

## Effect of Preslaughter Shackling on Stress, Meat Quality Traits, and Glycolytic Potential in Broilers

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### ABSTRACT

The aim of this study was to investigate the effects of preslaughter shackling durations on some blood parameters, breast and thigh meat quality traits, and muscle metabolites in broilers. The effects of shackling were determined in a group of Ross 308 broilers (240 birds) aged 42 days. Four shackling treatments were used in experimental tests: shackling of broilers for 10 (Group G<sub>10</sub>; as control), 30 (G<sub>30</sub>), 60 (G<sub>60</sub>), and 120 seconds (G<sub>120</sub>). Results showed that corticosterone (CORT) level (2314.79 pg ml<sup>-1</sup>) at 120 seconds shackling group increased (P < 0.01). Results indicated that kinase (CK) activity was the highest (2265.69 U l<sup>-1</sup>) in the 120 seconds shackling group while it was the lowest (1970.64 U l<sup>-1</sup>) in 10 s group according to the shackling duration (P < 0.05). The breast meat redness value increased due to increase in shackling duration (P < 0.05). It was revealed that shackling duration had decreased breast muscle glycogen level in all treatment groups (P < 0.001). Conversely, breast lactate level increased according to increase in shackling duration (P < 0.05). It was revealed that there was a negative relationship (r = -0.466) between breast meat ultimate pH and cooking loss (CL) value in male broilers (P < 0.01). These results indicated that the preslaughter shackling procedure might be a considerably stressful procedure for broilers, particularly exceeding 60 s. This study suggested that broilers could be at disadvantage due to more struggle during long duration shackling and accelerated postmortem glycolysis, which is detrimental to the quality of breast meat.

**Keywords:** Creatine kinase activity, Corticosterone, Glycogen level, Lactate level, Wing flapping.

### INTRODUCTION

Increasing demands to ensure animal welfare are also closely associated with increasingly strict requirements for meat quality. Preslaughter shackling alters both the metabolism and psychological state of animals, which may produce undesirable changes in meat quality (Kannan *et al.*, 1997; Berri *et al.*, 2005; Debut *et al.*, 2005). Concentrations of certain plasma hormones and enzymes such as corticosterone (CORT) and creatine kinase (CK) have been suggested to be sensitive parameters

indicating the level of stress and muscle damage in poultry (Nijdam *et al.*, 2005; Zhang *et al.*, 2009). In addition, preslaughter and postmortem glycogen metabolism and lactate accumulation from glycolysis affect important meat quality parameters (Debut *et al.*, 2005; Zhang *et al.*, 2009). The accumulation of lactic acid and resultant pH decline in meat are believed to be dependent mainly on glycogen present in the muscle at time of slaughter (Berri *et al.*, 2007). Debut *et al.* (2005) determined the lactate level in broiler as 50.1 and 54.1 μM g<sup>-1</sup> for 10 and 120 seconds shackling duration (P < 0.01).

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The glycolytic potential (GP) expresses the potential lactate formation in the muscles at exanguination and it is believed that GP at the moment of animal death is capable of predicting the final meat quality (Hartschuh *et al.*, 2002; Berri *et al.*, 2005). The GP is less sensitive than either glycogen or lactate measured alone (Hartschuh *et al.*, 2002).

The purpose of this study was to evaluate how pre-slaughter stress affects breast and thigh meat quality parameters such as biochemical (CORT and CK activity), muscle metabolites (glycogen, lactate and GP), and meat quality (pH, colour, cook and drip losses) in broilers.

## MATERIALS AND METHODS

A total of 272 (equal number of male and female broilers) 1-day old broiler chickens (Ross 308) obtained from a commercial hatchery were used in the study. From the first day, chicks were housed on deep litter of wood shavings in an experimental barn with controlled light, heating, and hygienic and feeding patterns according to standard breeding requirements for broilers. The feed supply was changed from starter (3,100 kcal ME kg<sup>-1</sup>; 22% crude protein) to finisher pellet (3,250 kcal ME kg<sup>-1</sup>; 21% crude protein) at 21 days of age. Free Access to feed and water throughout and constant photo-period of 24 hours was provided. The ambient barn temperature was gradually decreased from 32±1°C on day 1 to 23±1°C on the last day of fattening (day 42). The relative humidity varied between 50 to 60%.

