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## Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens

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**Abstract** — The respective effects of ambient temperature, dietary crude protein and feed intake were investigated in finishing chickens and the consequence of protein supplementation under high temperature conditions was analysed in particular. Heat-related reduction in growth was associated with decreased nitrogen retention (–30 or –35 % according to the diet), which could not be explained by the observed lower feed intake alone. Tissue samples performed in 5- to 6-week-old chicks showed varying effects of heat according to the muscles studied: at 32 °C, the proportion of *Pectoralis major* muscle (in percentage of body weight) appeared slightly reduced (reduction lower than 10 %), whereas the proportion of two leg muscles were increased (+10 to +15 % for the *Sartorius* muscle; +5 % for the *gastrocnemius* muscle). At 32 °C, providing a high protein diet significantly ( $P < 0.05$ ) increased weight gain and feed efficiency, and slightly improved whole body protein deposition. © Inra/Elsevier, Paris.

chronic heat exposure / dietary protein level / growth / nitrogen retention / muscle

**Résumé** — Influence de l'exposition chronique à la chaleur et de l'ingéré protéique sur les performances de croissance, la rétention azotée et le développement musculaire chez le poulet de chair. Nous avons étudié les effets respectifs de la température ambiante, du taux protéique du régime et de l'ingéré chez des poulets en finition et analysé en particulier l'incidence d'une supplémentation protéique lors de l'exposition à des températures élevées. La réduction de la croissance au chaud est associée à une diminution de la rétention azotée (–30 ou –35 % selon le régime) qui ne s'explique pas seulement par la baisse d'ingéré observée. Des prélèvements de tissus réalisés à 5–6 semaines montrent des effets différents de la chaleur selon les muscles : à 32 °C, la proportion de muscle *Pectoralis major* (en % du poids vif) apparaît légèrement réduite (réduction inférieure à

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10 %) alors que celles de deux muscles de la patte sont augmentées (+ 10 à 15 % pour le *Sartorius* ; + 5 % pour le *Gastrocnemius*). A 32 °C, la distribution du régime riche en protéines entraîne une augmentation significative ( $p < 0,05$ ) du gain de poids et de l'efficacité alimentaire, et une légère amélioration du dépôt protéique corporel. © Inra/Elsevier, Paris.

## exposition à la chaleur / taux protéique du régime / croissance / rétention azotée / muscle

### 1. INTRODUCTION

High ambient temperatures induce economic losses for the poultry industry both in hot countries and in regions with a temperate climate such as France: 40 million francs are lost per year during the summer [6]. Heat exposure decreases feed consumption in order to reduce metabolic heat production and maintain homeothermy, and also leads to a lower weight gain [3, 8, 13, 17]. Compared with pair-fed birds exposed to thermoneutrality, heat-exposed chickens still exhibit slower growth and decreased feed efficiency [14, 15].

The impact of heat exposure can be reduced by controlling environmental factors (house temperature and ventilation) or by breeding techniques such as fasting, water control and limited poultry density in the building. However, alternative strategies involving feeding need to be considered to improve chicken growth in hot environments, e.g. dietary supplementation with electrolytes or ascorbic acid, and modifications of dietary composition [3, 5, 10, 21, 24]. Another solution also consists in increasing dietary crude protein in order to limit the reduction in protein intake under heat stress conditions.

This strategy has been controversial since protein usually contributes more to the heat increment than carbohydrates or fat. Some authors have suggested minimising protein levels and improving the balance of amino acids by adding synthetic essential amino acids [3, 30, 31]. However, whereas the negative effect of excess protein is incontestable at thermoneutrality, increasing the dietary

protein level at 32 °C decreases the heat increment [14]. Moreover, providing high protein diets can improve chicken growth in hot environments [7, 20]. We therefore studied the effect of chronic heat exposure (32 versus 22 °C) and dietary crude protein (25 versus 20 %) on body and muscle growth of broiler chickens between 4 and 6 weeks of age. We also investigated the influence of decreased feed intake at both 22 and 32 °C.

