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## Behavioural and physiological responses of three chicken breeds to pre-slaughter shackling and acute heat stress

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**Abstract** 1. The aim of this study was to compare the behavioural and physiological responses to hanging and acute heat stress in three different chicken breeds. Chicks were obtained from a slow-growing French 'Label Rouge' line (SGL), a fast-growing standard line (FGL) and a heavy line (HL). The SGL, FGL and HL birds were slaughtered at their respective market ages of 12, 6 and 6 weeks, in an attempt to achieve similar body weights. Before stunning, birds were either shackled by their legs on the moving line for 2 min (shackling stress: SH) or placed in a room at 35°C and 60% of humidity for 3–5 h and then shackled for 2 min (acute heat stress plus shackling: H + SH) or subjected to minimal stress by shackling for 10 s before stunning (control group: C).

2. Bird physiological responses to the three pre-slaughter treatments were estimated by measuring blood corticosterone, glycaemia, creatine kinase activity, acid–base status and electrolyte concentration as well as lactate content and glycolytic potential in the breast (*Pectoralis major*) and thigh (*Ilio tibialis*) muscles. Behavioural responses to shackling stress were evaluated by measuring wing flapping duration, straightening up attempts and vocalisations.

3. Blood corticosterone was higher in SH and H + SH groups than in the C group, regardless of genotype. The struggling activity on the shackle line differed among chicken breeds. It was more intense and occurred more rapidly after hanging in the SGL birds than in both other breeds. Furthermore, SGL struggling activity was not affected by hanging duration while it increased with hanging duration in FGL and HL birds.

4. Wing flapping duration was negatively correlated with blood pH, bicarbonate concentration and positively correlated with breast muscle lactate content, indicating that struggling stimulated ante-mortem glycolysis activity in breast muscle. Acute heat stress affected blood Ca<sup>2+</sup> and Na<sup>+</sup> concentration and increased glycaemia and glycolytic potential of thigh muscle.

5. Both acute heat stress and shackling before slaughter were experienced as stressful events by all types of birds.

### INTRODUCTION

In poultry as in pigs, the quality of meat products results from complex interactions between the genotype and the environment, more especially the stresses undergone before slaughter (Berri, 2000; Debut *et al.*, 2003). Genetic variation in muscle and meat characteristics have been shown by several comparisons of genotypes in chicken and turkey (Xiong *et al.*, 1993; Gardzielewska *et al.*, 1995; Schreurs, 1995; Szalkowska and Meller, 1999; Berri *et al.*, 2001; Fernandez *et al.*, 2001; Lonergan *et al.*, 2003) and a few genetic studies in selected populations (Le Bihan-Duval *et al.*, 2001, 2003). Nevertheless, implication of

the stress reactivity in these genetic variations has been seldom investigated.

The purpose of this study was to characterise and compare the behavioural and physiological responses to pre-slaughter stress in chicken breeds differing in growth rate and muscle development. Currently the meat industry mainly uses standard chickens produced from fast-growing or even high breast yield lines. However, extensive methods of production using less productive chicken lines are now developing to answer consumers' demands for enhanced animal welfare and more tasty products (Sauveur, 1997). Therefore, a slow-growing Label line, a fast-growing standard line and

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a heavy line were selected for the study. Shackling and acute heat stress were chosen as pre-slaughter treatments as they are commonly encountered under commercial practice and, according to previous studies, were expected to have a negative impact on animal welfare (Gregory and Bell, 1987; Gregory, 1994; Sparrey and Kettlewell, 1994; Kannan *et al.*, 1997) and meat quality (Grey *et al.*, 1974; Froning *et al.*, 1978; Ngoka and Froning, 1982; Papinaho *et al.*, 1995; Kannan *et al.*, 1997; Sandercock *et al.*, 1999, 2001; Debut *et al.*, 2003). The bird response to stress was estimated by behavioural measurements on the shackle line as well as blood variables such as acid-base status, electrolyte, corticosterone, glucose content and creatine kinase activity. Effects on breast and thigh muscle metabolism were investigated by measuring lactate concentration and glycolytic potential early post-mortem. Moreover, the impact on meat quality is described in a companion paper (Berri *et al.*, 2005).