### Methods

All procedures used in the present study were approved by Adnan Menderes University Animal Experiments Local Ethics Committee (No:B.30.2.ADÜ.050.04/2011/079). When the 272 broilers were 42 days old, 240 birds were selected at random for tests related to shackling. Five handlers captured one

broiler each and transported it by hand to the test room (Bedanova *et al.*, 2007), where chickens were immediately inverted and simultaneously suspended from stationary shackles placed in a line. The shackle spacing of the line was 50 cm for the shackling test. The shackled broilers could see hear, and partially touch each other during the test and were allowed to flap freely. Four shackling treatments were used: shackling of broilers for 10 (Group G<sub>10</sub>; as control, 15 male, 15 female), 30 (G<sub>30</sub>; 15 male, 15 female), 60 (G<sub>60</sub>; 15 male, 15 female), and 120 seconds (G<sub>120</sub>; 15 male, 15 female). The shackling treatments were repeated twice with different birds for each test (meat quality traits and muscle metabolites) as described below.

### Blood Parameters Analysis

At 42 days of age, blood samples from a total of 80 birds were randomly selected (20 birds (10 male and 10 female for each replication) per test group G<sub>10</sub>, G<sub>30</sub>, G<sub>60</sub> and G<sub>120</sub> were used for CORT and CK levels. Immediately after shackling treatment, blood samples were taken from brachial vena of birds in each test group. Trunk blood samples were collected in heparinized tubes and temporarily stored on ice. The heparinized blood was centrifuged at 837×g for 10 minutes and plasma samples were stored at -80°C in Eppendorf test tubes until analyses were performed (within one week). CK activity was measured in a Shimadzu UV-1601 spectrophotometry using commercial test kit. The plasma CORT level was measured using a commercial CORT EIA Kit (Cayman Chemical, Ann Arbor, MI).

### Meat Quality Parameters and Muscle Glycolytic Metabolite Traits

On day 42, blood samples from the remaining 80 birds were randomly selected (10 male and 10 female for each replication)

per test group  $G_{10}$ ,  $G_{30}$ ,  $G_{60}$  and  $G_{120}$  and were used for determining the meat quality and muscle glycolytic metabolite traits. Immediately after shackling treatment, birds were slaughtered by exsanguination through a neck cut. The pH values were determined 15 minutes after slaughter (initial pH,  $pH_{15}$ ) and after chilling for 24 hours at 4°C in sealed plastic bags (ultimate pH,  $pH_u$ ), on the left *Pectoralis major* (breast) and *Ilio tibialis* (thigh) muscles of carcasses by using pH meter (Hanna Instrument HI 9124) with a penetration electrode (Hanna FC-200). Color measurements were made on the cranial portion of the dorsal surface left breast and thigh muscles. Color measurements of the skinless muscle surfaces were determined at 24 hours post slaughter using a portable Minolta colorimeter (Minolta CR-400, Ramsey, NJ) and reported according to the CIELAB Color System values of lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ). Drip loss (DL) of the breast and thigh muscles at 24 hours *post-mortem* was measured as described by Honikel (1998). Muscle samples were excised and weighed, then, samples were placed in an inflated plastic bag and suspended, ensuring that the sample did not make contact with the bag, and stored at 4°C. The muscle was removed from the bag 24 hours post slaughter, wiped, and weighed to evaluate DL, which was expressed as a percentage of the initial muscle weight. To obtain CL, samples were cooked individually in heat-and-seal bags immersed in 75°C water to internal temperature of 70°C. Temperature was measured with a penetrative probe (Hanna Instrument HI 7662) in a meat sample in a bag. Samples were weighed before and after cooking to determine cooking loss. Weight loss was expressed as a percentage of initial weight for cooking loss.

