

THE EFFECTS OF PRESLAUGHTER SHACKLING ON SOME STRESS PARAMETERS, FEAR, AND BEHAVIOURAL TRAITS IN BROILERS

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Abstract. The aim of this study was to investigate the effects of preslaughter shackling durations on some stress parameters, fear reactions and behavioural traits in broilers. Stress effects of shackling were determined in a group of Ross 308 broilers (total number: 272) aged 42 d. Four shackling treatments were used in experimental tests: shackling of broilers for 10 s (Group G₁₀; as control), 30 s (Group G₃₀), 60 s (Group G₆₀), and 120 s (Group G₁₂₀). Results showed that heterophil to lymphocyte (h/l) ratio (1.39) at 120 s shackling group increased ($P<0.01$). It was revealed that shackling had increased blood glucose and cholesterol levels in all treatment groups. The results indicated that shackling duration has no significant effect on Tonic Immobility (TI) duration. The straightening up of the body, vocalisation, and wing flapping activities increased due to increase in shackling duration. It was concluded that shackling duration over 60 s have negative effects on some stress parameters and behavioural traits in broilers; for that reason, broilers should be housed at lower preslaughter shackling durations.

Keywords: broiler, h/l ratio, shackling, stress, tonic immobility.

Introduction

The slaughter of poultry differs from the slaughter of all other meat-producing animals because the live birds are hung upside down in shackles preslaughter (Bedanova et al., 2007a). Broilers display struggling behaviour immediately after they are inverted and suspended by shackles, suggesting that they become distressed as a result of preslaughter process (Lines et al., 2011). There should be a time lapse between shackling and stunning or killing that is just long enough for the birds to stop wing flapping (Kannan et al., 1997; Bedanova et al., 2007a, b).

The response to stress is generally estimated by blood variables such as h/l ratio, glucose, triglyceride, lactate, cholesterol, total protein levels, aspartate aminotransferase activity, acid-base status (Debut et al., 2005; Bedanova et al., 2007a; Türkyılmaz et al., 2011). Nijdam et al. (2005) found that plasma glucose and lactate levels increased during shackling. Bedanova et al. (2007a, b) reported that longer shackling duration led to an increase in h/l ratio. Fear level in poultry is simply measured by TI test (Jones, 1996). TI is an anti-predator behaviour shown in situations where the chicken has been caught by a predator (Thompson and Liebreich, 1987). Zulkifli et al. (2000) observed a prolonged TI duration in response of broiler chicks to hanging in an inverted position and claimed augmented fearfulness.

The aim of this study was to evaluate the stress and fear parameters such as h/l ratio, biochemical (glucose, cholesterol, triglyceride, total protein, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities), acid-base status (pH, pCO₂, pO₂, sO₂, tCO₂, HCO₃⁻), hematocrit, electrolytes (Na⁺, K⁺, Ca²⁺), TI and to compare physiological and behavioural responses to preslaughter stress in broilers.

Materials and methods

Material

As a material, a total of 272 1-d old Ross 308 broiler chickens were used. From the first day after hatching, broilers 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 (3100 kcal ME/kg; 22% crude protein) to finisher pellet (3250 kcal ME/kg; 21% crude protein) at 21 days of age. The ambient barn temperature was gradually decreased from 32±1°C on d 1 to 23±1°C on the last day of fattening (d 42). The relative humidity varied from 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). On day 42, 240 birds were selected at random for tests related to shackling. Five experimentalists captured 1 broiler each and transported it by hand to the test room (Bedanova et al., 2007a), 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 shackled broilers could see, hear, and partially touch each other during the test and those were allowed to flap freely. Four shackling treatments were used in experimental tests: shackling of broilers for 10 s (Group G₁₀; as control, n=30), 30 s (Group G₃₀, n=30), 60 s (Group G₆₀, n=30), and 120 s (Group G₁₂₀, n=30). The shackling treatments were repeated twice for each test (biochemical parameters, h/l ratio, acid-base status and TI test) described below.