## MATERIALS AND METHODS

### Animals

Female chickens from three grand-parental pure lines selected by Hubbard (Châteaubourg, France) were used: a slow-growing French 'Label Rouge' line (SGL), a fast-growing standard line (FGL) selected for high body weight and a heavy line (HL) highly selected for rapid growth and breast meat yield. All the birds were reared in confinement in a conventional poultry house

at the Poultry Research Centre (Nouzilly, France). Birds were fed *ad libitum* with an appropriate diet for each line (Table 1). Ninety-nine birds of each breed were weighed and slaughtered at their respective market age of 6 weeks for FGL and HL and 12 weeks for SGL. Mean body weight at slaughter was 1883 g ( $\pm 128$  g), 2235 g ( $\pm 183$  g) and 1940 g ( $\pm 129$  g) for FGL, HL and SGL, respectively.

### Pre-slaughter and slaughter conditions

A total of 99 birds per line were slaughtered on the same day at the experimental processing plant of the INRA Poultry Research Centre. They were all submitted to a 7-h feed withdrawal. Prior to slaughter, birds were randomly allocated to one of the three ante-mortem treatments (33 birds/line/treatment): minimum stress (careful handling and shackling for 10 s before stunning) (C), shackling for 2 min (SH) and acute heat plus shackling stress (exposure to 35°C and 60% relative humidity for 3.5 h and shackling for 2 min before stunning) (H+SH). Birds allocated to the C and SH conditions were crated (10 animals per crate) 10 min before slaughter and immediately transported to the processing plant (2 to 3 min of transport). Birds allocated to the H+SH conditions were crated 3.5 h prior to slaughter then subjected to the heat conditions and transported to the experimental processing plant. For each ante-mortem condition, three series of slaughter procedures (in which the three chicken types were equally represented) were performed in order to

**Table 1.** Composition of diets distributed during rearing period for the three lines (SGL = slow-growing line; FGL = fast-growing line; HL = heavy line)

	SGL			FGL and HL	
Weeks	0-4	4-8	8-12	0-2	2-6
Composition (%)					
Maize	52.4	36.6	46.0	41.8	34.1
Wheat	12.9	38.4	30.7	12.0	30.0
Rapeseed oil	-	-	-	5.0	4.2
Soybean meal	30.4	18.7	16.2	30.6	24.1
Maize gluten meal	-	2.3	3.2	0.7	3.8
Calcium carbonate	1.3	1.36	1.4	1.3	1.2
Dicalcium phosphate	1.8	1.5	1.4	1.9	1.5
Sodium chloride	0.3	0.3	0.3	0.4	0.4
Vitamins	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.14	0.10	-	0.14	0.03
L-Lysine	0.06	0.14	0.15	-	-
Anticoccidial	0.05	0.05	0.05	0.05	0.05
Characteristics (calculated)					
Metabolisable energy (MJ/kg)	11.5	11.8	11.8	12.5	12.5
Crude protein (g/kg)	197	171	164	217	193
Lysine (g/kg)	10.6	8.6	8.0	11.5	9.0
Methionine + cystine (g/kg)	8.0	7.0	6.0	8.0	7.0
Calcium (g/kg)	12.6	11.9	11.6	13.2	11.4
Available phosphorus (g/kg)	4.1	3.9	3.6	4.4	3.8

alternate C, SH and H+SH groups along the whole slaughter process. Birds were electrically stunned (120 Hz AC, 60 mA/bird, 5 s) and killed by neck cutting in the experimental processing plant of the Poultry Research Centre. After evisceration, whole carcasses were chilled and kept at 2°C for one day.

### Behavioural measurements

Activity of the birds on the shackle line was estimated by different measurements: straightening up (SU) of the body (head over the legs) was recorded from hanging to electrical stunning and noted as a binary variable equal to 0 when the bird did not try to stand up (absence) and otherwise 1 (presence). Vocalisations were recorded when the bird was hung and were classified into 4 categories: 0 when the bird did not vocalise, 1 when the bird vocalised briefly and weakly, 2 when it vocalised weakly for a long time and 3 when the bird vocalised strongly for a longer time. The total duration of wing flapping (TDWF) was recorded from hanging to electrical stunning.