Muscle lactate and glycogen were determined by the method of Zhang *et al.* (2009). Briefly, a frozen muscle sample (0.5 g) was cut and homogenized for 1 minute in 4.5 mL of ice-cold perchloric acid solution (0.85 M  $HClO_4$ ). The homogenates were

centrifuged at 2,700×g at 4°C for 10 minutes, and the supernatant fraction was neutralized with 10 M KOH and then stored in separate tubes at -80°C for analysis. Glycogen and lactate levels were measured using commercially available kits (catalog #: E2GN-100, BioAssay Systems, Hayward, CA and catalog #: K607-100, Biovision Inc, Milpitas, CA for glycogen and lactate, respectively). Assays were adjusted for measurement in microtiter plates. Spectrophotometric analysis was used to determine from the absorbance at 570 nm the glycogen and lactate content of the homogenate using a Multiskan Spectrum mikroplate reader (ThermoLabsystems, Franklin, MA).

Glycogen assay was used as a single Working Reagent that combines the enzymatic break down of glycogen and the detection of glucose in one step. The levels of glucose-6-phosphate and glucose were not determined individually, but they are included in the glycogen determination. The Glycolytic potential (GP,  $\mu M g^{-1}$  of muscle), which represents an estimation of resting glycogen level at death, was calculated in both muscles according to the equation of Monin and Sellier (1985):  $GP = 2 \times (Glycogen + Lactate)$  and expressed as micromoles of lactate equivalent per gram of muscle.

#### Determination of Wing Flapping Duration

On day 42, a total of 240 birds of the ones on the shackle line were recorded for wing flapping duration (WFD) and the WFD from hanging to killing of the birds.

#### Statistical Analysis

Statistical analyses were performed by using software package Statistical Package for the Social Sciences for Windows (SPSS) 15.0 (SPSS Inc, Chicago, IL. USA). For all variables tested, normality was checked by



means of a Shapiro-Wilk test (Zar, 1999). In the case of non-normal data (corticosterone), logarithmic transformations were used for analysis of variance, though actual mean values are presented in the tables. The data was subjected to ANOVA using the GLM procedure with shackling duration and gender as the main effects along with their interactions included in the following model:

$$x_{ijk} = \mu + M_i + D_j + (MD)_{ij} + e_{ijk},$$

Where,  $x_{ijk}$  = Analyzed measurement,  $\mu$  = Overall mean,  $M_i$  = Effect of shackling duration (10, 30, 60 and 120 seconds),  $D_j$  = Effect of gender (male, female),  $(MD)_{ij}$  = Effect of interaction,  $e_{ijk}$  = Residual random error.

In analysis, GLM was designed to reveal the effects of preslaughter shackling duration and gender on blood parameters, meat quality traits, muscle metabolites, and wing flapping durations. The partial effects of shackling duration and gender for each factor were analyzed with Least Squares Means Test and multiple comparisons were performed with a Duncan test (Duncan, 1955). The correlations between muscle and meat traits were calculated using Pearson's correlation coefficients.

## RESULTS

Table 1 shows that shackling treatment resulted in an elevation of CORT level in  $G_{60}$  and  $G_{120}$  birds ( $P < 0.001$ ). It was determined that CORT level was the highest ( $2314.79 \pm 87.39$  pg ml<sup>-1</sup>) in the 120 seconds shackling group while it was the lowest ( $1642.28 \pm 87.39$  pg ml<sup>-1</sup>) in the control group according to the shackling duration. CK activity was found significantly increased in  $G_{120}$  broilers, when compared with  $G_{10}$  and  $G_{30}$  groups ( $P < 0.05$ ). The highest CK activity was found as  $2265.69 \pm 88.40$  U I<sup>-1</sup> in  $G_{120}$  shackling group and this was higher than the control and  $G_{30}$  bird groups ( $1970.64 \pm 95.07$  and  $1948.88 \pm 85.44$  U I<sup>-1</sup>, respectively). Breast and thigh meat quality traits and muscle metabolites, and WFD about shackling groups are also given