Blood Parameters Analysis

Blood samples from a total of 80 birds (20 birds (10 male and 10 female) for each test groups) were used for biochemical examination. Immediately after shackling treatment, birds were killed by exsanguination through a neck cut. The blood tubes were allowed to clot for 2 h at 37°C, and then serum was decanted and stored at -20°C for later analyses (Daneshyar et al., 2009). One milliliter of blood was injected in a blood gas/electrolyte analyzer (IRMA TRUpoint™ Blood Analysis Systematories, Edison, NJ, USA) in which specific microelectrodes measured the pH, partial pressure of CO₂ (pCO₂), partial pressure of O₂ (pO₂), oxygen saturation (sO₂), total carbon dioxide (tCO₂), bicarbonate (HCO₃⁻), hematocrit (Hct), hemoglobin (Hb) and electrolytes. These values were corrected to reflect body temperature of 41.5°C. Selected serum biochemical parameters were measured by a spectrophotometry (Shimadzu UV-1601, Duisburg, FR, Germany) using commercial test kits (Diasis Diagnostics Systems, Turkey).

Heterophil to Lymphocyte Ratio (h/l)

Blood samples from remaining of 80 birds (20 birds (10 male and 10 female) for each test groups) were used for determining the h/l ratio. After the shackling, the used birds were spray-painted on their backs and allowed to their groups gently. After 20 h, blood samples were taken from the vena basilica of broilers in each shackling group. Because the h/l ratio response to short-duration stress peaks after 20 h (Zulkifli et al., 2002). Blood films were prepared from the samples and painted with May-Grünwald and Giemsa dyes (Gross and Siegel, 1983). After 100 leucocytes were counted in light microscope with (x100) magnification, h/l ratio was calculated by dividing heterophil count to lymphocyte count.

Tonic Immobility Test

The last 80 birds (20 birds (10 male and 10 female) for each test groups) were used for TI test. Immediately following shackling treatment, birds were subjected to the TI test that was described by Benoff and Siegel (1976).

Behavioural Traits

All of the birds on the shackle line were used for behavioural traits. The 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 slaughter and noted as a binary variable equal to 0 when the bird did not try to stand up (absence) and otherwise 1 (presence). Vocalisation (VO) and wing flapping (WF) were recorded when the bird was hung and were classified into 3 categories. VO categories; 0 when the bird did not vocalise, 1 when the bird vocalised briefly, 2 when it vocalised for a long time. WF categories; 0 when the bird did not try to wing flapping (absence), 1 when the bird wing flapped briefly, 2 when it flapped for a long time.

Statistical Analysis

SPSS version 15.0 was used for analysis and a General Linear Model (GLM) was designed to reveal the effects of preslaughter shackling duration and gender on blood variables and TI and WF durations. The partial

effects of shackling duration and gender for each factor were analysed with Least Square Means Test and multiple comparisons were performed with Duncan test (Harvey, 1987; Sümbüloğlu and Sümbüloğlu, 1993). Kruskal-Wallis ANOVA was used for TI induction (Sümbüloğlu and Sümbüloğlu, 1993). After categorisation of SU, VO and WF variables into classes of equal size, the effects of shackling duration and gender on categorical behavioural variables was tested by chi-square test (Sümbüloğlu and Sümbüloğlu, 1993).

Results

As can be seen in Table 1, shackling treatment led to an increase in stress in broilers. It was determined that h/l ratio was the highest (1.39) in the 120 s shackling group while was the lowest (0.69) in 10 s group according to the shackling duration (P<0.01). Stress related blood parameters, TI and WF durations about 10, 30, 60 and 120 s shackling groups are given in Table 1. Glucose level, as an important blood parameter in stress, was found significantly (P<0.05) increased in G₁₂₀ broilers when compared with G₁₀ and G₃₀ groups. Unlike these findings, there was no significant differences between G₆₀ and G₁₂₀ groups. The highest cholesterol level was found as 3.45 mmol/L in G₁₂₀ shackling group and this was higher (P<0.05) than control and G₃₀ bird groups. Shackling duration has no statistically significant effect on triglyceride, total protein, LDH and AST activity. The results also revealed that, h/l ratio was found as 0.83 and 1.07 for male and female broilers respectively, and there is no statistical significance between two genders. The cholesterol level in males (3.41 mmol/L) was found higher than female counterparts (3.04 mmol/L) (P<0.01). Differences in LDH activities between gender groups were found statistically significant (P<0.05).

