter and 18.3% is lost as ammonia (Patterson and Adrizal, 2005), and as protein levels increase nitrogen excretion increases (Blair *et al.*, 1999; Aletor *et al.*, 2000). Faria Filho *et al.* (2006) reported that nitrogen excretion decreased in broilers fed low-protein diets at 20 or 25°C, but this effect was not observed in broilers reared under 32°C. Nevertheless, the interaction between high-protein diets and

Faria Filho *et al.*: Protein levels for heat-exposed broilers

Table 1: Pre-experimental diet (1 to 21 days of age) and experimental diets (21 to 42 days of age)

| Ingredients (%) | Pre-experimental diet | Experimental diets | | |
|------------------------------------|-----------------------|---------------------|--------|--------|
| | | 17% CP ¹ | 20% CP | 23% CP |
| Corn | 56.8 | 69.7 | 58.6 | 48.3 |
| Soybean meal, 45 | 36.3 | 21.6 | 32.4 | 41.2 |
| Soybean oil | 2.99 | 3.34 | 5.27 | 7.09 |
| Bicalcium phosphate | 1.82 | 1.91 | 1.85 | 1.80 |
| Calcitic limestone | 0.99 | 0.89 | 0.84 | 0.81 |
| Salt | 0.45 | 0.10 | 0.40 | 0.40 |
| DL-methionine | 0.23 | 0.32 | 0.23 | 0.15 |
| L-lysine | 0.14 | 0.45 | 0.13 | - |
| L-threonine | - | 0.20 | - | - |
| L-tryptophan | - | 0.02 | - | - |
| L-arginine | - | 0.16 | - | - |
| L-valine | - | 0.15 | - | - |
| L-isoleucine | - | 0.11 | - | - |
| Sodium bicarbonate | - | 0.46 | - | - |
| Potassium chloride | - | 0.27 | - | - |
| Choline chloride 60% | 0.10 | 0.14 | 0.10 | 0.07 |
| Coxistac 12%® | 0.05 | 0.05 | 0.05 | 0.05 |
| Zinc bacitracin 15% | 0.03 | 0.03 | 0.03 | 0.03 |
| Supplement ² (1 kg/ton) | 0.10 | 0.10 | 0.10 | 0.10 |
| Total | 100 | 100 | 100 | 100 |
| ----- Calculated composition ----- | | | | |
| Energy and nutrients | | | | |
| Metabolizable energy (kcal/kg) | 3,000 | 3,190 | 3,190 | 3,190 |
| Crude protein (%) | 21.40 | 17.00 | 20.00 | 23.00 |
| Calcium (%) | 0.96 | 0.90 | 0.90 | 0.90 |
| Available phosphorus (%) | 0.45 | 0.45 | 0.45 | 0.45 |
| Sodium (%) | 0.22 | 0.20 | 0.20 | 0.20 |
| Potassium (%) | 0.84 | 0.77 | 0.77 | 0.90 |
| Chloride (%) | 0.36 | 0.34 | 0.34 | 0.29 |
| Electrolytic balance (mEq/kg) | 209 | 188 | 188 | 217 |
| Choline (ppm) | 1,996 | 1,868 | 1,868 | 1,868 |
| Digestible amino acids | | | | |
| Lysine | 1.14 | 1.04 | 1.04 | 1.14 |
| Methionine+cystine | 0.82 | 0.78* | 0.78* | 0.85* |
| Tryptophan | 0.24 | 0.18* | 0.22 | 0.27 |
| Threonine | 0.73 | 0.73* | 0.76 | 0.81 |
| Arginine | 1.35 | 1.09* | 1.23 | 1.47 |
| Isoleucine | 0.84 | 0.70* | 0.77 | 0.91 |
| Valine | 0.89 | 0.80* | 0.82 | 0.95 |
| Leucine | 1.72 | 1.37 | 1.61 | 1.79 |
| Histidine | 0.53 | 0.40 | 0.49 | 0.56 |
| Phenylalanine+Tyrosine | 1.62 | 1.19 | 1.49 | 1.73 |

¹CP=Crude protein. ²Vitamin/mineral supplement - levels per kg of diet: vitamin A 1,500 IU; vitamin D3 500 IU; vitamin E 20 mg; vitamin K 0.5 mg; vitamin B₁ 2.0 mg; vitamin B₂ 6.6 mg; vitamin B₁₂ 20.0 mcg; folic acid 0.1 mg; pantothenic acid 10.0 mg; niacin 100.0 mg; antioxidant 125 mg; copper 10.0 mg; iron 50.0 mg; iodine 1.365 mg; manganese 88.00 mg; selenium 0.25 mg; zinc 100 mg. *Ideal proportion in relation to lysine (Baker and Han, 1994).

environmental temperature on nitrogen excretion should be further evaluated.