### Blood variables

Animals were killed by exsanguination through a neck cut. Individual blood samples were collected during bleeding in heparinised tubes and temporarily stored on ice. For the measurement of glycaemia, corticosterone and creatine kinase activity, plasmas were separated following centrifugation at 2000 g for 15 min at 4°C and stored at -20°C until used. The acid-base status, electrolyte concentration and haematocrit were measured on 11 birds per genotype and pre-slaughter condition. One millilitre of blood was injected in a Combo's chip of the IRMA SL 2000 apparatus (Diametrics Medical) in which specific microelectrodes measured the pH, partial pressure of dioxygen (pO<sub>2</sub>, mmHg), sodium (Na<sup>+</sup>, mM), potassium (K<sup>+</sup>, mM), ionised calcium (iCa, mM) and haematocrit (Hct, %) concentrations. The bicarbonate concentration (HCO<sub>3</sub><sup>-</sup>, mM) was derived from blood gas measurements. Plasma creatine kinase activity (CK, UI/l) was measured on all birds by spectrophotometry using CK-NAC reagent and normal human serum as control (Advanced Diagnostics, Plainfield, NJ, USA). Glycaemia concentration (GLYC, mg/100 ml) was measured on all birds by the glucose oxidase method with a Glucose Analyser (Beckman, Palo Alto, CA, USA). GLYC was measured twice or more if differences were found between the two first measurements. Plasma corticosterone concentration (CORT, ng/ml) was measured using a specific radio immunoassay as described by Etches (1976).

### Muscle temperature, lactate content and glycolytic potential

The temperature of breast muscle was measured just after stunning by introducing the probe (ANRITSU 513K, Japan) of a thermometer (Bioblock scientific, K-thermometer 16212) directly into the muscle. Glycogen, glucose-6-phosphate and lactate (Lact) concentrations (expressed in μM/g of muscle) were measured in the *Pectoralis major* breast muscle and in the *Ilio tibialis* thigh muscle according to Dalrymple and Hamm (1973), from 1 g of fresh tissue taken and homogenised in 10 ml of 0.55 moles perchloric acid 15 min post-mortem. Glycolytic potential (GP, μM/g of muscle), which represents an estimation of resting glycogen concentration at death, was calculated in both muscles according to the equation of Monin and Sellier (1985):

$$GP = 2[(\text{glycogen}) + (\text{glucose}) + (\text{glucose-6-phosphate})] + (\text{lactate})$$

and expressed as micromoles of lactate equivalent per gram of muscle.

### Statistical analyses

Effects of pre-slaughter condition and breed and their possible interaction on log(CORT), blood and muscle variables were tested by a two-way ANOVA using the GLM procedure of SAS (SAS Institute, 1999). Pairwise comparisons of means for each significant effect of ANOVA were performed by Scheffe test using the LSMeans statement of the GLM procedure. After categorisation of WF variables into three classes of equal size, effect of the breed and the pre-slaughter conditions on categorical behavioural variables was tested by chi-square test using the FREQ procedure of SAS (SAS Institute, 1999). The effect of the breed on WF durations was estimated with a test of comparison of medians (Kruskal-Wallis test) using the NPAR1WAY procedure of SAS (SAS Institute, 1999). Finally, relationships between blood or muscle variables on the one hand and WF duration (expressed in s) on the other hand were estimated by Spearman Rank correlations using the CORR procedure of SAS (SAS Institute, 1999).

## RESULTS

### Behavioural measurements

The proportions of birds in class 1 of SU and in class 2 of WF (which corresponded to the highest levels of activity) were largely increased in SH and H+SH condition groups compared to the C

group for FGL and HL birds (Table 2). For FGL birds, frequencies of classes 2 and 3 of VOCAL were higher under SH and H+SH conditions than in controls. In contrast, pre-slaughter condition did not affect struggle activity (SU and WF) or VOCAL of SGL birds. Regardless of the breed, WF duration was similar under SH and H+SH conditions (Table 3). Regardless of pre-slaughter condition, WF duration was the highest in SGL birds and lowest in HL birds.

