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## PROCESSING AND PRODUCTS

### The Effect of Thermal Preslaughter Stress on the Susceptibility of Broiler Chickens Differing with Respect to Growth Rate, Age at Slaughter, Blood Parameters, and Ascites Mortality, to Hemorrhages in Muscles

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**ABSTRACT** In this study we investigated the occurrence of hemorrhages in four groups of electrically stunned broilers, differing with respect to growth rate, age at slaughter, hemodynamic parameters, and ascites mortality. In addition, the effect of three thermal preslaughter conditions on hemorrhage occurrence in thigh and breast muscles was studied. Broilers were either reared at a thermoneutral or low temperature regimen, and were either restricted in their feed consumption or consumed feed *ad libitum*. Prior to slaughter the broilers were exposed for 2–0.5 h to either cold (4–2 C, RH 100%), moderate (19–2 C, RH: 70 to 80%), or warm (30–2 C, RH: 60 to 70%)

conditions. There was no effect of rearing group, nor was there an interaction between rearing group and preslaughter condition on hemorrhage scores in the thighs or breasts. Preslaughter conditions only affected hemorrhage scores in the left thigh. Scores were highest in broilers exposed to moderate preslaughter conditions. These data indicate that the cause of hemorrhages in muscles is multifactorial. Hemorrhage severity was not diminished in broilers retarded in growth. Pathological hemodynamic adaptations to low rearing temperatures, leading to ascites, did not increase hemorrhage severity, neither upon exposure to moderate nor to cold or warm preslaughter conditions.

(Key words: hemorrhage, broiler, rearing temperature, feed restriction, thermal preslaughter stress)

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## INTRODUCTION

Hemorrhages in muscle tissue are major quality defects of carcasses from slaughter animals (Warrington, 1974; Griffiths and Nairn, 1984; Bilgili, 1992; Veerkamp, 1992; Hillebrand, 1993). Electrical stunning prior to slaughter induces blood splash in carcasses of lambs (Kirton *et al.*, 1981), pigs (Lambooy and Sybesma, 1988), and broilers (Hillebrand *et al.*, 1996; Kranen *et al.*, 1996). Susceptibility for muscle hemorrhages induced by electrical stunning depends on the constitution of the individual bird, determined by the interaction of genetic and environmental factors, as well as the physical condition of the bird at the moment of stunning.

Fast-growing meat-type chickens are selected for high growth rate and an extensive musculature. They reach their slaughter weight at a young age. Growth of connective tissue may not have kept up with the growth of muscle fibers, as reported for turkey breast muscle (Swatland, 1990; Hillebrand, 1993). The degree of capillarization is relatively low, due to the large diameter of the muscle fibers (Griffin and Goddard,

1994). As a result, the diffusion distance for blood gasses and metabolites in the muscle tissue is long. The sarcoplasmic regulation of cellular free calcium is inefficient (Reiner *et al.*, 1995). Plasma levels of skeletal muscle enzymes creatine kinase and lactate dehydrogenase were higher in a fast-growing than in an unselected line (Mitchell and Sandercock, 1994), indicating an impaired integrity of the sarcolemma. Activity may overload and exhaust the muscle tissue and, consequently, initiate mechanisms of (severe) muscular damage (Knochel, 1993; Reiner *et al.*, 1995).

Fast-growing broilers consuming feed *ad libitum* are reported to display a rather passive and lethargic behavior (Deaton, 1995), probably due to a mild form of hypothyroidism (Scheele, 1996). Activity will be further decreased by an abundance of feed, a high flock density, and by musculoskeletal anomalies caused by the high growth rate (Savory, 1992, 1995; Hocking, 1994). These birds can be considered as untrained animals, predisposed to muscular damage induced by sudden strenuous muscular activity (Knochel, 1993; Poels and Gabreëls, 1993). Restriction of feed retards growth and changes metabolism as well as body composition. Feed-restricted broilers will display a more active behavior (Savory, 1992; Deaton, 1995). They will reach the slaughter weight at an older age. Efficiency of calcium

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regulation of the skeletal muscle cell and development of connective tissue will probably be improved, because these processes are dependent on growth rate and age (Reiner *et al.*, 1995). Feed restriction may eliminate the predisposition of broilers to muscle damage and reduce the susceptibility to hemorrhages and blood splash in muscle tissue, either induced by handling, (electrical) stunning, or both.