### 2. MATERIALS AND METHODS

#### 2.1. Birds and experimental design

Day-old ISA JV15 male broiler chicks ( $n = 350$ ) from a local hatchery (Sicamen, Bouloire, France) were reared in individual battery cages in controlled environment rooms. Water was available ad libitum. Up to 28 days of age, they received a complete starter diet of the following composition ( $\text{g}\cdot\text{kg}^{-1}$ ): maize 485, wheat 126, soyabean meal 220, meat meal 41, fat 40, maize gluten 50, dicalcium phosphate 12.7, calcium carbonate 12.4, sodium chloride 4, L-lysine 2.0, DL-methionine 0.9, vitamins and minerals 6. The 23 h/1 h light/dark cycle lighting program was maintained until the end of the experiment. The ambient temperature was gradually decreased from 32 °C when the birds were 1 day old to 22 °C when they were 4 weeks old. At 4 weeks of age, birds were fasted for 4 h, weighed, and then 216 of them were selected in order to form six groups of similar weights ( $1\ 165 \pm 12$  g). They were placed in individual battery cages in controlled environment rooms maintained at a constant temperature of either 32 or 22 °C and relative humidity was maintained at about 55 %. Birds were fed either a control diet (C) or a high protein diet (HP) from 4 to 6 weeks of age (table I). The analysed protein contents were 19.6 and

**Table I.** Composition of experimental control (C) and high protein (HP) diets (g·kg<sup>-1</sup>).

	C Diet	HP Diet
<b>Ingredients and analysis</b>		
Maize	624.0	240.0
Corn gluten meal (60 % CP)	17.5	12.0
Wheat		294.3
Soyabean meal (48 % CP)	278.0	296.7
Soyabean protein		65.0
Rapeseed oil	38.4	50.0
Calcium carbonate	11.6	11.7
Dicalcium phosphate	19.4	19.3
Salt	4.0	4.0
Trace mineral <sup>1</sup>	1.0	1.0
Vitamins <sup>1</sup>	5.0	5.0
DL-methionine	0.8	1.0
Lysine-HCL	0.3	
<b>Calculated analysis</b>		
Metabolisable energy (kcal·kg <sup>-1</sup> )	3 100	3 090
Crude protein	196	251
Crude fat	70	73
Lysine	10.1	13.8
Sulphur amino acids	7.6	9.5
Threonine	7.4	9.3
Valine	10.0	12.8
Tryptophan	2.2	3.1
Leucine	18.7	20.9
Isoleucine	9.1	12.2

<sup>1</sup> See Tesseraud et al. [27, 29].

24.7 % in C and HP diets, respectively. All the diets were isoenergetic, in pellet form (2.5 mm in diameter) and had the same proportions of amino acids in relation to lysine content. At both rearing temperatures, the control diet (C) was given either ad libitum (AL groups) or at a restricted feed intake rate (RF groups) corresponding to 80 % of ad libitum intake.

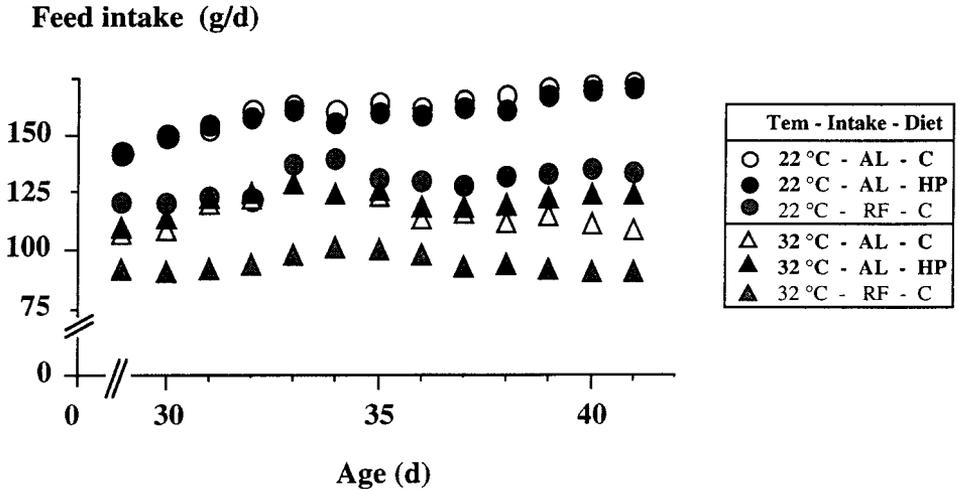
## 2.2. Measurements

Daily feed intake was individually measured at 0900 hours each morning. *Figure 1* shows the actual values for food intake. Note that relatively similar amounts of feed were consumed in the RF group fed the C diet and exposed to 22 °C and in the AL group fed the HP diet and exposed to 32 °C. Growth performance was determined during the experimental period (28 to 42 days of age). Body weights were recorded after a 4-h

period without feed ( $n = 25-30$  for AL groups and  $n = 18$  for RF groups).