in Table 1. The highest breast meat pH<sub>15</sub> ( $6.55 \pm 0.03$ ) was found in  $G_{10}$  shackling group ( $P < 0.01$ ). It was determined that breast  $a^*$  value was the highest ( $3.66 \pm 0.20$ ) in the 120 seconds shackling group while it was the lowest ( $2.90 \pm 0.20$ ) in 10 seconds group according to the shackling duration ( $P < 0.05$ ). Shackling duration has no statistically significant effect on breast and thigh meat pH<sub>u</sub>,  $L^*$ ,  $b^*$ , cooking, and drip losses. Shackling duration significantly affected the level of glycogen ( $P < 0.001$ ), lactate ( $P < 0.05$ ), and GP ( $P < 0.01$ ) in breast muscle. The level of lactate was higher in breast muscle ( $56.60 \pm 1.01$  μM g<sup>-1</sup>) than that in thigh muscle ( $41.94 \pm 1.70$  μM g<sup>-1</sup>), which would contribute to higher GP in breast muscle.

The results of the study revealed that, CORT level was found as 1939.42 and 1924.67 pg ml<sup>-1</sup> for male and female broilers, respectively. Differences in breast and thigh  $b^*$  values between gender groups were found statistically significant ( $P < 0.001$ ). A significant interaction between gender and shackling stress was observed for the pH<sub>u</sub> and  $a^*$  color value of breast meat as shown in Table 1 ( $P < 0.05$ ). However, the gender effect was found as non-significant for breast and thigh muscle metabolites. The correlations among breast and thigh pH values and quality traits within broiler chickens are presented in Tables 2 and 3. Highly positive and statistically significant correlations were determined between thigh meat pH<sub>15</sub> and pH<sub>u</sub> in male and female broilers ( $P < 0.001$ ). Breast meat pH<sub>u</sub> was moderately negatively related to cooking loss in male broilers.

## DISCUSSION

In our study, increasing shackling duration led to higher plasma CORT as reported by Kannan *et al.* (1997), Debut *et al.* (2005), and Bedanova *et al.* (2007) in chicken. Increased CK activity due to stress-inducing preslaughter processes in broilers were also reported by Mitchell *et al.* (1992). The study

**Table 1.** The least square means for some stress parameters, meat quality traits, muscle metabolites, and wing flapping durations.<sup>a</sup>