At low rearing temperatures, pathological blood circulation adaptations and ascites can occur (Julian, 1993; Kranen *et al.*, 1996; Scheele, 1996). Affected birds may not respond adequately to acute (thermal) stress occurring during handling, transport, and restraining prior to slaughter. They may be particularly vulnerable to mechanisms affecting muscle energy metabolism, leading to muscle damage and hemorrhages. Feed restriction is reported to cancel out detrimental effects of low rearing temperatures (Albers *et al.*, 1990; Julian, 1993).

Broiler breeders reduce their metabolic rate per unit of weight upon feed restriction (McLeod *et al.*, 1993). They are able to regulate their body temperature adequately at high ambient temperatures. According to Freeman (1984) and Hocking *et al.* (1994), feed restriction makes chickens more resistant to acute heat stress. The broilers are probably less susceptible to detrimental effects of hyperthermia, such as disturbances of the electrolyte and acid-base balance, (muscular) metabolism, and sarcolemmal integrity (Smith, 1986; Chen *et al.*, 1994; Mitchell and Sandercock, 1995). We hypothesize that feed-restricted broilers will be less susceptible to muscle damage and hemorrhages than broilers that consume feed *ad libitum*, upon exposure to warm preslaughter conditions.

In this study, broilers were either restricted in their feed consumption or consumed feed *ad libitum*. They were subjected to thermoneutral or low temperatures rearing conditions. As a result, four groups of broilers were created, differing with respect to growth rate, age at slaughter, blood circulation, and susceptibility to ascites. The purpose of this study was to investigate the effects of thermal stress prior to slaughter on hemorrhages in muscle tissue of broilers from these four groups.

## MATERIALS AND METHODS

### *Rearing and Processing*

A factorial experiment was performed with four broiler groups differing with respect to growth rate, age at slaughter, blood circulation, and susceptibility to ascites, and three preslaughter conditions, cold, moderate, and warm. The experiment was designed to slaughter all broilers at 1 d, having equal body weights, in order to avoid effects of day of slaughter.

TABLE 1. Rearing groups

Group	Feed regimen		Temperature regimen	
	<i>Ad libitum</i>	Restricted	Thermo-neutral	Low temperature
1	*		*	
2	*			*
3		*	*	
4		*		*

\*Treatment period.

For this experiment, Hybro<sup>2</sup> broilers either consumed feed *ad libitum* or were restricted in their feed consumption. They were reared under thermoneutral or low temperatures conditions. Group 1 consumed feed *ad libitum* and was reared at a thermoneutral regimen, Group 2 consumed feed *ad libitum* as well, but was reared at a low temperatures regimen, Group 3 was restricted in its feed consumption, and reared at a thermoneutral regimen, and Group 4 was feed-restricted and reared at a low temperatures regimen (Table 1). Rearing of feed-restricted broilers started at Day 0, whereas that of *ad libitum* broilers started at Day 9. All groups were transferred to individual cages at Day 31.

At Day 0, the day of hatching of the broilers to be restricted in their feed consumption (Groups 3 and 4), 352 male broilers were placed in two climate rooms, one climate room per temperature regimen. In each room, 16 floor pens were placed. Each pen (1.0 × 0.75 m) contained 11 broilers. Half of the pens within a climate room accommodated the experimental broilers, either from Group 3 or 4. The other half contained *ad libitum* broilers, used as a reference for the feed-restricted broilers. Feed restriction started at Day 7. At Day 9, 176 newly hatched male (Groups 1 and 2) and 176 female broilers were placed in two climate rooms, each containing 16 floor pens. Each pen accommodated 11 broilers, as on Day 0. All these broilers consumed feed *ad libitum*. The hens were placed in order to have the same density of birds per climate room as the feed-restricted birds of Groups 3 and 4.

At Day 0 and 9, respectively, a thermoneutral temperature regimen, i.e., a linear reduction of the ambient temperature from 33 C at the day of hatching to 19 C at Day 40 remaining constant until slaughter, was established in one of the two climate rooms. In the other climate room, a low temperature regimen was applied: a linear reduction of the ambient temperature from 30 C at the day of hatching to 15 C at Day 31, then constant until slaughter. The first 3 d continuous light was given to all groups. After that time, a regimen of 2 h light and 1 h dark was applied. Relative humidity of the air was kept constant at 60%. Water was provided for *ad libitum* consumption. Dead birds were removed daily. Necropsy was carried out the following day. Birds were judged to be ascitic upon exhibition of a dilated, hypertrophic heart, a hydropericardium, and an edema of the abdomen.