Nitrogen retention, i.e. whole body protein deposition, was estimated in 7-8 chickens per treatment at 5.5 weeks of age by the difference between nitrogen intake and excretion over a 2-day period (between 38 and 40 days of age). The chickens selected displayed growth performance similar to the mean of the group. Excreta were collected, weighed and freeze-dried. Nitrogen contents of diet and excreta were measured by the Kjeldahl procedure.

Finally, between 5 and 6 weeks of age, 14 (in AL groups) and 9-10 (in RF groups) birds were killed after pentothal injection and exsanguinated. These chickens displayed growth performances similar to the mean of their group. Three types of skeletal muscles were chosen on the basis of their histochemical properties: the *Pectoralis major* (a breast muscle, entirely fast-twitch glycolytic fibre type), the *Sartorius* and the *Gastrocnemius*



**Figure 1.** Daily feed intake of male chickens exposed to 22 or 32 °C and fed a control diet (C) or a high-protein diet (HP) from 4 to 6 weeks of age. Values are means. For details of diets and procedures, see *table 1* and Materials and methods section. AL, ad libitum; RF, restricted feed; Tem, temperature.

(two leg muscles, mixed-fibre type) muscles. The left *Pectoralis major*, *Sartorius* and *Gastrocnemius* muscles were excised, weighed, frozen in liquid nitrogen and finally stored at -20 °C until analysis. Frozen muscles were finely ground in liquid nitrogen and the procedures were performed as described previously [27–29]. Tissue protein content was measured according to Smith et al. [26] by the colorimetric reaction with bicinchoninic acid (Pierce, Rockford, USA). Tissue RNA content was measured on the basis of the ultraviolet (UV) absorbance at 260 nm, with a correction for peptide material based on the UV absorbance at 232 nm, as described by Munro and Fleck [23]. The ribosomal capacity, i.e. the capacity for protein synthesis, was estimated as the ratio of RNA to protein [28, 29].

### 2.3. Statistical analysis

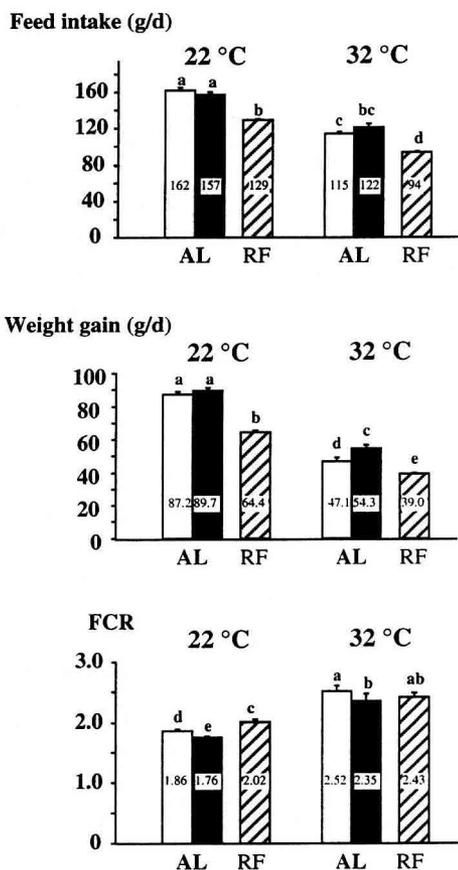
Values are given as means with their standard errors (SE). SE often differed between the six groups since feed restriction reduced variability and high temperature increased it. Due to the heterogeneity of variance between treatments (determined by the Bartlett test), data were analysed using the Kruskal-Wallis non-parametric test [19] and means were compared by the Mann-

Whitney U test (see Materials and methods sections of [1, 2, 15]). This heterogeneity was not observed for muscle proportions and ribosomal capacities; these findings were analysed using a classical variance analysis procedure (parametric test). Three-way ANOVA was performed to discriminate between the effects of temperature, diet and intake level, and their interactions.