While preslaughter shackling duration had a statistically significant (P<0.05) effect on WF duration, there was no significant effect on TI duration. As related to fear, the highest TI duration (414.29 s) was determined in female broilers (P<0.05). In other words, female broilers had been feared more than males. In this study, the statistically non-significant interaction was determined between shackling duration and gender groups for blood variables and TI and WF durations. The number of TI inductions in G₁₀, G₃₀, G₆₀ and G₁₂₀ broilers were found as 1.2, 1.3, 1.2 and 1.1, respectively (P>0.05). As measured by a number of attempts to induce TI, gender had a non-significant effect on susceptibility to TI.

In the study, statistically difference was found only for tCO₂ and HCO₃⁻ levels (P<0.05). However, the gender effect was found non-significant for blood gas parameters, Hct, Hb, and electrolytes (Table 2).

The effect of shackling duration on straightening up, vocalisation, and wing flapping are given in Table 3. For male broilers, frequencies of classes 1 and 2 of VO were higher under G₃₀, G₆₀ and G₁₂₀ conditions than in G₁₀. In contrast, preslaughter shackling duration did not affect WF activity of female birds, and it did not affect SU activity of male broilers.

Table 1. The least square means for TI and WF durations, some stress parameters¹

Parameters	Expected mean (μ) (n=80)	Shackling treatment (S)				Gender (G)		Pooled SED	Significant		
		G ₁₀ (n=20)	G ₃₀ (n=20)	G ₆₀ (n=20)	G ₁₂₀ (n=20)	Male (n=40)	Female (n=40)		S	G	SXG
Hematological											
h/l ratio	0.95	0.69 ^b	0.79 ^b	0.93 ^b	1.39 ^a	0.83	1.07	0.07	**	NS	NS
Biochemical											
Glucose (mmol/L)	13.30	12.81 ^b	13.09 ^b	13.14 ^{ab}	14.15 ^a	13.30	13.29	0.18	*	NS	NS
Cholesterol (mmol/L)	3.22	3.02 ^b	3.03 ^b	3.38 ^{ab}	3.45 ^a	3.41 ^a	3.04 ^b	0.06	*	**	NS
Triglyceride (mmol/L)	0.85	1.01	1.06	0.66	0.66	0.67	1.02	0.10	NS	NS	NS
Total protein (g/L)	31.34	31.53	30.14	30.57	33.12	31.75	30.93	0.51	NS	NS	NS
LDH (U/L)	833.93	792.21	811.85	851.54	880.09	753.54 ^b	914.31 ^a	37.03	NS	*	NS
AST (U/L)	323.93	331.15	288.68	320.01	355.88	305.80	342.06	16.31	NS	NS	NS
TI duration (s)	361.93	298.96	396.94	358.27	393.55	309.57 ^b	414.29 ^a	20.75	NS	*	NS
WF duration (s)	4.90	4.69 ^{ab}	3.61 ^b	5.08 ^{ab}	6.21 ^a	5.04	4.76	0.28	*	NS	NS

¹Data presented as the least square means; ^{ab} Means with different superscript letters in the same row differ (P<0.05), NS: Not significant, *: P<0.05, **: P<0.01

