Stress in Broilers Resulting from Shackling

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ABSTRACT The aim of this study was to assess stress response of broilers to different periods of shackling. Stress effects of shackling were monitored in a group of male Ross 308 broilers (total number: 400) aged 42 d. Three shackling treatments were used in our experiment: shackling of broilers for 30 s (group T_{30}), 60 s (group T_{60}), and 120 s (group T_{120}). Corticosterone plasma concentration was elevated in T_{60} broilers ($P < 0.05$) and in T_{120} birds ($P < 0.01$); glucose plasma concentration was increased ($P < 0.05$) in both T_{60} and T_{120} broilers when compared with nonshackled control. Lactate concentrations increased in T_{30} birds ($P < 0.05$) and in both T_{60} and T_{120} birds ($P < 0.01$). Furthermore, T_{120} broilers exhibited an increase ($P < 0.01$) in heterophil counts and heterophil:lymphocyte ratio. Duration of tonic immobility was increased ($P < 0.05$) in T_{60} and T_{120} broilers. Number of attempts to induce tonic immobility decreased ($P < 0.01$) in all test groups (T_{30}, T_{60}, T_{120}). Duration of shackling period was positively correlated ($P < 0.001$) with corticosterone, glucose and lactate level, tonic immobility duration, and heterophil:lymphocyte ratio. The number of inductions was negatively correlated ($P < 0.001$) with duration of the shackling period. According to the results of our study, the act of shackling is a considerable traumatic procedure for broilers, and its stress effect is markedly dependent on duration of shackling period that the broiler chickens experience. It follows from our study that the optimal shackling period should be less than 60 s.

Key words: broiler, shackling, stress, welfare

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INTRODUCTION

Despite increasing efforts that have gone into ensuring animal welfare and eliminating stress in birds during the whole preslaughter processing of poultry, until now, only minor attention has been devoted to potential stress associated with shackling and whether welfare is affected by factors such as the length of time the bird hangs on the shackles. A time lapse between shackling and stunning is unavoidable in commercial processing plants. Council Directive 93/119/EC (European Union, 1993) on the protection of animals at the time of slaughter or killing requires that poultry are in a sufficiently relaxed state on the shackles for stunning to be carried out effectively. Therefore, it is recommended that there should be a time lapse between shackling and stunning that is just long enough for the birds to stop wing flapping. Gregory and Bell (1987) suggested that chickens should not be put through the stunner for a period of 12 s after shackling. Maximum time lapse is not strictly set by European Commission directives. The aim of this study was to assess stress response of broilers to different periods of shackling in conditions similar to practice at slaughterhouses.

Corticosterone concentration in blood plasma is widely used as a measurement of environmental stress in birds. Corticosterone is the principal glucocorticoid released by the avian adrenal gland, and elevated plasma corticosterone is therefore an accepted indicator of stress condition in birds (McFarlane and Curtis, 1989). Shackling the birds in an inverted position on the shackles would probably also increase the plasma corticosterone response, because holding broilers by their legs in an inverted position has this effect (Kannan and Mench, 1996). Kannan et al. (1997) stated that duration of shackling had a significant influence on plasma corticosterone concentrations in experiments with male broilers. Birds were transported (for a duration of 5 min) by truck to the processing facility for the shackling experiments. During the experiment, shackling was done by gently picking up every bird and inverting it just before shackling. Shackling significantly ($P < 0.05$) elevated the concentration of plasma corticosterone in accordance with the duration of shackling. Korte et al. (1997) studied the effect of manual restraint in chickens on plasma corticosterone concentrations and found plasma corticosterone level significantly higher ($P < 0.001$) during manual restraint (time-dependent elevation) compared with resting birds. An increase in the level of plasma corticosterone in broilers resulting from inverted handling for 2 min was reported by Kannan and Mench (1997). The authors also found that corticosterone levels were highest immediately after handling. Nijdam et al.

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(2005) evaluated stress parameters in broilers during processing. The dynamics of corticosterone, glucose, and lactate levels showed a similar pattern. Plasma levels increased at the start of catching, and they further increased during transportation, shackling, and stunning.