## 3. RESULTS

### 3.1. Growth performance

At 22 °C, 6-week body weights were  $2\,389 \pm 31$  and  $2\,421 \pm 28$  g for AL groups fed C and HP diets, respectively, and  $2\,069 \pm 18$  g for the RF group fed the C diet. At 32 °C, they were  $1\,821 \pm 27$  and  $1\,925 \pm 34$  g for AL groups fed C and HP diets, respectively, and  $1\,708 \pm 16$  g for the RF group fed the C diet. Average values for feed intake, weight gain and feed conversion ratio (grams of feed per gram of gain) are presented in *figure 2*. Chronic heat exposure significantly decreased feed intake and weight gain ( $P < 0.001$ ); for feed intake, -29 and -23 % with C and HP diets, respec-



**Figure 2.** Feed intake, weight gain and feed conversion ratio (FCR) of male chickens exposed to 22 or 32 °C and fed a control diet (C) (□) or a high-protein diet (HP) (■) from 4 to 6 weeks of age. Values are means  $\pm$  SE ( $n = 25\text{--}30$  for AL groups;  $n = 18$  for RF groups); means within parameter with no common letter differ significantly ( $P < 0.05$ ); AL, ad libitum; RF, restricted feed.

tively; for weight gain,  $-46$  and  $-39\%$ , respectively. Since weight gain was more reduced than feed intake, the feed conversion ratio was increased under high temperatures ( $P < 0.001$ ) (approximately  $+35\%$  irrespective of diet).

Feed restriction by about  $20\%$ , irrespective of diet, significantly decreased

weight gain:  $-26$  and  $-17\%$  at 22 and 32 °C, respectively. The feed conversion ratio was thus increased at 22 °C ( $+9\%$ ,  $P < 0.05$ ), whereas it was unchanged at 32 °C ( $-4\%$ ,  $P = 0.50$ ).

The HP diet did not significantly alter feed intake at the two ambient temperatures (figure 2). Note, however, that even though there was no effect of protein level on feed intake at 22 °C, the HP diet increased feed intake in the second week at 32 °C (figure 1). It increased weight gain at 32 °C ( $+15\%$ ,  $P < 0.05$ ) but there was no change at 22 °C (variation lower than  $5\%$ ,  $P = 0.38$ ). Finally, it resulted in significantly reduced feed conversion ratios ( $-5$  and  $-7\%$  at 22 and 32 °C, respectively;  $P < 0.05$ ).

### 3.2. Nitrogen balance

Irrespective of diet, heat exposure decreased nitrogen intake expressed both in g/d (approximately  $-27\%$ ,  $P < 0.01$ ) and in g/d per kg BW (about  $-13\%$ ,  $P < 0.05$ ) (table II). In contrast, it did not modify nitrogen excretion ( $P > 0.05$ ) and, as a result, it very significantly reduced nitrogen retention ( $P < 0.01$ ):  $-47$  and  $-40\%$  with C and HP diets, respectively, when expressed in g/d;  $-37$  and  $-29\%$ , respectively, when expressed in g/d per kg BW.

Irrespective of ambient temperature, feed restriction decreased nitrogen intake by approximately  $20\%$  when expressed in g/d or by  $10\text{--}15\%$  when expressed in g/d per kg BW. It also reduced nitrogen excretion, mainly under high temperatures, with reductions of  $30$  to  $35\%$  depending on the mode of expression (g/d or g/d per kg BW). It therefore resulted in significantly decreased nitrogen retention at 22 °C ( $-10$  to  $-20\%$  depending on the mode of expression) without any clear modification at 32 °C (variations lower than  $10\%$ ,  $P > 0.20$ ). Note that the chickens exposed to 32 °C and fed the C diet had lower nitrogen retention than the feed-restricted chickens kept at thermoneu-

**Table II.** Nitrogen balance of male chickens exposed to 22 or 32 °C and fed a control diet (C) or a high protein diet (HP) from 4 to 6 weeks of age.