**Variation of blood and muscle variables in relation to pre-slaughter condition**

As reported in Tables 4 and 5, blood and muscle variables were similarly affected by pre-slaughter treatments in the different breeds (no breed × pre-slaughter treatment interaction). By comparison to the C condition, the CORT concentration increased in both stress treatments, the highest value being observed for H+SH. Moreover, GLYC concentration was increased under H+SH by comparison to both the other pre-slaughter conditions. Blood HCO<sub>3</sub><sup>-</sup> concentration decreased in SH and H+SH compared to C. The K<sup>+</sup> concentration was also reduced in the stress treatments, the difference being significant only under H+SH condition. The H+SH treatment induced an increase in Na<sup>+</sup> and a decrease in iCa concentration compared to the alternative treatments. Under H+SH, the temperature of breast muscle and the GP of thigh muscle increased (Table 5). Finally, breast muscle lactate content was higher under SH than under both C and H+SH conditions.

**Within breed relationships between wing flapping duration and physiological measurements**

A moderate positive correlation of 0.28 (*P*=0.006) was observed between WF duration and CORT concentration in the SGL birds. Regardless of the breed, WF duration was highly negatively correlated with blood pH (*r*-values of -0.66, -0.76, and -0.68 for SGL, FGL and HL, respectively) and with HCO<sub>3</sub><sup>-</sup> concentration (*r*-values of -0.66, -0.42 and -0.49, respectively). Strong positive correlations of 0.67, 0.66 and 0.58 were also obtained between WF duration and breast muscle lactate content in SGL, FGL and HL, respectively. In thigh muscle, moderate correlations of 0.24 and 0.22 were obtained between WF and lactate content in FGL and SGL breeds, respectively.

**Table 3.** Wing flapping duration<sup>1</sup> (expressed in s) of birds between hanging and stunning according to pre-slaughter treatment (C = control group; SH = shackling; H + SH = heat + shackling) and breed (SGL = slow-growing line; FGL = fast-growing line; HL = heavy line) and probabilities of the Kruskal-Wallis test

	C	SH	SH + H	Breed
SGL	7	12	7	
FGL	1	5	5	<i>P</i> <0.0001
HL	0	3	3	
Pre-slaughter treatment	<i>P</i> =0.0002			

<sup>1</sup>Data presented as medians.

**Table 2.** Frequencies (%) per class of behavioural variables (SU = straightening up; VOCAL = vocalisations; WF = duration of wing flapping) and chi-square probabilities according to pre-slaughter treatment (C = control group; SH = shackling; H + SH = heat + shackling) and breed (SGL = slow-growing line; FGL = fast-growing line; HL = heavy line)

SU	C			SH				H + SH				<i>P</i>	
	0		1	0		1		0		1			
	0	1	2	3	0	1	2	3	0	1	2		3
SGL	45.7	54.3			44.1	55.9			54.5	45.5			0.60
FGL	73.5	26.5			31.2	68.8			36.4	63.6			<0.001
HL	84.8	15.1			58.8	41.2			72.7	27.3			0.05
<i>P</i>	0.002			0.08				0.01					

  

VOCAL	C				SH				H + SH				<i>P</i>
	0		1		0		1		0		1		
	0	1	2	3	0	1	2	3	0	1	2	3	
SGL	40.6	6.2	9.4	43.7	26.5	17.6	20.6	35.3	37.9	24.1	3.4	34.5	0.18
FGL	40.6	31.2	25.0	3.13	12.9	16.1	41.9	29.0	15.1	15.1	42.4	27.3	0.007
HL	33.3	20.0	46.7	0.0	18.2	18.2	39.4	24.2	27.3	12.1	45.4	15.1	0.16
<i>P</i>	<0.0001				0.54				0.008				

  