The present study was carried out to evaluate the effects of crude protein levels and environmental temperature on performance, nutrients digestibility and energy and protein metabolism in broiler chickens from 21 to 42 days of age.

Materials and Methods

In the pre-experimental period (1 to 21 days of age), chicks were fed the same diet (Table 1) and were kept in environmentally controlled rooms under thermoneutral conditions: 30.2±2.8°C (1 to 7 days of age), 27.3±2.6°C (8 to 14 days of age) and 26.3±3.0°C (15 to 21 days of age). Relative humidity was kept at 56 ± 12%. At 21 days

of age, 96 male Cobb-500® broilers (body weight = 899.6±3.8 g) were housed in battery cages, and assigned randomly in a 3 x 2 factorial arrangement, as follows: crude protein levels (17, 20 and 23%) and environmental temperatures (21.9±0.9°C and 32.2±1.3°C). There were four repetitions (cages) per treatment with four birds each.

Water and feed were provided *ad libitum*. Corn soybean meal-based diets (Table 1) were formulated using Rostagno *et al.* (2000) for ingredient composition and nutrient requirements. The low-protein diet (17%) was formulated based on the ideal protein concept (Baker and Han, 1994) where digestible amino acid requirements were expressed as percentages of digestible lysine, as follows: methionine+cystine 75%,

Table 2: Observed means and analysis of variance for feed intake, weight gain and feed conversion of broilers between 21 and 42 days of age

| Source of variation | Feed intake (g) | Body weight gain (g) | Feed conversion g/g |
|---------------------|-------------------------|-----------------------|------------------------|
| Temperature (°C) | | | |
| 22 | 3,007±35 ^a | 1,786±20 ^a | 1.68±0.03 ^b |
| 32 | 2,219±36 ^b | 1,145±34 ^b | 1.95±0.04 ^a |
| Crude protein (%) | | | |
| 17 | 2,687±163 ^a | 1,416±120 | 1.93±0.06 ^a |
| 20 | 2,627±150 ^{ab} | 1,505±127 | 1.77±0.05 ^b |
| 23 | 2,523±147 ^b | 1,475±129 | 1.75±0.07 ^b |
| Probabilities | | | |
| Temperature (T) | < 0.0001 | < 0.0001 | < 0.0001 |
| Protein (P) | 0.02 | 0.07 | 0.01 |
| Interaction T x P | 0.65 | 0.37 | 0.99 |

Means ± standard errors followed by similar letters in the column, within each factor, are not statistically different by the Test of Tukey (p>0.05).

threonine 70%, tryptophan 17%, valine 77%, arginine 105% and isoleucine 67%. In the control and high-protein diets, which contained 20 and 23% of crude protein, respectively, only methionine+cystine followed the ideal proportion (Baker and Han, 1994).

Performance was assessed by feed intake, weight gain and feed conversion between 21 and 42 days of age.

A digestibility trial was conducted according to the traditional method of total excreta collection during 27 to 31 days of age after a six-day adaptation period (21 to 26 days of age). In this trial, the digestibility coefficient of dry matter, crude protein, ether extract and the apparent metabolizable energy corrected for nitrogen balance (AMEn) were determined. Excreta were collected using plastic covered trays under the cages. On the first and the last sampling days, 1% of ferric oxide was added to the diets in order to identify the excreta produced from experimental diets. Samplings were performed twice daily and the excreta were separated per repetition and immediately frozen (-4°C). At the end of the trial, the amount of ingested food and the total of excreta were determined. After thawing at room temperature, excreta were homogenized and dried in a forced ventilation oven at 55 ± 2°C for 72 hours and then ground. Diets and excretas were analyzed for dry matter, crude protein, ether extract and gross energy (GE) levels (Silva and Queiroz, 2002).

The AMEn was calculated as follows:

$$\text{AMEn (kcal/kg natural matter)} = \{[(\text{GE ingested}) - (\text{GE excreted})] / (\text{kg natural matter ingested})\} - 8.22 \times \text{NB}$$

Where NB corresponds to the nitrogen balance that was determined by the difference between the ingested and the excreted nitrogen, expressed in kilograms. The ingested and excreted gross energy was expressed per kg of natural matter.