Temperature	22 °C				32 °C			
	AL		RF		AL		RF	
	C (n = 7)	HP (n = 8)	C (n = 8)	RF (n = 7)	C (n = 8)	HP (n = 8)	C (n = 8)	RF (n = 8)
5.5-week body weight (BW) (g)	2 051 ± 26 <sup>a</sup>	2 068 ± 31 <sup>a</sup>	1 811 ± 25 <sup>b</sup>		1 706 ± 29 <sup>c</sup>	1 721 ± 37 <sup>c</sup>	1 573 ± 26 <sup>d</sup>	
Nitrogen intake (g/d)	5.15 ± 0.15 <sup>b</sup>	5.98 ± 0.15 <sup>a</sup>	4.13 ± 0.01 <sup>c</sup>		3.66 ± 0.25 <sup>d</sup>	4.41 ± 0.22 <sup>c</sup>	2.88 ± 0.01 <sup>e</sup>	
(g/d/kg BW)	2.51 ± 0.05 <sup>b</sup>	2.90 ± 0.08 <sup>a</sup>	2.28 ± 0.03 <sup>c</sup>		2.15 ± 0.14 <sup>c</sup>	2.56 ± 0.10 <sup>b</sup>	1.83 ± 0.03 <sup>d</sup>	
Nitrogen excretion (g/d)	2.24 ± 0.10 <sup>b</sup>	2.97 ± 0.08 <sup>a</sup>	1.80 ± 0.02 <sup>c</sup>		2.11 ± 0.21 <sup>bc</sup>	2.60 ± 0.16 <sup>ab</sup>	1.36 ± 0.04 <sup>d</sup>	
(g/d/kg BW)	1.09 ± 0.04 <sup>b</sup>	1.44 ± 0.04 <sup>a</sup>	0.99 ± 0.02 <sup>c</sup>		1.25 ± 0.13 <sup>abc</sup>	1.51 ± 0.08 <sup>ab</sup>	0.87 ± 0.04 <sup>d</sup>	
Nitrogen retention (g/d)	2.91 ± 0.09 <sup>a</sup>	3.01 ± 0.10 <sup>a</sup>	2.34 ± 0.01 <sup>b</sup>		1.55 ± 0.19 <sup>c</sup>	1.80 ± 0.15 <sup>c</sup>	1.52 ± 0.05 <sup>c</sup>	
(g/d/kg BW)	1.42 ± 0.04 <sup>a</sup>	1.46 ± 0.05 <sup>a</sup>	1.29 ± 0.01 <sup>b</sup>		0.90 ± 0.10 <sup>c</sup>	1.04 ± 0.07 <sup>c</sup>	0.96 ± 0.02 <sup>c</sup>	

Means ± SE; values measured at 5.5 weeks of age.

Means in the same horizontal row with different superscript letters were significantly different ( $P < 0.05$ ).

AL, ad libitum and RF, restricted feed.

trality ( $-30\%$ ,  $P < 0.05$ ), although nitrogen intake, expressed in g/d per BW, did not significantly differ between these two groups ( $P = 0.86$ ). This suggests that the heat-related reduction in nitrogen retention could not be explained by decreased feed intake under hot conditions.

The HP diet increased nitrogen intake (+ 15 to + 20 % according to the ambient temperature) and nitrogen excretion (+ 20 to + 30 %) whatever the mode of expression (g/d or g/d per kg BW). Nitrogen retention was not changed at 22 °C (only + 3 % of variation), whereas it was increased at 32 °C (+ 16 %); however, this diet-related difference did not reach statistical significance.

### 3.3. Muscle characteristics

Muscle weights, protein and RNA contents are presented in *table III*. They were decreased by chronic heat exposure ( $P < 0.01$ ) and slightly increased by the HP diet ( $P > 0.05$ ). Muscle proportions and ribosomal capacities are presented in *figure 3*. High temperatures slightly decreased the proportion of *Pectoralis major* muscle (reduction lower than 10 %,  $P = 0.06$ ), whereas the proportions of the two leg muscles were significantly increased (about + 10 to 15 % for the *Sartorius* muscle, + 5 % for the *Gastrocnemius* muscle). Three-way ANOVA showed a significant effect of the intake level (i.e. feed restriction) only for the *sartorius* muscle ( $P > 0.25$  for the other muscles). It showed a diet effect ( $P < 0.05$ ) on *Gastrocnemius* muscle proportions. There was only a tendency to a diet effect or no effect at all for the two other muscle proportions. Finally, in terms of ribosomal capacity (Cs), there was no significant effect of diet or intake level, irrespective of muscle. Conversely, there was a very significant ambient temperature effect ( $P < 0.001$ ) and heat-exposed chickens had lower Cs than birds kept at thermoneutrality, irrespective of diet or intake level. It is of note that the interactions between diet and temperature and between

temperature and intake level were not significant for Cs or muscle proportion, irrespective of muscles studied.

## 4. DISCUSSION

As reported in the literature, heat-exposed chickens showed decreased growth [3, 8, 13, 17]. Moreover, the reduction in growth was greater than the reduction in feed intake, resulting in increased feed to gain ratio, in agreement with previous data [1, 15, 17]. Chronic heat exposure appears therefore to have a depressive effect which is independent of the decrease in feed intake. A similar conclusion has been drawn comparing chickens reared at 22 and 32 °C and receiving the same feed intake [14, 15]. Conversely, in rats and piglets an improvement in feed efficiency was observed at high temperatures [9, 25]. We also showed that heat reduced nitrogen retention, this effect being independent of feed intake. According to Geraert et al. [15], chronic exposure to 32 °C also decreases protein gain ( $-54\%$ ) and protein retention efficiency ( $-46\%$ ).