Parameters	Shackling treatment (S)			Gender (G)		Pooled SEM <sup>b</sup>	Significance			
	G <sub>10</sub> (n= 20)	G <sub>30</sub> (n= 20)	G <sub>60</sub> (n= 20)	G <sub>120</sub> (n= 20)	Male (n= 40)		Female (n= 40)	S	G	S×G
<b>Biochemical</b>										
CORT (pg ml <sup>-1</sup> )	1642.28 <sup>c</sup>	1819.50 <sup>b,c</sup>	1951.60 <sup>b</sup>	2314.79 <sup>a</sup>	1939.42	1924.67	45.07	***	-	-
CK (U l <sup>-1</sup> )	1970.64 <sup>b</sup>	1948.88 <sup>b</sup>	2144.41 <sup>a,b</sup>	2265.69 <sup>a</sup>	2123.12	2041.69	45.19	*	-	-
<b>Meat Quality</b>										
<i>Breast Meat</i>										
pH <sub>1,5</sub>	6.55 <sup>a</sup>	6.47 <sup>a,b</sup>	6.40 <sup>b</sup>	6.41 <sup>b</sup>	6.46	6.46	0.02	**	-	-
pH <sub>u</sub>	6.14	6.11	6.15	6.14	6.12	6.15	0.01	-	-	*
L*	58.58	58.44	57.93	58.50	59.78 <sup>a</sup>	56.94 <sup>b</sup>	0.38	-	***	-
a*	2.90 <sup>b</sup>	3.03 <sup>b</sup>	3.01 <sup>b</sup>	3.66 <sup>a</sup>	3.19	3.11	0.10	*	-	*
b*	3.69	4.07	4.67	3.65	4.36 <sup>b</sup>	3.68 <sup>b</sup>	0.18	-	*	-
CL (%)	28.45	28.66	27.96	27.25	28.97 <sup>a</sup>	27.19 <sup>b</sup>	0.40	-	*	-
DL (%)	4.43	3.93	3.64	3.24	3.91	3.71	0.16	-	-	-
<i>Thigh Meat</i>										
pH <sub>1,5</sub>	6.57	6.59	6.61	6.58	6.59	6.58	0.01	-	-	-
pH <sub>u</sub>	6.47	6.49	6.54	6.51	6.52	6.49	0.01	-	-	-
L*	57.43	59.63	58.03	56.95	59.64 <sup>a</sup>	56.38 <sup>b</sup>	0.45	-	***	-
a*	5.59	6.07	5.66	5.71	5.74	5.77	0.17	-	-	-
b*	4.81	6.51	6.08	5.22	6.45 <sup>a</sup>	4.87 <sup>b</sup>	0.24	-	***	-
CL (%)	30.33	30.88	28.81	27.87	29.72	29.22	0.46	-	-	-
DL (%)	3.78	3.42	3.01	3.65	3.57	3.36	0.13	-	-	-
<b>Muscle Metabolites (µM g<sup>-1</sup>)</b>										
Breast glycogen	38.57 <sup>a</sup>	37.69 <sup>a</sup>	36.00 <sup>a</sup>	32.77 <sup>b</sup>	36.65	35.87	0.52	***	-	-
Breast lactate	52.54 <sup>b</sup>	54.73 <sup>a,b</sup>	55.00 <sup>a,b</sup>	56.60 <sup>a</sup>	54.26	55.18	0.50	*	-	-
Breast GP	130.13 <sup>a</sup>	130.11 <sup>a</sup>	127.00 <sup>a,b</sup>	121.74 <sup>b</sup>	127.65	126.84	0.90	**	-	-
Thigh glycogen	18.69 <sup>a</sup>	18.18 <sup>a</sup>	17.21 <sup>a,b</sup>	15.76 <sup>b</sup>	17.57	17.35	0.36	*	-	-
Thigh lactate	40.30	41.50	41.46	41.94	41.02	41.58	0.83	-	-	-
Thigh GP	77.67 <sup>a</sup>	77.85 <sup>a</sup>	75.89 <sup>b</sup>	73.46 <sup>c</sup>	76.16	76.28	0.35	***	-	-
WFD (s)	4.69 <sup>a,b</sup>	3.61 <sup>b</sup>	5.08 <sup>a,b</sup>	6.21 <sup>a</sup>	5.04	4.76	0.28	*	-	-

<sup>a</sup> Data presented as the least square means, <sup>a,b,c</sup> Means with different superscript letters in the same row differ (P<0.05). \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001, -; Non-significant. <sup>b</sup> Standard Error Mean.



**Table 2.** Pearson correlation coefficients among Pectoralis major muscle pH and meat quality traits in broilers.

Parameters <sup>a</sup>		Pectoralis major muscle					
Male	pH <sub>15</sub>	pH <sub>u</sub>	L*	a*	b*	CL (%)	DL (%)
pH <sub>15</sub>	-						
pH <sub>u</sub>	-0.005	-					
L*	0.125	-0.212	-				
a*	-0.169	-0.020	-0.023	-			
b*	0.166	0.256	0.042	-0.441**	-		
CL (%)	-0.032	-0.466**	0.315*	0.066	-0.050	-	
DL (%)	0.004	-0.198	0.326*	-0.049	0.024	0.179	-
Female	pH <sub>15</sub>	pH <sub>u</sub>	L*	a*	b*	CL (%)	DL (%)
pH <sub>15</sub>	-						
pH <sub>u</sub>	0.414**	-					
L*	-0.076	-0.208	-				
a*	-0.014	-0.005	-0.066	-			
b*	0.011	0.137	-0.005	0.043	-		
CL (%)	0.260	-0.221	0.177	-0.001	0.043	-	
DL (%)	0.045	-0.216	-0.028	0.122	-0.014	0.104	-