The digestibility coefficients of dry matter, ether extract and crude protein were calculated based on the formula:

$$\text{Digestibility (\%)} = \{[(\text{ingested nutrients}) - (\text{excreted nutrients})] / (\text{Ingested nutrients})\} \times 100$$

Energy and protein metabolism were determined by the comparative slaughter method (Blaxter, 1989).

Energy metabolism was assessed by apparent metabolizable energy intake, heat production, energy retained as fat and as protein, and energy retention efficiency. Apparent metabolizable energy intake was calculated by multiplying the feed intake by the dietary metabolizable energy levels. In order to determine retained energy, birds were slaughtered after 24 hours fasting at 21 days of age (n=10) and at 42 days of age (n=2 per repetition). Whole carcasses were frozen (-4°C) with feathers, blood and viscera, and then ground and dried in a forced ventilation oven at 55 ± 2°C for 72 hours. Afterwards, they were ground using a ball mill, and samples were used to determine dry matter, crude protein, ether extract and crude energy contents (Silva and Queiroz, 2002). The retained body energy was calculated as the difference in energy contents between 42 and 21 days of age. Heat production was determined as the difference between the apparent metabolizable energy intake and the retained energy. Energy retention as protein was calculated by multiplying the retained protein (difference in protein contents between 42 and 21 days of age) by 5.66 kcal/g. The energy retained as fat was calculated as the difference between the total retained energy and the energy retained as protein (Swennen *et al.*, 2004). The energy retention efficiency was obtained as the ratio between the retained energy and the ingested energy.

Protein metabolism was evaluated by nitrogen ingestion, retention, excretion, and retention efficiency. Nitrogen ingestion was assessed by multiplying the feed intake by the dietary nitrogen levels. Nitrogen retention was calculated as the difference between the retained nitrogen at 42 and 21 days of age. Nitrogen excretion was estimated as the difference between the ingested nitrogen and the retained nitrogen. The efficiency of nitrogen retention was obtained as the ratio between retained nitrogen and ingested nitrogen.

The data were checked for outliers, and after the normal distribution of studentized errors (Cramer-Von Mises test) and homogeneity of variances (Brown-Forsythe test) was assessed. After the assumptions had been attended, the data were submitted to analysis of variance using General Linear Model procedure of SAS® (Littell *et al.*, 2002). Statistically different means (P<0.05) were compared by Tukey's test at 5% of probability.

Results and Discussion

Performance: There was no interaction between protein levels and environmental temperature on feed intake, weight gain and feed conversion (Table 2).

Birds exposed to 32°C showed feed intake 26% lower than those reared at 22°C, which might represent a

Faria Filho *et al.*: Protein levels for heat-exposed broilers

Table 3: Observed means and analysis of variance for the digestibility coefficient of dry matter (DM), crude protein (CP) and ether extract (EE) and apparent metabolizable energy corrected for nitrogen balance (AMEn)

| Source of variation | DM | CP | EE | AMEn |
|---------------------|-----------------------|-----------------------|------------------------|--------------------------------|
| | ----- % ----- | | | ---- kcal/kg ¹ ---- |
| Temperature (°C) | | | | |
| 22 | 74.5±0.8 | 64.3±0.9 | 80.4±1.4 | 3,024±15 |
| 32 | 74.8±0.9 | 63.3±0.8 | 82.4±0.9 | 3,037±19 |
| Crude protein (%) | | | | |
| 17 | 77.9±0.3 ^a | 66.2±0.8 ^a | 79.0±0.9 ^b | 3,075±10 ^a |
| 20 | 74.7±0.4 ^b | 64.5±0.8 ^a | 81.0±1.6 ^{ab} | 3,043±16 ^a |
| 23 | 71.4±0.4 ^c | 60.6±0.6 ^b | 84.1±1.3 ^a | 2,973±16 ^b |
| Probabilities | | | | |
| Temperature (T) | 0.45 | 0.23 | 0.17 | 0.42 |
| Protein (P) | <0.0001 | <0.0001 | 0.03 | 0.0002 |
| Interaction T x P | 0.46 | 0.10 | 0.22 | 0.17 |

Means ± standard errors followed by similar letters in the column, within each factor, are not statistically different by the test of Tukey ($p>0.05$). ¹on natural matter.

means of avoiding heat production associated with food consumption (Koh and Macleod, 1999). Body weight gain and feed conversion were poorer (-36% and +16%, respectively) in broilers submitted to 32°C in comparison to those reared at 22°. As observed, the proportion of reduction in body weight gain was greater than the proportion of reduction in feed intake (-36 versus -26%) for heat-exposed broilers, leading to poor feed conversion, which are in close agreement with previous works (Ain Baziz *et al.*, 1996; Geraert *et al.*, 1996). Furthermore, these results indicate that the decreased body weight is not only due to the lower feed intake, but also to a direct effect of environmental temperature on broilers physiology/metabolism.