Under high environmental temperatures, chickens had a slightly lower proportion of *Pectoralis major* muscle ( $P = 0.06$ ), whereas the proportion of the two leg muscles was significantly increased ( $P < 0.001$  for the *Sartorius* muscle and  $P < 0.05$  for the *Gastrocnemius* muscle). Howlader and Rose [18] similarly found a reduced breast meat yield. These results are also in agreement with those of Aïn Baziz et al. [1] who observed a decreased proportion of breast muscle ( $-12\%$ ) and a slight but significant increase in the proportion of leg muscles, i.e. thigh plus drumstick proportion (+ 6 %). Note that the heat-related modifications of proportion of muscle could not be explained by a lower feed intake since there was either no effect of feed intake level ( $P = 0.43$  for the *Pectoralis major* muscle and  $P = 0.29$  for the *Gastrocnemius* muscle) or an opposite effect between heat exposure and feed intake for the *Sartorius* muscle. Indeed, as

**Table III.** Muscle characteristics of male chickens exposed to 22 or 32 °C and fed a control diet (C) or a high protein diet (HP) from 4 to 6 weeks of age.

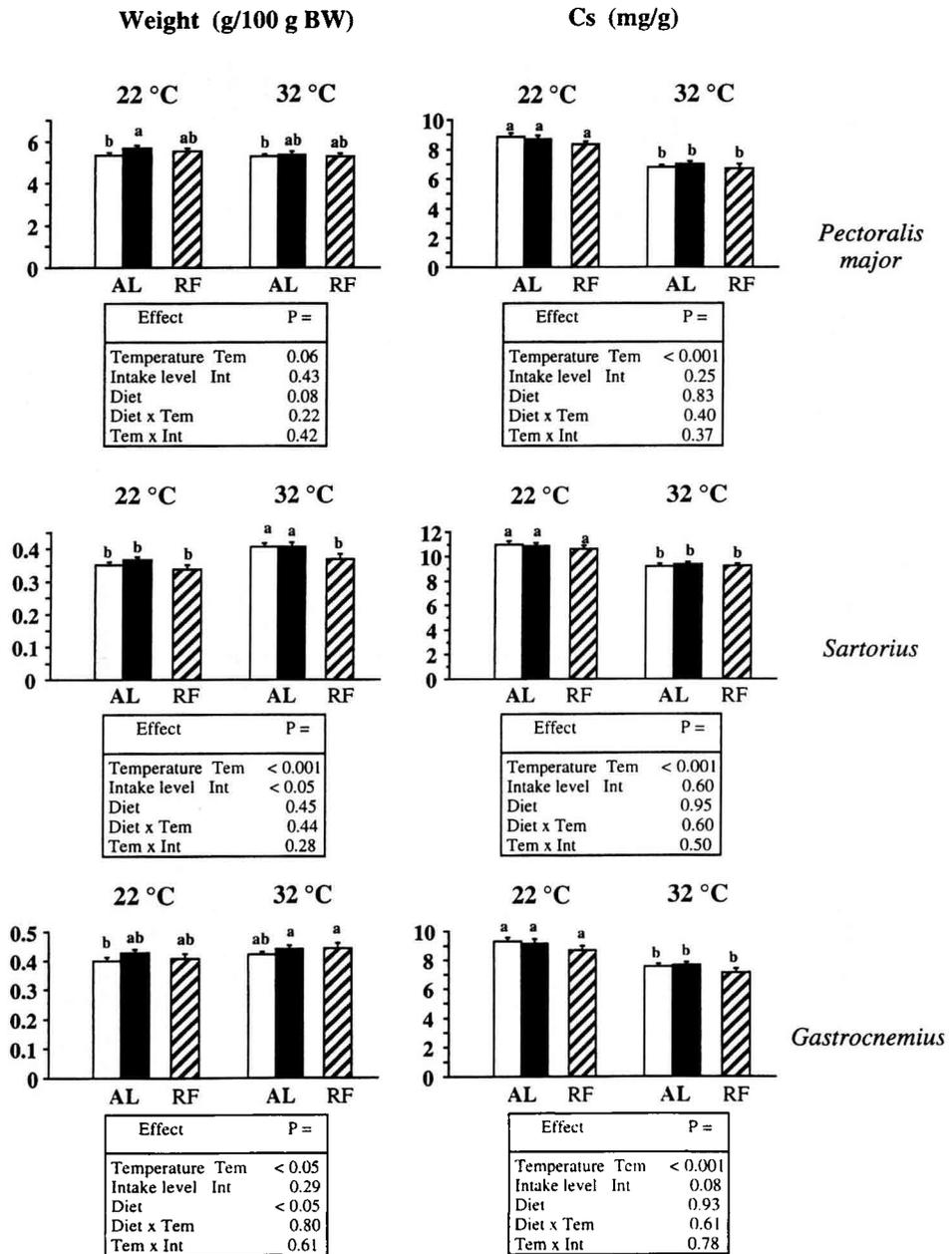
Temperature	22 °C				32 °C			
	Intake level	AL		RF	AL		RF	
		C (n = 14)	HP (n = 14)		C (n = 10)	C (n = 14)		HP (n = 14)
Body weight*	(g)	2 045 ± 75 <sup>a</sup>	2 070 ± 76 <sup>a</sup>	1 821 ± 76 <sup>b</sup>	1 679 ± 35 <sup>bc</sup>	1 728 ± 46 <sup>bc</sup>	1 573 ± 53 <sup>d</sup>	
<i>Pectoralis muscle</i>								