At Day 31, 288 experimental broilers (72 per group) were transferred to individual layer cages in four climate

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rooms, two rooms per temperature regimen. The 72 broilers from each group were distributed over two climate rooms. The redundant experimental broilers, as well as all reference broilers and hens were euthanatized and disposed of.

At Day 49, the day of slaughter, the broilers were subjected to thermal stress prior to slaughter. They were exposed for 1.5 to 2.5 h to one of three different preslaughter conditions: 1) cold: 4 °C, RH: 100% (code c); 2) moderate: 19 °C, RH: 70 to 80% (code m); 3) warm: 30 °C, RH: 60 to 70% (code w). The broilers were placed in individual cages in climate cells, one cell per preslaughter condition. They were subjected to thermal stress and subsequent slaughter in six batches of 48 broilers (16 broilers per batch per climate cell, i.e., 4 broilers per batch per group per climate cell).

Per group by preslaughter condition combination, 16 of the 24 broilers were individually stunned in a water bath for 4 s at 100 V (AC, 50 Hz). After neck cutting (unilateral jugular vein severance), they were bled for 3 min, scalded, plucked, and eviscerated. At 1 d post-mortem, both pectoral (breast) muscle pairs were excised and legs were separated from the carcasses. The remaining eight broilers were used to collect blood from, just before and after stunning and subsequent neck cutting. Feed was withdrawn from Group 1 and 2, 8 h before slaughter of the batch concerned. Broilers from Group 3 and 4 received their last feed 8 h before slaughter of the first batch of birds.

At Day 14 a *Salmonella enteritidis* infection was detected in the *ad libitum* group. All broilers were treated with Methoxazol-T<sup>3</sup> from Day 15 to 20. The antibiotic was administered with the drinking water (2‰, vol/vol).

## Measurements

Feed-restricted broilers and their reference groups were weighed three times a week. *Ad libitum* broilers were weighed on Day 17, 24, 29, and 31, and after Day 31, three times a week. At Day 48 all broilers were weighed individually. Feed consumption of the reference groups was measured simultaneously with body weight. The amount of feed to be provided to the restricted Groups 3 and 4, was set on 95% of that consumed by their reference groups, corrected for body weight. Half of the daily allowance was given in the morning, the rest in the evening. Adjustments were made immediately after each measurement. Feed consumption and body weight were determined consistently after the first feed turn of the feed restricted groups. After Day 31 the amount of feed to be provided to the feed-restricted groups was calculated by extrapolating the feed consumption of the reference groups, with respect to body weight.

From Day 8 to 31, mean body weight was derived from the pooled weight of all birds within a pen. From Day 32 to 45, the body weight of 18 individual broilers per group was measured. Body weight data as determined pen-wise from Day 8 to Day 31, could adequately be described by an exponential equation:  $y = y_8 e^{kt}$ ; where  $y$  = mean body weight per pen (grams);  $y_8$  = mean body weight at an age of 8 d (grams);  $k$  = growth rate parameter (grams per day);  $t$  = age (days). Values for  $r^2$  varied between 0.97 to 0.99. From Day 32 to 48, body weight gain could be described by a linear equation:  $y = y_{32} + bt$ ; where  $y_{32}$  = weight of the individual birds at Day 32; and  $b$  = growth rate parameter (slope: grams per day). Linear regression was significant, with  $r^2$  values between 0.97 and 0.99. The growth rate parameters  $k$  and  $b$  were subjected to statistical analysis. Growth retardation was calculated according to the equation:  $R = (D_i - 39)/39 \cdot 100$ , where  $R$  = growth retardation (percentage);  $D_i$  = the rearing period of the  $i$ th group to reach a weight of 2,065 g (the average weight of the broilers from Group 1 at the age of 39 d).