In regard to protein levels, feed intake was similar in broilers fed diets containing 17 and 20% of crude protein. It is known that feed intake is controlled not only by protein levels, but also by protein quality (Harper *et al.*, 1970). The diet with 17% of crude protein had a high-quality protein, since it had ideal proportions of methionine, threonine, tryptophan, arginine, valine and isoleucine (Baker and Han, 1994), which might have contributed for the similar feed intake in comparison to the diet with 20% of crude protein. Broilers fed 23% of crude protein have lower feed intake compared to broilers fed 17% of crude protein, which might be due to the excess of essential amino acids in the 23% crude protein diet. The excess of some essential amino acids (methionine, histidine, threonine, tryptophan, and lysine) has an appetite suppressant activity (Acar *et al.*, 2001). Body weight gain was not affected by protein levels, but there was a tendency ($p = 0.07$) of improvement in broilers fed with 20 or 23% of crude protein compared those fed 17% crude protein. Feed conversion was better in broilers fed with 20 or 23% of crude protein in comparison to those fed 17% of crude protein.

The absence of interaction between the protein levels and environmental temperature on the performance of broilers indicates that the protein level to be adopted is independent of environmental temperature. In previous studies, we have found similar results with broiler fed

high-protein diets (Faria Filho, 2006) or low-protein diets (Faria Filho, unpublished results). On the other hand, other studies in our lab showed a clear interaction between low-protein diets and environmental temperature (Faria Filho, 2003; Faria Filho *et al.*, 2006). In both studies where interaction was seen, the use of low-protein diets impaired broilers performance under 32°C, but these diets were technically feasible for broiler reared under thermoneutral conditions. These contradictory results might be attributed to differences in severity and timing of heat exposure, as discussed by Gonzalez-Esquerra and Lesson (2005, 2006).

Nutrients digestibility: There was no interaction between protein levels and environmental temperature on nutrients digestibility and apparent metabolizable energy corrected for nitrogen balance (AMEn) (Table 3). Environmental temperature did not affect the digestibility of dry matter, crude protein, ether extract, and AMEn values. This indicates that the reduced performance of broilers kept at 32°C is not related to poorer utilization of nutrients. The literature is unclear whether the ability of broilers to metabolize nutrients is affected by high temperatures. Geraert *et al.* (1992) have reported that dietary metabolizable energy is not changed due to heat exposure of broilers, whereas Keshavarz and Fuller (1980) showed higher contents and Yamazaki and Zi-Yi (1982) found lower metabolizable energy levels. In regard to digestibility of nutrients, Wallis and Banalve (1984) have shown that heat exposure decreased the dietary amino acids digestibility, but such effect was seen mostly in females. Bonnet *et al.* (1997) reported lower coefficient of fat and protein digestibility in heat-exposed broilers. Once again, such different results might be attributed to specific experimental conditions in each study (e.g. strain, nutritional levels, ingredients, gender, among others), and especial attention should be paid to the severity and timing of heat exposure, as mentioned by Gonzalez-Esquerra and Lesson (2005, 2006).

Dietary crude protein levels affected both nutrients

Faria Filho *et al.*: Protein levels for heat-exposed broilers

Table 4: Observed means and analysis of variance for apparent metabolizable energy intake (AMEI), heat production (HP), energy retained as crude protein (ER-CP) and energy retained as fat (ER-EE) and the energy retention efficiency (efficiency) in broilers from 21 to 42 days of age