<sup>a</sup> pH<sub>15</sub>= pH measured 15 minutes *post-mortem*; pH<sub>u</sub>= pH measured 24 hours *post-mortem*; L\*= Lightness; a\*= Redness; b\*= Yellowness; CL= Cooking Loss (% of the initial muscle weight), DL= Drip Loss (% of the initial muscle weight). \*: P< 0.05; \*\*: P< 0.01, \*\*\*: P< 0.001.

**Table 3.** Pearson correlation coefficients among Ilio tibialis muscle pH and meat quality traits in broilers.

Parameters <sup>1</sup>		Ilio tibialis muscle					
Male	pH <sub>15</sub>	pH <sub>u</sub>	L*	a*	b*	CL (%)	DL (%)
pH <sub>15</sub>	-						
pH <sub>u</sub>	0.649***	-					
L*	0.114	0.147	-				
a*	0.181	-0.005	-0.320*	-			
b*	0.066	0.090	0.402*	0.040	-		
CL (%)	-0.183	-0.156	-0.026	-0.053	0.042	-	
DL (%)	-0.186	0.046	-0.318*	0.108	-0.230	0.017	-
Female	pH <sub>15</sub>	pH <sub>u</sub>	L*	a*	b*	CL (%)	DL (%)
pH <sub>15</sub>	-						
pH <sub>u</sub>	0.527***	-					
L*	-0.069	-0.119	-				
a*	0.123	0.000	-0.274	-			
b*	0.034	0.070	0.275	0.012	-		
CL (%)	-0.313*	-0.226	0.299	-0.243	0.389*	-	
DL (%)	-0.089	-0.158	0.016	-0.035	-0.187	0.223	-

<sup>a</sup> pH<sub>15</sub>= pH measured 15 minutes *post-mortem*; pH<sub>u</sub>= pH measured 24 hours *post-mortem*; L\*= Lightness; a\*= Redness; b\*= Yellowness; CL= Cooking Loss (% of the initial muscle weight), DL= Drip Loss (% of the initial muscle weight). \*: P< 0.05; \*\*: P< 0.01, \*\*\*: P< 0.001.

has shown that preslaughter shackling treatment were experienced as stressful events of broilers, as indicated by the rising

concentration of the CORT and CK activity after treatment. This confirms the

importance of restricting preslaughter stress in order to increase the animal welfare.

In the study, increase in shackling duration promoted bird wing flapping and, as a result, hastened breast muscle  $pH_{15}$  drop after death. Berri *et al.* (2005) indicated that the decrease in  $pH_{15}$  was 0.06 pH units compared to 10 and 120 seconds shackling duration groups (6.48 vs 6.42). In the present study, the  $pH_{15}$  values of stressed birds ( $G_{60}$  and  $G_{120}$  birds) remained relatively similar (around 6.40) compared to those reported in the study by Berri *et al.* The WFD reported in the study was parallel to (between 3.61 and 6.21 seconds according to the shackling condition) the preslaughter shackling applied in the Debut *et al.* (2005) study (1 to 5 seconds). In this study, the 120 seconds shackling duration resulted in higher  $a^*$  value than the 10, 30, and 60 seconds shackling durations ( $P < 0.01$ ). Similarly, Kannan *et al.* (1997), Berri *et al.* (2005), and Schneider *et al.* (2012) indicated that there was an increase in redness due to increase in shackling duration in broilers.