At Day 45, venous blood pressure was measured in the *vena ulnaris* of nine broilers per group, using a canula filled with heparinized water connected to a signal transducer and a digital electromanometer.<sup>4</sup> At Day 49, the day of slaughter, the birds were stunned, dried, and weighed. Blood loss was derived from the difference in body weight measured before, and 3 min after neck cutting and was expressed as a percentage of the body weight before bleeding. Hearts were collected after evisceration. The relative heart weight was determined as described by Kranen *et al.* (1996). Hemorrhages of breast muscles and thighs were scored independently by four observers. For classification a threshold model, consisting of a 5-point scale with 4 cutoff points, was used. Cutoff points were formed by photographs of breast and thigh muscles, showing a particular severity of hemorrhages; class 1: hemorrhage-free; class 5: numerous and extensive hemorrhages.

Blood samples were taken from the ulnar vein prior to stunning. Two milliliters of blood was immediately transferred to tubes containing 200  $\mu$ L 0.18 M sodium citrate and centrifuged for 15 min, 1,400 g, at 4 C. The plasma was frozen at -20 C until determination of the prothrombin time. Immediately after stunning, the jugular vein was exposed and severed. Approximately 10 mL of blood was collected in tubes containing 120  $\mu$ L 50 mg EDTA/mL, and stored on ice until measurement of blood viscosity and hematocrit.

Prothrombin time was determined using brain extract from 3-wk-old broilers as thromboplastin source. Brain extract was prepared as described by Doerr *et al.* (1975). The citrated plasma was thawed and 0.1 mL was pipetted into a polystyrene tube and incubated for 2 min at 40 C. Subsequently, 0.1 mL prewarmed brain extract, twice diluted in 25 mM CaCl<sub>2</sub>, was added, and the contents mixed. Fibrin formation (clot) was detected

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TABLE 2. Performance parameters

Rearing group	Growth rate parameters				Growth retardation (%)
	Exponential part <sup>1</sup>		Linear part <sup>2</sup>		
	k	n	b	n	
	(g/d)		(g/d)		
1	0.118 <sup>a</sup>	8	77.8 <sup>a</sup>	18	. . .
2	0.115 <sup>a</sup>	8	66.0 <sup>b</sup>	18	10.2
3	0.091 <sup>b</sup>	8	78.3 <sup>a</sup>	18	13.8
4	0.093 <sup>b</sup>	8	66.9 <sup>b</sup>	18	16.4
LSD	0.003		7.7		

<sup>a,b</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Growth rate parameter (k) of exponential part of the body weight gain curve.

<sup>2</sup>Growth rate parameter (b) of the linear part of the body weight gain curve.

manually. Blood viscosity and hematocrit was measured as described by Kranen *et al.* (1996).

### Statistical Analysis

All data were analyzed with the statistical package Genstat 5 (Genstat Committee, 1993). Growth rate parameters, as well as body weight, blood pressure, blood viscosity, hematocrit, relative heart weight, and blood loss data were analyzed with an analysis of variance model. For analysis of growth rate parameters and body weight and blood pressure data, group was introduced into the model as a fixed term and climate room as a random term. For blood viscosity, hematocrit, relative heart weight, and blood loss data, fixed effects in the model were main effects of group and preslaughter condition and their interaction. Effects of batch were accounted for by random terms. Data were analyzed with Restricted Maximum Likelihood (REML). Tests for interactions and main effects were based on Wald statistics. Least significant differences were approximate.

Prothrombin time was analyzed applying Mann-Whitney tests. Hemorrhage scores were analyzed following a linear mixed model for ordinal responses, as described by Kranen *et al.* (1996). Main effects and interactions of group, preslaughter condition, and ob-

server were analyzed. Batch effects were introduced as random terms.

## RESULTS

### Performance, Blood Pressure, and Ascites Mortality

The four broiler groups studied differed with respect to growth rate, dependent on the part of the growth curve. In the exponential part, Groups 1 and 2 were growing faster than Groups 3 and 4, as indicated by growth rate parameter k presented in Table 2. In the linear part, growth rates of Groups 1 and 3 were significantly higher than those of Groups 2 and 4, as indicated by growth rate parameter b (Table 2). Compared to Group 1, growth was retarded in Groups 2, 3, and 4.