| Source of variation | AMEI | HP | ER-CP | ER-EE | Efficiency % |
|---------------------|--|------------------------|-----------------------|-----------------------|-----------------------|
| | ----- kcal/kg ^{0.75} /day ----- | | | | |
| Temperature (°C) | | | | | |
| 22 | 327.2±5.4 | 200.7±3.6 | 60.7±1.4 ^a | 65.7±4.5 ^b | 37.3±1.3 ^a |
| 32 | 339.2±6.4 | 212.2±5.7 | 53.4±1.5 ^b | 73.2±4.6 ^a | 31.7±1.3 ^b |
| Crude protein (%) | | | | | |
| 17 | 353.6±4.1 ^a | 219.9±5.8 ^a | 52.5±1.8 ^b | 81.2±2.5 ^a | 29.8±1.2 ^b |
| 20 | 329.2±5.1 ^b | 202.6±3.9 ^b | 58.1±1.6 ^a | 68.4±4.7 ^a | 35.4±1.1 ^a |
| 23 | 316.8±6.1 ^b | 196.8±5.5 ^b | 60.5±2.1 ^a | 51.9±2.8 ^b | 38.4±1.7 ^a |
| Probabilities | | | | | |
| Temperature (T) | 0.06 | 0.06 | 0.0001 | 0.02 | <0.0001 |
| Protein (P) | 0.0002 | 0.008 | 0.0015 | 0.0001 | <0.0001 |
| Interaction T x P | 0.69 | 0.40 | 0.076 | 0.46 | 0.78 |

Means ± standard errors followed by similar letters in the column, within each factor, are not statistically different by the test of Tukey (p>0.05).

digestibility and AMEn. The digestibility coefficients of dry matter and protein, and AMEn decreased as dietary protein levels increased. The low protein utilization for broilers fed high-protein diets has been reported (Blair *et al.*, 1999; Aletor *et al.*, 2000; Swennen *et al.*, 2004), and might be associated with the oxidation of amino acid excesses. The lower AMEn values also observed is related the poor utilization of protein. On the other hand, ether extract digestibility increased with the protein levels, which could be attributed to the high inclusion of soybean oil in the high-protein diets (Table 1). It is well known that high-oil diets have the extra-caloric effect that generates a better metabolization of fat (Mateos and Sell, 1980).

Energy metabolism: There was no interaction between protein levels and environmental temperature on apparent metabolizable energy (AME) intake, heat production and energy retained as protein or fat (Table 4).

The AME intake and heat production were not affected by environmental temperature, but these variables tended to increase (p = 0.06) when the birds were exposed to 32°C. Nevertheless, it was expected lower heat production in broilers reared at 32°C (Macleod, 1992), since birds reared under such conditions show lower basal metabolism and lower activity (Ain Baziz *et al.*, 1996). Broilers exposed to 22°C showed greater energy retention as protein and energy retention efficiency. Also, they had lower energy retention as fat compared to birds reared at 32°C. It is known that heat exposure reduces body protein deposition and increases body fat deposition (Ain Baziz *et al.*, 1996; Geraert *et al.*, 1996), and such effects were also observed in the breast, thigh+drumstick and wings (Faria Filho, 2006). The lower deposition of energy as protein in heat-exposed broilers might be related to a metabolism adaptation to maintain thermal homeostasis, since the greater protein deposition causes greater heat production (Macleod, 1997), which is not desirable for heat-exposed broilers.

Moreover, the lower basal metabolism and activity of heat-exposed broiler (Ain Baziz *et al.*, 1996) explain the higher deposition of energy as fat for broilers reared at 32°C.

Heat production and AME intake were lower, and energy retention as protein and energy retention efficiency was greater in broilers fed 20% or 23% of crude protein compared to birds fed 17%. Energy retention as fat was lower in broilers fed 23% of crude protein compared with their 20 or 17% of crude protein counterparts.

Some studies, as the present findings, have shown that low-protein diets increased heat production (Buyse *et al.*, 1992; Nieto *et al.*, 1997; Swennen *et al.*, 2004), whereas others have reported no effects (Macleod, 1990, 1992, 1997). The greater heat production of low-protein diets has been attributed to increased serum levels of the thyroid-derived hormone triiodothyronine (T₃) (Buyse *et al.*, 1992), which has a calorogenic effect. The low-protein diets used by Buyse *et al.* (1992), Nieto *et al.* (1997) and Swennen *et al.* (2004) were amino acid deficient, which is associated to the greater plasma levels of T₃. Diets deficient in arginine, lysine, isoleucine, methionine (Carew *et al.*, 1997) and tryptophan (Carew *et al.*, 1983) increased T₃ plasma levels. Conversely, the low-protein diet in the present study had ideal amino acid profile (Baker and Han, 1994) for methionine, threonine, tryptophan, arginine, valine and isoleucine. The AME intake was greater in broilers fed with 17% of crude protein, and also, the energy:protein relation was greater in this diet since the energy content was kept constant. Therefore, increased heat production and greater fat retention were the mechanisms used by broilers to compensate for the excessive energy intake, as observed by Swennen *et al.* (2004). Such findings explain the mechanism by which there is greater fat deposition when broilers are fed with low-protein diets (Aletor *et al.*, 2000). The lower energy retention efficiency of broilers fed 17% of crude protein is also associated with the mechanism mentioned before.