WF	C			SH			H + SH			<i>P</i>
	0		1	0		1	0		1	
	0	1	2	0	1	2	0	1	2	
SGL	31.4	11.4	57.1	20.6	14.7	64.7	36.4	12.1	51.5	0.71
FGL	38.2	58.8	2.9	11.8	50.0	38.2	9.1	57.6	33.3	<0.001
HL	63.6	30.3	6.1	29.4	44.1	26.5	30.3	33.3	36.4	0.007
<i>P</i>	<0.0001			0.004			0.002			

**Table 4.** Blood variables<sup>1</sup> for the three breeds (SGL = slow-growing line; FGL = fast-growing line; HL = heavy line) and the three pre-slaughter conditions (C = control group; SH = shackling; H + SH = heat + shackling) and probabilities of the ANOVA

Variable <sup>1</sup>	Breed			Pre-slaughter treatment			Pooled SED	Breed	Pre-slaughter treatment	Breed × pre-slaughter treatment
	SGL	FGL	HL	C	SH	H + SH				
PH	7.32 <sup>a</sup>	7.34 <sup>ab</sup>	7.37 <sup>b</sup>	7.37	7.33	7.34	0.01	**	NS	NS
Oxygen (pO <sub>2</sub> , mmHg)	48.02 <sup>b</sup>	40.49 <sup>a</sup>	38.71 <sup>a</sup>	40.54	43.15	43.53	1.08	***	NS	NS
Bicarbonate (HCO <sub>3</sub> <sup>-</sup> , mM)	20.81 <sup>b</sup>	22.60 <sup>b</sup>	25.51 <sup>a</sup>	25.33 <sup>a</sup>	21.85 <sup>b</sup>	20.77 <sup>b</sup>	0.47	***	***	NS
Sodium (Na <sup>+</sup> , mM)	144.94 <sup>a</sup>	143.71 <sup>a</sup>	143.91 <sup>a</sup>	142.23 <sup>a</sup>	143.30 <sup>a</sup>	147.04 <sup>b</sup>	1.08	NS	**	NS
Potassium (K <sup>+</sup> , mM)	7.23 <sup>a</sup>	6.45 <sup>b</sup>	6.40 <sup>b</sup>	7.04 <sup>a</sup>	6.59 <sup>ab</sup>	6.44 <sup>b</sup>	0.14	***	**	NS
Ionised calcium (Ca <sup>2+</sup> , mM)	1.83 <sup>a</sup>	1.76 <sup>a</sup>	1.74 <sup>a</sup>	1.82 <sup>b</sup>	1.83 <sup>b</sup>	1.67 <sup>a</sup>	0.03	NS	***	NS
Corticosterone (log ng/ml)	1.80 <sup>a</sup>	2.11 <sup>b</sup>	2.3 <sup>c</sup>	1.72 <sup>a</sup>	2.14 <sup>b</sup>	2.38 <sup>c</sup>	0.06	***	***	NS
Haematocrit (Hct, %)	27.87 <sup>b</sup>	23.54 <sup>a</sup>	28.05 <sup>b</sup>	26.11	27.5	25.85	0.80	***	NS	NS
Creatine kinase activity (CK, UI/l)	3605	3290	3623	3547	3593	3377	135	NS	NS	NS
Glycaemia (GLYC, mg/100 ml)	2.14 <sup>a</sup>	1.99 <sup>b</sup>	2.00 <sup>b</sup>	2.00 <sup>a</sup>	2.04 <sup>a</sup>	2.10 <sup>b</sup>	0.02	***	**	NS

<sup>1</sup>Data presented as Least Square Means.

<sup>a-c</sup>LSmeans with different superscript letters in the same row differ ( $P < 0.05$ ).

\*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; NS = not significant.