Heterophil:lymphocyte (**H:L**) ratio is also used as an index of stress status in birds. The reliability of H:L ratio as a biological index of stress in avian species has been comprehensively reviewed (Maxwell, 1993). Gross and Siegel (1983) stated that the number of heterophil cells per unit of blood increases and the number of lymphocytes decreases in birds under stress, but the ratio of these cell types is less variable and thus a better measure than individual cell numbers. A normal ratio is about 0.4, but this can rise to 8 in birds under severe stress. Siegel and Gross (2000) stated that under extended periods of higher levels of stress, H:L ratios range from 0.6 to 1.2. An H:L ratio above 1.3 usually indicates a disease in progress. A study, which focused on monitoring the effects of different handling methods on stress reactions in the blood of broilers, was published by Zulkifli et al. (2000). Irrespective of the method used, subjecting chicks to a brief handling procedure resulted in an increase of elevated H:L ratios for up to 20 h, indicating a stress response.

The preslaughter handling is a potentially traumatic process; the tonic immobility (**TI**) fear reactions of broilers to various preslaughter treatments are therefore measured. According to Jones (1989), TI is thought to provide a useful measure of general fearfulness, because close relations have been found between TI reactions and its responsiveness to a variety of fear-eliciting situations in poultry. Scott et al. (1998) stated that frightened birds could be put into TI, an unlearned, catatonic state, the duration of which is positively related to the level of fear of the birds. Rough handling prolongs the latency until the first alert head movement and the duration of TI, and it also increases susceptibility to TI in broiler chickens (Jones, 1992). Changes in TI in response to handling in broilers have also been reported by Nicol (1992), Newberry and Blair (1993), Fluck et al. (1997), Zulkifli et al. (2000, 2002), and Zulkifli and Azah (2004).

Increasing demands to ensure animal welfare are also closely associated with increasingly strict requirements for meat quality. A relationship between preslaughter stress and meat quality has already been proven (Mengert and Fehlhaber, 1996; Debut et al., 2003; Vecerek et al., 2006). Meat quality changes due to shackling were described by Kannan et al. (1997).

This experiment was performed to study the particular effect of different periods of shackling in broilers with the elimination of other possible concurrent stress factors (e.g., preslaughter transport, crating, and ambient disturbances). The stress response of broilers to shackling was assessed using conventional stress and fear parameters: biochemical (corticosterone, glucose, triglycerides, lactate, aspartate aminotransferase), hematological (H:L ratio), and behavioral (TI).

MATERIALS AND METHODS

Birds and Their Treatment

Stress effects of shackling were monitored in a group of male Ross 308 broilers (total number: 400) aged 42 d. 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 meat hybrid poultry. The ambient barn temperature was gradually decreased from 30 ± 1°C on d 1 to 20 ± 1°C on the last day of fattening (d 42). Depending on temperatures, RH levels ranged from 20 to 60%. When the broilers were 42 d old, 240 chickens were selected at random for tests. Five experimentalists captured 1 broiler each and transported it by hand to the test room next door, where chickens were immediately inverted and simultaneously suspended from stationary shackles placed in a line. The test room was lit by strip lighting (fluorescent tubes, white light) with the intensity of 160 lx, and shackle spacing of the line was ~60 cm. The shackled broilers therefore could see, hear, and partially touch each other during the test and were allowed to flap freely. The aim of the study was to particularly assess the stress response of broilers to shackling; therefore, other stress factors (e.g., preslaughter transport, crating, and ambient disturbances) were eliminated. Three shackling treatments were used in our experimental tests: shackling of broilers for 30 s (group T_{30}, n = 30), 60 s (group T_{60}, n = 30), 120 s (group T_{120}, n = 30), and there was a control group of nonshackled broilers (n = 30). The shackling treatments were repeated twice for each test (biochemical examination, H:L ratio, and TI tests) described below.