Body weight as determined the day before slaughter was not equal for all groups (Table 3). For blood pressure, as measured at Day 45, there was a significant effect of group. Blood pressure of the feed-restricted Groups 3 and 4 was significantly lower than that of Group 1. A considerable number of the broilers from Group 2 and only a small number of the broilers from Group 1 died of ascites (Table 3), and 9 and 12.5% of the broilers of Groups 1 and 2, respectively, died of salmonellosis. None of the

TABLE 3. Body weight, venous blood pressure and mortality due to ascites

Rearing group	Body weight <sup>1</sup>		Blood pressure <sup>2</sup>		Ascites mortality (%)
	$\bar{x}$	n	$\bar{x}$	n	
	(g)		(mm Hg)		
1	2,079 <sup>b</sup>	72	17.4 <sup>a</sup>	9	1.1
2	1,890 <sup>c</sup>	59	11.4 <sup>ab</sup>	9	13.6
3	2,323 <sup>a</sup>	72	8.6 <sup>b</sup>	9	. . .
4	2,171 <sup>b</sup>	71	7.6 <sup>b</sup>	9	. . .
LSD	121		6.9		

<sup>a-c</sup>Means within a column with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Mean body weight of all broilers within a group, as determined on Day 48.

<sup>2</sup>Blood pressure measured in the ulnar vein, at Day 45.

TABLE 4. Viscosity and hematocrit of venous blood, relative heart weight and blood loss at slaughter

Variable	Blood viscosity		Hematocrit		Relative heart weight		Blood loss at slaughter	
	(mPas)	(n)	(%)	(n)	(%)	(n)	(%)	(n)
Rearing group								
1	2.13 <sup>bc</sup>	24	30.7 <sup>b</sup>	17	0.46 <sup>b</sup>	48	3.10 <sup>bc</sup>	48
2	2.49 <sup>a</sup>	20	35.6 <sup>a</sup>	14	0.57 <sup>a</sup>	37	4.37 <sup>a</sup>	37
3	2.09 <sup>c</sup>	24	30.7 <sup>b</sup>	14	0.39 <sup>c</sup>	47	2.76 <sup>c</sup>	47
4	2.27 <sup>b</sup>	23	33.8 <sup>a</sup>	14	0.49 <sup>b</sup>	47	3.23 <sup>b</sup>	47
LSD	0.16		2.3		0.03		0.40	
Preslaughter condition <sup>1</sup>								
c	2.33 <sup>a</sup>	30	34.2 <sup>a</sup>	19	0.47 <sup>a</sup>	59	3.33 <sup>a</sup>	59
m	2.22 <sup>ab</sup>	30	32.0 <sup>b</sup>	21	0.49 <sup>a</sup>	59	3.38 <sup>a</sup>	59
w	2.18 <sup>b</sup>	31	31.8 <sup>b</sup>	19	0.47 <sup>a</sup>	61	3.38 <sup>a</sup>	61
LSD	0.12		1.5		0.02		0.34	

<sup>a-c</sup>Means within a column of rearing group or preslaughter condition with no common superscript differ significantly ( $P < 0.05$ ).

<sup>1</sup>Preslaughter condition: c = cold, 4 C, RH 100%; m = moderate, 19 C, RH 70 to 80%; w = warm, 30 C, RH 60 to 70%.

feed-restricted broilers of Groups 3 and 4 died of ascites or salmonellosis.

### Blood Viscosity, Hematocrit, Prothrombin Time, Relative Heart Weight, and Blood Loss at Slaughter

Viscosity and hematocrit of the blood collected at slaughter were dependent on group and preslaughter condition (Table 4). There was no interaction. Blood viscosity and hematocrit of Group 2 were significantly higher than those of Group 1 and those of Group 3 were higher than those of Group 4. Blood viscosity was highest for Group 2. Blood of broilers exposed to cold preslaughter conditions was more viscous than that of broilers exposed to warm conditions. The hematocrit of broilers exposed to cold conditions was significantly higher than that of broilers exposed to moderate and warm conditions. There were no significant differences with respect to prothrombin time. Prothrombin time varied pre-

dominantly between 20 s and 1 min. Some plasmas, however, did not coagulate at all.

Relative heart weight as well as blood loss at slaughter were affected by group, but not by preslaughter condition. There was no interaction for either parameters (Table 4). Relative heart weight and blood loss of Groups 2 and 4 were higher than those of Groups 1 and 3, respectively. Relative heart weight was highest for Group 2 and lowest for Group 3. Broilers of Group 2 lost the most blood within the first 3 min following neck cutting.