Other important point to consider is that broilers reared

Faria Filho *et al.*: Protein levels for heat-exposed broilers

Table 5: Observed means and analysis of variance for nitrogen ingestion, retention, excretion and efficiency of retention in broilers between 21 and 42 days of age.

| Source of variation | Ingestion | Retention | Excretion | Efficiency % |
|---------------------|-------------------------------------|------------------------|------------------------|-----------------------|
| | -----g/kg ^{0.75} /day----- | | | |
| Temperature (°C) | | | | |
| 22 | 3.24±0.09 | 1.72±0.04 ^a | 1.53±0.08 ^b | 53.1±1.1 ^a |
| 32 | 3.31±0.09 | 1.51±0.04 ^b | 1.80±0.07 ^a | 45.6±1.0 ^b |
| Crude protein (%) | | | | |
| 17 | 2.95±0.04 ^c | 1.48±0.05 ^b | 1.46±0.08 ^b | 50.5±2.1 |
| 20 | 3.25±0.04 ^b | 1.64±0.05 ^a | 1.61±0.07 ^b | 50.6±1.7 |
| 23 | 3.64±0.05 ^a | 1.71±0.06 ^a | 1.93±0.07 ^a | 47.0±1.7 |
| Probabilities | | | | |
| Temperature (T) | 0.18 | 0.0001 | 0.0004 | <0.0001 |
| Protein (P) | <0.0001 | 0.0015 | <0.0001 | 0.09 |
| Interaction T x P | 0.31 | 0.76 | 0.65 | 0.66 |

Means ± standard errors followed by similar letters in the column, within each factor, are not statistically different by the test of Tukey ($p>0.05$).

under 32°C and fed high-protein diet did not modify protein synthesis (Temim *et al.*, 2000b). Since heat production is highly associated to protein accretion (Macleod, 1997), such results indicate that high-protein diets have not increased heat production. The present findings corroborate this hypothesis. The low heat production supports the practice of feeding high-protein diets to broilers in high environmental temperatures.

Protein metabolism: There was no significant interaction between protein levels and environmental temperature on nitrogen intake, retention, excretion and retention efficiency (Table 5).

Environmental temperature did not affect nitrogen ingestion corrected for metabolic weight (kg^{0.75}). Nevertheless, birds exposed to 22°C showed nitrogen excretion 15% smaller than the birds reared at 32°C. Temim *et al.* (1999) has reported a decrease of 27% in nitrogen excretion for broilers under the same conditions. The greater nitrogen excretion results from the lower efficiency of nitrogen retention in broilers reared at 32°C, as observed by Faria Filho *et al.* (2006) between 42 and 49 days of age in broilers exposed to 32°C. In the present study, approximately 49% of the nitrogen was retained in the carcasses, similarly to the value of 51.1% reported by Patterson and Adrizal (2005). Nitrogen intake, retention and excretion increased with protein levels in the diet. The efficiency of nitrogen retention tended ($p = 0.09$) to decrease in birds fed 23% of crude protein compared with their 17 or 20% counterparts. Protein utilization is generally better in broilers fed low-protein diets (Blair *et al.*, 1999; Aletor *et al.*, 2000; Swennen *et al.*, 2004) and this fact might be a metabolism adjustment in order to use protein better when it is provided in limited quantities. Broilers fed with 20 and 17% of crude protein decreased nitrogen excretion in 17 and 24% in comparison to the ones fed with 23% of crude protein. These values are similar to those reported by Blair *et al.* (1999), who concluded that it might be possible to reduce nitrogen excretion between 10 and 27% by decreasing dietary protein

levels. Faria Filho *et al.* (2006) reported that nitrogen excretion decreased in birds kept at 20 or 25°C and fed low-protein diets, but such effect was not observed at 32°C. Although it has been shown in the present study that the low-protein diets have decreased nitrogen excretion, broiler performance has been impaired.