Table 2. The least square means for blood gas parameters¹

Gas Parameters	Expected mean (μ) (n=80)	Shackling treatment (S)				Gender (G)		Pooled SED	Significant		
		G ₁₀ (n=20)	G ₃₀ (n=20)	G ₆₀ (n=20)	G ₁₂₀ (n=20)	Male (n=40)	Female (n=40)		S	G	SXG
pH	7.31	7.32	7.31	7.30	7.31	7.31	7.31	0.01	NS	NS	NS
pCO ₂ (mmHg)	46.38	47.13	47.88	45.48	45.04	46.18	46.58	0.73	NS	NS	NS
pO ₂ (mmHg)	73.44	72.75	73.56	73.99	73.45	74.15	72.72	1.61	NS	NS	NS
sO ₂ (%)	84.32	84.83	83.73	84.55	84.20	85.10	83.55	1.03	NS	NS	NS
tCO ₂ (mEq/L)	23.04	24.18 ^a	23.63 ^{ab}	22.00 ^b	22.34 ^b	22.81	23.27	0.28	*	NS	NS
HCO ₃ ⁻ (mEq/L)	21.87	22.98 ^a	22.43 ^{ab}	20.84 ^b	21.22 ^b	21.64	22.10	0.27	*	NS	NS
Hct (%)	24.15	23.57	24.84	23.05	25.13	24.59	23.70	0.58	NS	NS	NS
Hb (g/dl)	8.27	8.26	8.45	7.85	8.54	8.36	8.19	0.19	NS	NS	NS
Electrolytes											
Na ⁺ (mEq/L)	143.38	144.55	142.77	143.49	142.73	143.26	143.51	0.31	NS	NS	NS
K ⁺ (mEq/L)	5.17	5.37	5.18	5.12	5.00	5.00	5.33	0.08	NS	NS	NS
Ca ²⁺ (mEq/L)	1.62	1.47	1.53	1.62	1.85	1.54	1.70	0.09	NS	NS	NS

¹Data presented as the least square means; ^{ab} Means with different superscript letters in the same row differ (P<0.05), NS: Not significant, *: P<0.05

Table 3. Frequencies (%) per class of behavioural variables (SU, VO, and WF) and chi-square results by shackling duration and gender

SU		G ₁₀		G ₃₀		G ₆₀		G ₁₂₀		P				
		0	1	0	1	0	1	0	1					
	Male	53.3	46.7	56.7	43.3	60.0	40.0	30.0	70.0	0.083				
	Female	63.3	36.7	83.3	16.7	66.7	33.3	36.7	63.3	0.002				
P		0.600		0.049		0.789		0.784						
VO		G ₁₀			G ₃₀			G ₆₀			G ₁₂₀			P
		0	1	2	0	1	2	0	1	2	0	1	2	
	Male	53.3	36.7	10.0	40.0	40.0	20.0	33.3	43.3	23.3	10.0	43.3	46.7	0.007
	Female	46.7	43.3	10.0	53.3	36.7	10.0	53.3	36.7	10.0	13.3	30.0	56.7	0.000
P		0.861			0.446			0.207			0.560			
WF		G ₁₀			G ₃₀			G ₆₀			G ₁₂₀			P
		0	1	2	0	1	2	0	1	2	0	1	2	
	Male	36.7	36.7	26.6	50.0	26.7	23.3	50.0	26.7	23.3	10.0	46.7	43.3	0.030
	Female	43.3	40.0	16.7	50.0	33.3	16.7	36.7	36.7	26.7	13.3	46.7	40.0	0.073
P		0.637			0.757			0.561			0.913			

SU: straightening up, VO: vocalisation, WF: wing flapping

Discussion

As parallel to an increase in shackling periods (10-120 s), the h/l ratio was increased from 0.69 to 1.39. These results are in agreement with the findings of other researchers (Zulkifli et al., 2000, 2002; Bedanova et al., 2007a, b). According to Siegel and Gross (2000), who stated that h/l ratios ranging from 0.6 to 1.2 indicate a higher level of stress, we can deduce that the shackling of broilers for a 120 s duration is a very stressful procedure.