### Hemorrhage Scores

Hemorrhage scores of the breast and the left and right thigh are presented in Table 5. There was a marked main effect of observer for the left thigh and the breast (not shown). The effect of observer did not interact with group or preslaughter condition, nor were there any other interaction for all items scored. Scores of the left thigh were affected by preslaughter condition. Scores for broilers exposed to warm or cold conditions were

TABLE 5. Hemorrhage scores

Item	Breast			Left thigh			Right thigh		
	$\bar{x}$	Fitted $\bar{x}$	n	$\bar{x}$	Fitted $\bar{x}$	n	$\bar{x}$	Fitted $\bar{x}$	n
General mean									
Mean	6.4	4.1	182	5.4	3.5	182	6.0	3.8	182
SE	0.6			0.5			0.5		
Preslaughter condition									
c	6.8 <sup>a</sup>	4.3	61	5.1 <sup>a</sup>	3.4	61	5.7 <sup>a</sup>	3.7	61
m	6.2 <sup>a</sup>	4.0	61	5.9 <sup>b</sup>	3.8	61	6.2 <sup>a</sup>	3.9	61
w	6.2 <sup>a</sup>	4.0	60	5.0 <sup>a</sup>	3.3	60	6.0 <sup>a</sup>	3.8	60
LSD	1.1			0.7			0.7		

<sup>a,b</sup>Means within a column of preslaughter condition with no common superscript differ significantly ( $P < 0.05$ ).

$\bar{x}$  = predicted values for general mean or means for preslaughter condition; fitted  $\bar{x}$  = predicted means fitted on the original scale.

significantly lower than for broilers exposed to moderate preslaughter conditions. There were no effects of preslaughter condition on breast and right thigh. Neither was there an effect of group for all items scored.

## DISCUSSION

The experiment was designed to retard growth by feed restriction and to slaughter all experimental groups at equal body weights. Feed restriction retarded growth within the exponential phase, not in the linear phase. Rearing at low temperatures retarded growth considerably in the *ad libitum* group, but only slightly in the feed-restricted group. As a result, body weight the day before slaughter was highest in the feed-restricted groups and lowest in the group that consumed feed *ad libitum* and that was reared at low temperatures.

The four rearing groups also differed with respect to susceptibility to ascites, blood parameters, relative heart weight, and blood loss at slaughter. Compared to the groups reared at thermoneutral conditions, the groups reared at low temperatures had a higher blood viscosity, hematocrit, relative heart weight, and blood loss at slaughter. These results indicate that these groups adapted to increased oxygen consumption, resulting from the higher metabolic rate to produce heat, caused by the low temperatures (Julian, 1993; Scheele, 1996). The hemodynamic adaptations were detrimental to many broilers of Group 2. Ascites mortality was considerable, growth was retarded and relative heart weight, blood viscosity and blood loss at slaughter were highest in this group. Ascites is believed to be due to increased venous blood pressure (Julian, 1993; Scheele, 1996). Blood pressure in the ulnar vein, however, was not increased in the group most susceptible to ascites. Apparently, a rise in venous blood pressure occurred only in chickens that actually developed ascites. Blood loss from the jugular vein at slaughter was not related to venous blood pressure. The increased blood loss in the groups reared at low temperatures is therefore believed to be due to differences in blood distribution throughout the body. In a previous study (Kranen *et al.*, 1996), we reported that Hybro broilers were hardly affected by low rearing temperatures. They exhibited only minor adaptations to low rearing temperatures, and their body weight at slaughter was not affected, indicating that they grew normally. None of these birds died of ascites or displayed any symptoms of the syndrome. Apparently, within the present study, the individual accommodation made the Hybro broilers more susceptible to the detrimental effects of the low rearing temperatures. An (additional) effect of salmonellosis, however, cannot be excluded. Chickens infected with *S. enteritidis* at a young age are reported to suffer from pericarditis (Gorham *et al.*, 1994; Suzuki, 1994). Heart function may be impeded, making the birds more susceptible to backward failure of the heart and ascites.

Adaptations in circulation of broilers from Group 4 must have been adequate to meet the increased need for

oxygen. None of the birds died of ascites. Feed restriction is reported to be a powerful procedure to reduce high pulmonary pressure and susceptibility to ascites, which is in accordance with our results (Albers *et al.*, 1990; Arce *et al.*, 1992; Julian, 1993).