Conclusions:

- 1 Heat exposure impairs broilers performance and increases nitrogen excretion, but do not change nutrients digestibility;
- 2 High-protein diets are technically feasible and promote lower heat production for broilers reared under thermoneutral or hot environments, however, high-protein diets increases nitrogen excretion.

Acknowledgements

The authors thank to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Process 03/06804-2) for the scholarship granted to Daniel Emygdio de Faria Filho.

References

- Acar, N., P.H. Patterson and G.F. Barbato, 2001. 1. Appetite suppressant activity of supplemental dietary amino acids and subsequent compensatory growth of broilers. *Poult. Sci.* 80: 1215-1222.
- Ain Baziz, H., P.A. Geraert, J.C.F. Padilha and S. Guillaumin, 1996. Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. *Poult. Sci.*, 75: 505-513.
- Aletor, V.A., I.I. Hamid, E. Nieb and E. Pfeffer, 2000. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole body composition and efficiencies of nutrient utilization. *J. Sci. Food Agri.*, 80: 547-554.
- Alleman, F. and B. Leclercq, 1997. Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. *Br. Poult. Sci.*, 38: 607-610.

- Baker, D.H. and Y. Han, 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. *Poult. Sci.*, 73: 1441-1447.
- Blair, R., J.P. Jacob, S. Ibrahim and P. Wang, 1999. A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. *J. Appl. Poult. Res.*, 8: 25-47.
- Blaxter, K.L., 1989. Energy metabolism in animals and man. University Press, Cambridge.
- Bonnet, S., P.A. Geraert, M. Lessire, B. Carre and S. Guillaumin, 1997. Effect of high ambient temperature on feed digestibility in broilers. *Poult. Sci.*, 76: 857-863.
- Buyse, J., E. Decuyper, L. Berghman, E.R. Kuhn and F. Vandersande, 1992. Effect of dietary protein content on episodic growth hormone secretion and on heat production of male broiler chickens. *Br. Poult. Sci.*, 33: 1101-1109.
- Carew, L.B., F.A. Alster, D.C. Foss and C.G. Scanes, 1983. Effect of a tryptophan deficiency on thyroid gland, growth hormone and testicular functions in chickens. *J. Nutr.*, 113: 1756-1765.
- Carew, L.B., K.G. Evarts and F.A. Alster, 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. *Poult. Sci.*, 76: 1398-1404.
- Cheng, T.K., M.L. Hamre and C.N. Coon, 1999. Effect of constant and cyclic environmental temperatures, dietary protein, and amino acid levels on broiler performance. *J. Appl. Poult. Res.*, 8: 426-439.
- Faria Filho, D.E., 2003. Efeito de dietas com baixo teor protéico, formuladas usando o conceito de proteína ideal, para frangos de corte criados em temperaturas fria, termoneutra e quente. Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil (Master Theses).
- Faria Filho, D.E., 2006. Aspectos produtivos, metabólicos, econômicos e ambientais da nutrição protéica para frangos expostos ao calor. Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP, Brazil (PhD Dissertation).
- Faria Filho, D.E., R.S. Rosa, D.F. Figueiredo, F. Dahlke, M. Macari and R.L. Furlan, 2006. Dietas de baixa proteína no desempenho de frangos criados em diferentes temperaturas. *Pesq. Agrop. Bras.* 41: 101-106.
- Geraert, P.A., S. Guillaumin and L.M. Zuprizal, 1992. Effect of high ambient temperature on dietary ME value in genetically lean and fat chickens. *Poult. Sci.*, 71: 2113-2116.
- Geraert, P.A., J.C.F. Padilha and S. Guillaumin, 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. *Br. J. Nutr.*, 75: 195-204.
- Gonzalez-Esquerro, R. and S. Lesson, 2006. Physiological and metabolic responses of broilers to heat stress – implications for protein and amino acid nutrition. *World's Poult. Sci. J.*, 62: 282-295.
- Gonzalez-Esquerro, R. and S. Lesson, 2005. Effects of acute versus chronic heat stress on broiler response to dietary protein. *Poult. Sci.*, 84: 1562-1569.
- Harper, A.E., N.J. Benevenga and R.M. Wohlhueter, 1970. Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev.*, 50: 428-558.
- Hruby, N., M.L. Hamre and C.N. Coon, 1995. Predicting amino acid requirements for broilers at 21.1°C and 32.2°C. *J. Appl. Poult. Res.*, 4: 395-401.
- Keshavarz, K. and H.L. Fuller, 1980. The influence of widely fluctuating temperatures on heat production and energetic efficiency of broilers. *Poult. Sci.*, 59: 2121-2128.
- Koh, K. and M.G. Macleod, 1999. Effects of ambient temperature on heat increment of feeding and energy retention in growing broilers maintained at different food intakes. *Br. Poult. Sci.*, 40: 511-516.
- Littell, R.C., W.W. Stroup and R.J. Freund, 2002. SAS For Linear Models. SAS Institute, Cary, DC.
- Macleod, M.G., 1990. Energy and nitrogen intake, expenditure and retention at 20°C in growing fowl given diets with a range of energy and protein contents. *Br. J. Nutr.*, 64: 625-637.
- Macleod, M.G., 1992. Energy and nitrogen intake, expenditure and retention at 32°C in growing fowl given diets with a range of energy and protein contents. *Br. J. Nutr.*, 67: 195-206.
- Macleod, M.G., 1997. Effects of amino acid balance and energy: protein ratio on energy and nitrogen metabolism in male broiler chickens. *Br. Poult. Sci.* 38: 405-411.
- Mateos, G.G. and J.L. Sell, 1980. Influence of carbohydrate and supplemental fat source on the metabolizable energy of the diet. *Poult. Sci.*, 59: 2129-2135.
- Musharaf, N.A. and J.D. Latshaw, 1999. Heat increment as affected by protein and amino acid nutrition. *World's Poult. Sci. J.*, 55: 233-240.
- Nieto, R., J.F. Aguilera, I. Fernández-Fígares and C. Pietro, 1997. Effect of a low protein diet on the energy metabolism of growing chickens. *Arch. Anim. Nutr.*, 50: 105-119.
- Patterson, P.H. and Adrizal, 2005. Management strategies to reduce air emissions: emphasis-dust and ammonia. *J. Appl. Poult. Res.*, 14: 638-650.
- Rostagno, H.S., L.F.T. Albino, J.L. Donzele, P.C. Gomes, A.S. Ferreira, R.F. Oliveira and D.C. Lopes, 2000. Tabelas Brasileiras para Aves e Suínos: Composição de alimentos e exigências nutricionais. UFV, Viçosa, MG, Brazil.
- Silva, D.J. and A.C. Queiroz, 2002. Análise de Alimentos: Métodos químicos e biológicos. UFV, Viçosa, MG, Brazil.