Weight	(g)	109.1 ± 5.5 <sup>ab</sup>	118.5 ± 6.0 <sup>a</sup>	100.8 ± 4.4 <sup>bc</sup>	89.3 ± 2.9 <sup>d</sup>	93.8 ± 4.5 <sup>cd</sup>	83.3 ± 3.1 <sup>d</sup>	
Protein content	(g)	15.00 ± 0.80 <sup>ab</sup>	15.98 ± 0.85 <sup>a</sup>	13.52 ± 0.61 <sup>bc</sup>	11.83 ± 0.32 <sup>d</sup>	12.26 ± 0.56 <sup>cd</sup>	10.94 ± 0.53 <sup>d</sup>	
RNA content	(mg)	130.8 ± 5.2 <sup>a</sup>	136.4 ± 6.6 <sup>a</sup>	112.1 ± 4.0 <sup>b</sup>	79.4 ± 2.3 <sup>c</sup>	86.2 ± 4.6 <sup>c</sup>	73.4 ± 4.3 <sup>c</sup>	
<i>Sartorius muscle</i>								
Weight	(g)	7.2 ± 0.3 <sup>a</sup>	7.6 ± 0.3 <sup>a</sup>	6.2 ± 0.4 <sup>bc</sup>	6.9 ± 0.2 <sup>ab</sup>	7.1 ± 0.4 <sup>ab</sup>	5.7 ± 0.4 <sup>c</sup>	
Protein content	(g)	0.77 ± 0.03 <sup>ab</sup>	0.80 ± 0.03 <sup>a</sup>	0.68 ± 0.03 <sup>bc</sup>	0.73 ± 0.02 <sup>abc</sup>	0.79 ± 0.04 <sup>a</sup>	0.63 ± 0.04 <sup>c</sup>	
RNA content	(mg)	8.4 ± 0.4 <sup>a</sup>	8.6 ± 0.4 <sup>a</sup>	7.3 ± 0.4 <sup>b</sup>	6.7 ± 0.2 <sup>bc</sup>	7.3 ± 0.4 <sup>b</sup>	5.8 ± 0.4 <sup>c</sup>	
<i>Gastrocnemius muscle</i>								
Weight	(g)	8.2 ± 0.4 <sup>ab</sup>	9.0 ± 0.5 <sup>a</sup>	7.5 ± 0.5 <sup>b</sup>	7.1 ± 0.2 <sup>b</sup>	7.7 ± 0.3 <sup>b</sup>	7.0 ± 0.4 <sup>b</sup>	
Protein content	(g)	0.99 ± 0.05 <sup>ab</sup>	1.08 ± 0.06 <sup>a</sup>	0.89 ± 0.07 <sup>bc</sup>	0.83 ± 0.02 <sup>c</sup>	0.93 ± 0.05 <sup>bc</sup>	0.87 ± 0.07 <sup>bc</sup>	
RNA content	(mg)	9.1 ± 0.3 <sup>a</sup>	9.7 ± 0.4 <sup>a</sup>	7.7 ± 0.5 <sup>b</sup>	6.2 ± 0.2 <sup>c</sup>	7.0 ± 0.3 <sup>bc</sup>	6.2 ± 0.5 <sup>c</sup>	

\* Measured on the day of the experiment (between 5 and 6 weeks of age).