**Table 5.** Muscle variables<sup>1</sup> for the three breeds (SGL = slow-growing line; FGL = fast-growing line; HL = heavy line) and the three pre-slaughter conditions (C = control group; SH = shackling; H + SH = heat + shackling) and probabilities of the ANOVA

Variable <sup>1</sup>	Breed			Pre-slaughter treatment			Pooled SED	Breed	Pre-slaughter treatment	Breed × pre-slaughter treatment
	SGL	FGL	HL	C	SH	H + SH				
Temperature (°C)	41.1 <sup>b</sup>	40.4 <sup>a</sup>	40.6 <sup>a</sup>	40.5 <sup>a</sup>	40.1 <sup>a</sup>	41.5 <sup>b</sup>	0.13	***	***	NS
Breast lactate (µM/g of muscle)	61.1 <sup>c</sup>	50.2 <sup>b</sup>	43.0 <sup>a</sup>	50.1 <sup>a</sup>	54.1 <sup>b</sup>	50.3 <sup>a</sup>	1.18	***	**	NS
Breast glycolytic potential (µM/g of muscle)	134.8 <sup>b</sup>	115.8 <sup>a</sup>	129.5 <sup>b</sup>	127.8	123.4	128.8	2.21	***	NS	NS
Thigh lactate (µM/g of muscle)	45.7 <sup>b</sup>	36.5 <sup>a</sup>	37.7 <sup>a</sup>	39.6	40.5	39.8	0.74	***	NS	NS
Thigh glycolytic potential (µM/g of muscle)	75.1 <sup>ab</sup>	74.1 <sup>a</sup>	78.8 <sup>b</sup>	73.9 <sup>a</sup>	71.3 <sup>a</sup>	82.9 <sup>b</sup>	1.37	**	***	NS

<sup>1</sup>Data presented as Least Square Means.

<sup>a-c</sup>Means with different superscript letters in the same row differ ( $P < 0.05$ ).

\*\* $P \leq 0.01$ ; \*\*\* $P \leq 0.001$ ; NS = not significant.

### Breed variations of blood and muscle variables

The blood CORT concentration differed among lines, with the highest value for the HL, the lowest for SGL, and FGL birds were intermediate (Table 4). FGL birds exhibited a lower Hct than the two other breeds, while SGL birds exhibited a higher GLYC. The blood pH was lower in SGL than in HL birds, FGL birds being intermediate for this trait. The SGL birds exhibited higher blood pO<sub>2</sub> and K<sup>+</sup> concentrations than both other breeds. HL birds were characterised by a higher blood HCO<sub>3</sub><sup>-</sup> concentration than both other lines.

The SGL birds exhibited the highest breast muscle temperature and lactate content at 15 min post-mortem (Table 5). The lactate content was the lowest in breast muscle of HL birds, FGL birds exhibiting intermediate values. In thigh muscle, SGL birds also showed the highest lactate concentration. A lower GP was observed in the breast muscle of FGL birds. FGL birds also exhibited a lower thigh muscle GP than HL birds.

## DISCUSSION

### Heat stress

Exposure to acute heat stress is likely to lead to various metabolic changes. An early reaction to high ambient temperature is the increased body temperature (Sandercock *et al.*, 1999), which can be used as an indicator of the extent of thermoregulatory effort (Hocking *et al.*, 1994). Furthermore, according to Edens (1978), chickens exposed to high environmental temperatures (43°C) showed a rising plasma corticosterone concentration early in the heating episode (before 90 min), afterwards a significant fall signifying the Acute Adrenal Cortical Insufficiency (AACI) syndrome. As reported by Edens and Siegel (1976) or Edens (1978), this syndrome is associated with a loss of plasma glucose, cholesterol, total calcium and inorganic phosphate and decreased plasma sodium to potassium ratio. Finally, birds developing a severe hyperthermia are characterised by an increased blood pH which indicates a respiratory alkalosis (Hocking *et al.*, 1994; Sandercock *et al.*, 1999, 2001). In the present study, despite observing an increase of breast muscle temperature and plasma corticosterone, we found no evidence of changes in blood variables associated with the AACI syndrome. Indeed, glycaemia and Na<sup>+</sup>/K<sup>+</sup> ratio were increased and no effect of heat on blood acid-base balance (pH and HCO<sub>3</sub>) was found in our study. This could be due to the degree of heat stress applied in this study, of no more than 35°C *vs* 43°C in Edens (1978).