Faria Filho *et al.*: Protein levels for heat-exposed broilers

- Swennen, Q., G.P.J. Janssens, E. Decuyper and J. Buyse, 2004. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: energy and protein metabolism and diet-induced thermogenesis. *Poult. Sci.*, 83: 1997-2004.
- Temim, S., A.M. Chagneau, S. Guillaumin, J. Michel, R. Peresson, P.A. Geraert and S. Tesseraud, 1999. Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. *Reprod. Nutr. Dev.*, 39: 145-156.
- Temim, S., A.M. Chagneau, S. Guillaumin, J. Michel, R. Peresson and S. Tesseraud, 2000 a. Does excess dietary protein improve growth performance and carcass characteristics in heat-exposed chickens? *Poult. Sci.*, 79: 312-317.
- Temim, S., A.M. Chagneau, R. Peresson and S. Tesseraud, 2000 b. Chronic heat exposure alters protein turnover of three different skeletal muscles in finishing broiler chickens fed 20 or 25% protein diets. *J. Nutr.*, 130: 813-819.
- Wallis, I.R. and D. Banalve, 1984. The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broiler chickens. *Poult. Sci.*, 25: 401-407.
- Yamazaki, M. and Z. Zi-Yi, 1982. A note on the effect of temperature on true and apparent metabolizable energy values of a layer diet. *Br. Poult. Sci.*, 23: 447-450.
- Zarate, A.J., E.T. Moran and D.J. Burnham, 2003a. Exceeding essential amino acid requirements and improving their balance as a means to minimize heat stress in broilers. *J. Appl. Poult. Res.*, 12: 33-44.
- Zarate, A.J., E.T. Moran and D.J. Burnham, 2003 b. Reducing crude protein and increasing limiting essential amino acid levels with summer-reared, slow- and fast-feathering broilers. *J. Appl. Poult. Res.*, 12: 160-168.