As a blood parameter, it was revealed that glucose level was increased in broilers as parallel to increase in shackling duration. Increasing blood glucose levels, due to the effect of glucocorticoids (Simon, 1984), are described as an important indicator of stress condition. It is known that basal glucose level in chickens is ranging from 11.10 to 13.88 mmol/L (Karagül et al., 2000). Increased glucose levels due to preslaughter processing of broilers were also reported by Kannan et al. (1997), Nijdam et al. (2005), Bedanova et al. (2007a) Chloupek et al. (2011). Cholesterol level was increased to 3.45 mmol/L in 120 s shackling group due to an increasing shackling duration while cholesterol level in control group was measured as 3.02 mmol/L. Karagül et al. (2000) reported that basal cholesterol level is varying from 3.24 to 5.18 mmol/L in chickens. Similarly, Türkyılmaz et al. (2011) indicated that there was an increase in cholesterol level due to high preslaughter stress conditions in broilers. On the other hand, Daneshyar et al. (2009) found no changes in cholesterol levels of broilers at cold stress. In this study, it was determined that shackling duration did not affect serum triglyceride, total protein levels, LDH, and AST enzyme activities. Bedanova et al. (2007a) and Chloupek et al. (2011) revealed that stress has no significant effect on total protein and triglyceride levels, while Daneshyar et al. (2009) also reported that LDH and AST were not affected by cold stress. The LDH activity result of our study is consistent with those of Khajali and Quejck (2005), who did not observe any significant difference for LDH activity between healthy and ascitic broilers. Blood HCO_3^- and tCO_2 concentrations decreased in G_{60} and G_{120} broilers compared to G_{10} ($P < 0.05$). It can be assumed that decreased HCO_3^- level was related to the intense struggle on the shackling line. Decreased tCO_2 level that represents solved CO_2 in plasma could lead to a decrease in HCO_3^- level. In the study, the differences between shackling groups for electrolyte levels were found as statistically insignificant. Debut et al. (2005), reported similar findings on Na^+ , K^+ , Ca^{++} levels related to shackling duration. The mentioned parameters indicated a high level of stress in broiler chickens during long-lasting shackling at the slaughter line unless they were slaughtered immediately after shackling.

The differences between shackling duration groups for TI duration and TI induction were found as statistically non-significant. It is indicated that there was no change in the level of fear in shackled broilers due to the extension of the shackling period. Zulkifli et al. (2000), indicated no significant effect of handling in inverted position on susceptibility to TI in broilers.

However, by Bedanova et al (2007a) a significant positive correlation between shackling period and TI duration was found in broilers.

It was determined that an increasing hanging duration promoted bird WF. It can be said that vigorous WF can be seen as an escape behaviour and indicator of discomfort. Reduction in struggling and WF during the brief period immediately after live-bird hanging and before slaughter may also reduce discomfort and, thereby, improve the well-being of the birds. The proportions of birds in class 1 of SU and in class 2 of VO were largely increased in G_{120} shackling compared to the G_{10} , G_{30} and G_{60} groups for female birds. The increase in SU, VO, and WF activities due to stress-inducing preslaughter processes in broilers was similar to other studies (Satterlee et al., 2000; Debut et al., 2005).

Gender has a statistically significant ($P < 0.01$) effect on cholesterol level in broilers which had been shackled before slaughter. In other words, male birds were more susceptible to shackling than female birds. Similarly, Türkyılmaz et al. (2011) observed an increased level of cholesterol in male broilers due to acute preslaughter stress. Bowes et al. (1989) reported that the normal level of cholesterol was 3.23 and 3.13 mmol/L for male and female, respectively.

The effect of gender on TI showed that males had shorter TI duration (309.57 s) than females (414.29 s). These finding was similar to the study performed by Wang et al. (2008). However, no gender differences in TI duration were found in broilers (Türkyılmaz et al., 2011).

Conclusions

This study has shown that shackling before slaughter was experienced as a stressful event by broilers, as indicated by the rising h/l ratio, glucose, cholesterol after treatment. And stressful preslaughter conditions led to more wing flapping and vocalisation. Briefly, it can be recommended that optimum shackling period should range from 10 to 60 s in poultry processing plants.

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