Similarly, Altan *et al.* (2000) did not report variation in blood pH and electrolyte concentration in broilers exposed to 38°C for 2 h. Moreover, while Edens (1978) reported that birds exposed to 43°C for 2 h developed irreversible heat prostration and cardiovascular failure, in the present study no mortality was noted and birds rapidly recovered at the end of heat exposure as shown by the extent of their struggling activity on the shackle line.

Acute heat stress before slaughter increased the GP of the *Ilio tibialis* thigh muscle but did not affect that of *Pectoralis major* breast muscle. One can expect that under our experimental conditions bird physical activity during heat exposure was lowered, which may prevent glycogen depletion, at least in the thigh muscle. As already demonstrated in pork, increasing muscle glycolytic potential is likely to decrease meat ultimate pH and therefore affect meat quality. Recently, Debut *et al.* (2003) reported that acute heat stress in chickens induced a lower final pH in thigh muscle, which is consistent with the increased thigh glycolytic potential that we observed in birds exposed to heat treatment.

### Hanging stress

Most of the studies of struggling on the shackling line have been investigated in the context of animal welfare (Gregory and Bell, 1987; Gregory, 1994; Sparrey and Kettlewell, 1994). Kannan and Mench (1996) reported that hanging broilers in an inverted position is experienced as a stressful event which leads to an increase in plasma corticosterone concentration. In our study, increasing shackling time led to higher plasma corticosterone as already reported by Kannan *et al.* (1997) in chicken. Vigorous wing flapping can be seen as an escape behaviour and an indicator of discomfort (Sparrey and Kettlewell, 1994). Reaction to hanging has been already shown to be intensified by environmental factors such as rough hanging, noise, bright light, unsuitable shackles or separation from familiar counterparts (Gregory and Bell, 1987). In accordance with Debut *et al.* (2003), the present study demonstrated that stress reaction at slaughter is also genotype dependent. Struggling activity was more immediate and intense for SGL birds than for both standard lines. Furthermore, differences were observed among standard breeds, the HL birds being less active than FGL ones. However, no relationship was found within breed between body weight and WF duration (not shown), which did not support the hypothesis that variations in weight may directly explain the variations in struggle activity.



Regardless of bird type, intense struggling led to blood acidification and decreased bicarbonate concentration. This may explain the slight blood acidosis which occurred in the most active SGL birds and the reduced blood bicarbonate concentration under SH and H + SH conditions, which favour intense struggle on the shackling line. As described by Dejours (1930), blood acidification could be the result of an excess of lactic acid in muscle. The present study showed that muscle post-mortem metabolic changes were influenced by pre-slaughter activity, especially in breast muscle where lactate concentration at 15 min post-mortem increased with struggling activity. This confirms the results already obtained in chicken (Grey *et al.*, 1974; Papinaho *et al.*, 1995) and turkey (Froning *et al.*, 1978; Ngoka and Froning, 1982) and shows for the first time that differences in struggling activity could explain breed variations of post-mortem glycolysis. Our results also showed that breast muscle was more sensitive to struggling activity than thigh muscle in which lactate concentration was barely affected. This could be related to the glycolytic status of the breast muscle and its association with WF activity.

In conclusion, this study has shown that both acute heat stress and shackling before slaughter were experienced as stressful events by all types of birds, as indicated by the rising concentration of plasma corticosterone after treatment. This confirms the importance of limiting pre-slaughter stress in order to increase animal welfare. On the other hand, both stressful pre-slaughter conditions led to various metabolic changes, which were more marked when bird responsiveness was high. In particular, because of their intense struggling activity on the shackling line, slow-growing birds were shown to be more likely to develop fast rates of post-mortem pH fall, while heavy birds appeared to be less sensitive. Implications for meat quality have been described in the complementary study of Berri *et al.* (2005). From our results, one can conclude that optimisation of pre-slaughter conditions should be investigated in relation to bird type. Gas stunning methods which avoid hanging of conscious birds before stunning should be more appropriate for more active birds, such as slow-growing chickens. The potential interest for animal welfare and meat quality of such alternative stunning methods have now to be investigated and should take into account the diversity of the genetic lines that are currently produced.

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