In summary, the four groups of broilers can be characterized as follows: Group 1, fast-growing broilers with low ascites mortality, but a relatively high blood pressure; Group 2, broilers retarded in growth, with pathological hemodynamic adaptations to low rearing temperatures; Group 3, broilers retarded in growth due to feed restriction; Group 4, broilers retarded in growth due to feed restriction, with nonpathological hemodynamic adaptations to low rearing temperatures. The groups therefore can be used to test the following hypotheses:

1) Growth retardation by feed restriction, and hence, slaughter at a higher age, eliminates the predisposition to muscular damage and reduces the susceptibility to hyperthermia induced by high ambient temperatures. As a result, susceptibility to hemorrhages and blood splash will be reduced.

2) Broilers with pathological hemodynamic adaptations to low rearing temperatures are not able to respond adequately to acute (thermal) preslaughter stress. They become more vulnerable to mechanisms affecting muscle metabolism, leading to muscular damage and hemorrhages.

In contrast to the first hypothesis, hemorrhage scores were not reduced in thighs and breasts of feed-restricted broilers retarded in growth, and, hence, slaughtered at a higher age. It indicates that *ad libitum* broilers are not predisposed to muscular damage and hemorrhaging. It also indicates that changes in body and muscle composition due to feed restriction, as reported to occur in pigs upon limitation of the energy intake (Harrison *et al.*, 1996), do either not occur or not affect the susceptibility to hemorrhages in muscles.

Feed-restricted chickens are reported to be more resistant to acute heat stress and detrimental effects of hyperthermia than chickens that eat *ad libitum* (Freeman, 1984; Smith, 1986; Chen *et al.*, 1994; Hocking *et al.*, 1994). Feed-restricted chickens were expected to be less susceptible to rhabdomyolysis and hemorrhages caused by handling, shackling, and electrical stunning, upon exposure to heat stress. The absence of an unambiguous effect of preslaughter condition and group on hemorrhage severity, however, indicates that differences in tolerance to heat, if present, do not affect susceptibility to hemorrhages, either in thighs or in breasts.

In contradiction to the second hypothesis, the group of broilers most susceptible to ascites had no higher hemorrhage scores upon exposure to acute thermal stress prior to slaughter. Either thermoregulation of this group of broilers was not affected, despite clear signs of circulatory disorders, or an attenuated thermoregulatory response did not affect susceptibility to hemorrhages.

Cold preslaughter conditions increased hematocrit and blood viscosity, independent of the rearing group,

the increased hematocrit probably reflects a decreased plasma volume due to acute hemoconcentration induced by hypothermia (Pinder and Smits, 1993; Danzl and Pozos, 1994). Hemoconcentration did not affect hemorrhage severity. There was no significant correlation between venous blood pressure or prothrombin time and hemorrhage severity. Apparently, changes in hemodynamic parameters such as blood pressure, viscosity, hematocrit, coagulation, and relative heart weight are not responsible for the variation in susceptibility of electrically stunned broilers to muscle hemorrhages. This result is in accordance with our previous results (Kranen *et al.*, 1996).

Effects of preslaughter condition were significant for the left thigh only. Broilers exposed to cold or warm thermal preslaughter stress had lower scores than broilers exposed to moderate preslaughter conditions, independent of the group. Apparently, effects of preslaughter condition affected the severity of hemorrhages induced by electrical stunning different from those induced by other factors such as handling. The broilers were grounded to both legs during electrical stunning at shackles.

In conclusion, Hybro broiler chickens differing with respect to growth rate, age at slaughter, hemodynamic adaptations to low rearing temperatures, and susceptibility to ascites, do not differ with respect to susceptibility to hemorrhages in breast and thigh muscles upon electrical stunning and subsequent slaughter. Growth retardation, and, hence, slaughter at older age, does not diminish hemorrhage severity, neither upon exposure to moderate nor to warm preslaughter conditions. Broilers from the rearing group with pathological hemodynamic adaptations to low rearing temperatures, which are exposed to cold or warm preslaughter stress, are as susceptible to hemorrhages as the other rearing groups tested. The difference between the left and right thigh with respect to the effect of preslaughter condition on hemorrhage scores indicates that cause of hemorrhages in muscles is multifactorial.

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