The GP values reported for normal breast meat of  $G_{10}$  (control),  $G_{30}$ , and  $G_{60}$  shackled birds in this study was similar to GP values reported for breast meat of broilers exposed to minimal stress preslaughter ( $127.8 \mu M g^{-1}$ ) by Debut *et al.* (2005). The present study showed that muscle *post-mortem* metabolic changes were influenced by preslaughter activity, especially in breast muscle where lactate level at *post-mortem* increased with preslaughter struggling activity. Significant effects of preslaughter durations on meat characteristics and some interactions with the gender were observed in the present study. However, preslaughter durations had higher effect than gender and interactions were limited to thigh characteristics.

This study results also showed that breast muscle was more sensitive to struggling activity than thigh muscle in which lactate level was barely affected. These results suggest that breast meat is more sensitive to environmental factors than thigh meat. This could be related to the glycolytic status of

the breast muscle and its association with wing flapping activity. Depletion of glycogen reserves from skeletal muscles due to preslaughter stress has been reported to affect meat quality (Bee *et al.*, 2006). As a result of shackling stress, glycogenolysis in breast muscle was increased dramatically, which might cause accumulation of lactate and could further induce a lower  $pH_{15}$  and higher  $a^*$  color values. In the present study, the accelerated rate of pH decline (low  $pH_{15}$ ) was mainly associated with higher values of  $a^*$  of the meat. It can be said that pale color, soft texture, and high water loss of meat was related to increased rate of early *post-mortem* glycolysis. Similarly, Kannan *et al.* (1997), Berri *et al.* (2001), and Debut *et al.* (2003) indicated that as a result of lactate accumulation, glycolysis lowered  $pH_u$ , which further influenced meat color and drip loss. This study suggested that slaughtering the broilers with longer shackling duration could be disadvantage due to excess struggling and also accelerated *post-mortem* glycolysis, which is detrimental for the quality of breast meat. It can be said that the shackling duration should not exceed 60 seconds in processing plants for animal welfare and meat quality point of view.

It was determined that there were increased breast and thigh  $L^*$  and  $b^*$  values in male broilers due to before-slaughter shackling stress. Regarding meat quality, an increase in fiber cross-sectional area corresponded to meat with a darker color (lower  $L^*$ ). In the same way, muscles of female chickens, which contain larger fibers than those of males, are also characterized by darker color. This result was in agreement with Berri *et al.* (2007), who observed the color  $L^*$  values of 55.54 and 54.25 for male and female, respectively ( $P < 0.001$ ). The reported effect of gender on breast and thigh meat  $L^*$  values indicated that males had higher ( $P < 0.01$ )  $L^*$  value than females (Yetişir *et al.*, 2008). However, some researchers have reported no effects of gender on breast muscle color  $L^*$  and  $b^*$  attributes in birds (Anadon, 2002; Salakova *et al.*, 2009; Lopez *et al.*, 2011). Schneider



et al. (2012) noted that breast meat  $L^*$  and  $b^*$  values were higher in female broilers than male.

In this study, the effect of gender on breast meat cooking loss showed that males had higher loss (28.97 %) than females (27.19 %). This finding was similar to the study performed by Salakova et al. (2009). However, no gender differences in breast meat cooking loss were found in some other studies (Schneider, 2009; Lopez et al., 2011; Schneider et al., 2012). In this study, it was determined that gender did not affect breast and thigh muscle metabolites. Berri et al. (2007) reported similar findings on breast muscle metabolite levels related to gender.

In this study, breast meat  $pH_{15}$  was significantly correlated with  $pH_u$  (0.414) in female broilers. This correlation agrees with those reported by Anadon (2002) who observed that the correlation between breast meat pH at 4 hours and pH at 24 hours postmortem was significant in male. It can be said that the early rate of postmortem pH declined during bleeding and this effect was most likely due to wing flapping during this period. It was determined that a negative correlations existed between chicken breast meat  $pH_{15}$  and  $a^*$  values; breast meat between  $pH_u$  and  $L^*$  values; breast meat between  $pH_u$  and  $DL$  values in male and female broiler. A similar relationship between breast meat  $pH_u$  and  $L^*$  values has also been reported, with decreasing  $pH_u$  associated with increasing  $L^*$  values (Debut et al., 2003; Berri, 2005; Berri et al., 2007; Salakova et al., 2009). Debut et al. (2003) reported that negative correlations of 0.52 was observed between  $pH_u$  and  $DL$  values of the breast meat in the female broiler. In this study, thigh  $L^*$  value was correlated with higher  $b^*$  value, in agreement with a previous study (0.31) on the thigh meat quality (Debut et al., 2003).