Means ± SE; values measured between 5 and 6 weeks of age.

Means in the same horizontal row with different superscript letters were significantly different ( $P < 0.05$ ).

AL., ad libitum and RF, restricted feed.



**Figure 3.** Muscle proportions and ribosomal capacities (Cs) of male chickens exposed to 22 or 32 °C and fed a control diet (C) (□ ▨) or a high-protein diet (HP) (■) from 4 to 6 weeks of age. Values are means ± SE ( $n = 14$  for AL groups;  $n = 9-10$  for RF groups); measurements performed between 5 and 6 weeks of age; means within parameter with no common letter differ significantly ( $P < 0.05$ ); AL, ad libitum; RF, restricted feed; BW, body weight. Tables summarise results of statistical analysis (three-way ANOVA).

for *sartorius* muscle proportions, there was a significant reduction ( $P < 0.05$ ) with a lower feed intake and conversely a significant increase ( $P < 0.001$ ) induced by high temperatures. In the present experiment, the *Pectoralis major* muscle was the most sensitive to high temperatures. The differential response to heat exposure in individual muscles could be characteristic of the fibre type composition of that muscle. The response of the *Pectoralis major* muscle, which is an entirely fast-twitch glycolytic fibre type muscle, is greater to nutritional factors such as lysine deficiency than the mixed fibre type *Sartorius* muscle [27]. In particular, differences might be related to the energy characteristics of these muscles and thus to their respective substrates: glucose and fatty acids.

We found a direct effect of heat exposure on muscle Cs. At high temperatures, the reduced capacity for protein synthesis represented changes in protein metabolism which could explain reduced gain in muscle protein and therefore in muscle development. Further investigations of muscle protein metabolism, including the measurement of in vivo protein synthesis by an appropriate technique, e.g. the flooding dose method developed by Garlick et al. [12] and the determination of proteolysis, need to be undertaken to provide a better understanding of the regulation of muscle protein deposition.

In the present study, the HP diet appeared to be beneficial at 32 °C since it reduced the adverse effects of high temperatures on growth and feed efficiency. This is in agreement with results obtained by Kubena et al. [20]. This effect might, however, be dependent on the genotype since birds with different growth rates and fatness respond differently to dietary protein level under heat stress [7]. In suggesting a positive effect of a high protein diet in hot conditions, our results contradict the idea that lowering dietary protein content with suitable supplementation with essential amino acids represents a way to improve chicken perfor-

mance in this stress situation [3, 30, 31]. On the contrary, recent findings have shown that providing a low protein diet (16 % crude protein with added lysine, methionine, threonine, arginine and valine versus 20 %) does not prevent a negative heat effect, and it even worsens performance [2].

The enhanced growth due to the high protein diet was not related to an increase in feed intake in hot conditions. Note, however, that the HP diet did not reduce feed intake at 32 °C, whereas metabolic heat production could be increased, subsequently limiting feed consumption. In fact, while heat production expressed as a proportion of ME intake was increased by higher dietary protein content (23 versus 19 % crude protein) at 22 °C, it was depressed by a high protein diet at 32 °C [14]. This result observed in experimental lines is consistent with the findings of a previous study by McLeod [22] in broilers.

In the present study, increasing dietary protein content in hot conditions limited the heat-related reduction in protein intake: 30.1 g protein per day at 32 °C with the HP diet compared to 22.5 g protein per day at 32 °C with the C diet, the value at 22 °C with the C diet being equal to 31.8 g protein per day. Chick growth became better despite remaining lower than that recorded at thermoneutrality. Note that our results did not allow determination of protein requirement under chronic heat exposure. This requires further investigation. Moreover, several authors have suggested that requirements in some amino acids other than lysine and methionine can be increased by high temperatures [2, 4, 5, 11, 16].

In conclusion, our results indicate that it is possible to limit the adverse effects of high ambient temperatures using high protein diets. The poorly understood mechanisms that regulate chicken metabolism under heat exposure need to be elucidated in order to provide a basis for diet formulation (protein requirement and amino acid supplementation) and to better adapt nutrition to high environmental temperatures.

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