## CONCLUSIONS

As a conclusion, this study has shown that shackling before slaughter was experienced

as a stressful event by birds, as indicated by the rising level of plasma CORT and CK activity after preslaughter shackling treatment. The results demonstrated that *ante-mortem* struggling and glycogen level in muscle at death were key factors associated with the onset and extent of postmortem pH decline and, therefore, the quality decline of broiler breast and thigh meats. Reduction in struggling and WFD in the brief period immediately after live-bird shackling and before slaughter may also reduce discomfort and, thereby, improve the well-being of the birds. For this reason, to reach fewer physiological stress response and better meat quality in broiler production, it is suggested that optimum shackling period should range from 10 to 60 s.

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## اثر قلاب بندی قبل از کشتار روی تنش، کیفیت گوشت و مقدار گلیکوژن (Glycolytic Potential) جوجه های گوشتی

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### چکیده

هدف این پژوهش بررسی اثر مدت قلاب بندی قبل از کشتار روی برخی پارامترهای خون، صفات کیفیت گوشت ران و سینه، و متابولیت های ماهیچه در جوجه های گوشتی (کبابی) بود. به این منظور، اثرات قلاب بندی روی یک گروه جوجه ۴۲ روزه شامل ۲۴۰ جوجه ی کبابی از نژاد راس ۳۰۸ (Ross 308) تعیین شد. چهار تیمار قلاب بندی در این آزمون استفاده شد: قلاب بندی به مدت ۱۰ ثانیه (گروه  $G_{10}$ )، ۳۰ ثانیه ( $G_{30}$ )، ۶۰ ثانیه ( $G_{60}$ ) و ۱۲۰ ثانیه ( $G_{120}$ ). نتایج نشان داد که مقدار کورتیکوسترون (CORT) برابر  $2314.79 \text{ pg/ml}$  در تیمار ۱۲۰ ثانیه به طور معنی داری افزایش یافت ( $P < 0.01$ ). نیز بر اساس نتایج، فعالیت کیناز (CK) در تیمار ۱۲۰ ثانیه به طور معنی داری از همه بیشتر ( $2265.69 \text{ U/I}$ ) و در گروه ۱۰ ثانیه ( $1970.64 \text{ U/I}$ ) از همه کمتر ( $P < 0.05$ ) بود. همچنین قرمزی گوشت سینه با افزایش مدت قلاب بندی زیاد شد ( $P < 0.05$ ). نیز آشکار شد که مدت زمان قلاب بندی مقدار گلیکوژن ماهیچه سینه را در همه تیمار ها کاهش داد ( $P < 0.05$ ). بر عکس، مقدار لاکتیت در سینه با افزایش زمان قلاب بندی زیاد شد ( $P < 0.05$ ). نتایج نشان داد که بین پی اچ نهایی گوشت سینه و تلفات پخت (cooking loss) در جوجه های نر یک رابطه منفی ( $r = -0.466$ ) وجود داشت ( $P < 0.05$ ). به گواهی این نتایج می توان گفت که قلاب بندی قبل از کشتار ممکن است روشی بسیار تنش دار برای جوجه ها باشد به ویژه اگر بیشتر از ۶۰ ثانیه طول بکشد. بر اساس این آزمون پیشنهاد می شود که طولانی بودن زمان قلاب بندی جوجه ها مضر است زیرا با دست و پا زدن بیشتر آنها و تشدید گلیکولیز، کیفیت گوشت سینه کاهش می یابد.