Biochemical Examination

A total of 80 birds (20 birds per test group T_{30}, T_{60}, T_{120} + 20 control nonshackled birds) were used for biochemical examination. Immediately after shackling treatment, blood samples were taken from the vena basilica of broilers in each test group and also in the other 20 randomly selected broilers that were kept undisturbed for the whole preceding period (control group). Blood samples that were not collected within a maximum time of 90 s were discarded. The whole blood collection process occurred from 0700 to 0900 h each sampling day to take into account the diurnal levels of monitored biochemical indices. The heparinized blood was centrifuged at 837 × *g* for 10 min, and plasma samples were stored deep-frozen (–80°C) in Eppendorf test tubes until analyses were performed (within 1 wk). Selected plasma biochemical indices, glucose, lactate, aspartate aminotransferase, and triglycerides were measured by a Cobas EMira biochemical analyzer using commercial test kits (BioVendor, Laboratorni Medicina AS, Modrice, Czech Republic). Plasma corticosterone concentration was measured using commercial corticosterone EIA kit (Cayman Chemical, Ann Arbor, MI).

Table 1. Biochemical parameters of broilers shackled for 30 s (T_{30}, n = 20), 60 s (T_{60}, n = 20), 120 s (T_{120}, n = 20), and nonshackled control broilers (n = 20)[1]

	Group			
Parameter	Control	T_{30}	T_{60}	T_{120}
Corticosterone (ng/mL)	0.61 ± 0.12[b]	1.65 ± 0.22[a,b]	2.37 ± 0.26[a]	5.51 ± 0.83[a]
Aspartate aminotransferase (μkat/L)	3.60 ± 0.37	3.20 ± 0.12	4.04 ± 0.39	3.40 ± 0.26
Triglycerides (mmol/L)	1.05 ± 0.10	1.10 ± 0.14	0.85 ± 0.19	0.96 ± 0.09
Glucose (mmol/L)	13.30 ± 0.32[b]	13.71 ± 0.38[a,b]	14.90 ± 0.49[a]	14.70 ± 0.25[a]
Lactate (mmol/L)	7.61 ± 0.81[b]	10.75 ± 0.62[a]	11.48 ± 1.01[a]	11.82 ± 0.69[a]

[a,b]Means in the same row with no common superscript differ significantly ($P < 0.05$).

[1]Mean ± SEM.

H:L Ratio

A total of 80 birds (20 birds per test group T_{30}, T_{60}, T_{120} + 20 control nonshackled birds) were used for determining the proportion of H:L. After shackling, the broilers were released, differently marked with paint, and left undisturbed to move freely in the barn. After 20 h, blood samples were taken from the vena basilica of broilers in each test group and also from the other 20 randomly selected broilers that were kept undisturbed for the whole preceding period (control group). Blood samples that were not collected within a maximum time of 90 s were discarded. For hematological examinations, the samples were stabilized by heparin. Blood samples were taken after 20 h, because the H:L ratio response to short-duration stress peaks after 20 h (Gross, 1990; Zulkifli et al., 2002). Blood smears were prepared using a coverslip technique and were air-dried. The Pappenheim method of biphasic staining with May-Grünwald and Giemsa-Romanowski stains was used (Doubek, 2003). Number of heterophils and lymphocytes was counted to a total of 200 cells with the use of a microscope with an immersion lens.

TI Tests

A total of 80 birds (20 birds per test group T_{30}, T_{60}, T_{120} + 20 control nonshackled birds) were used for testing for the duration of TI. Immediately after shackling treatment, 20 birds from each group (T_{30}, T_{60}, T_{120}) and 20 nonshackled birds (control) were individually carried to a separate room and subjected to TI measurements according to a modified Benoff and Siegel (1976) procedure. Tonic immobility was induced by laying the bird down on its right side and gently restraining it by hand for 15 s. Then, the hand was removed, and the experimentalist retreated approximately 1 m out of sight of the bird and remained silent. The time was measured from withdrawal of the hand until the bird straightened up. If the bird straightened up in less than 10 s, it was restrained repeatedly. If TI was not induced after 3 attempts, the duration of TI was considered 0 s. If the bird did not straighten up within 10 min, it was removed and given the maximum duration of 600 s. The number of inductions required to attain TI was also recorded for each bird.

Statistics

Results were analyzed using the statistical package Unistat 5.1. (Unistat Ltd., London, UK). Data with homogeneous variances (triglycerides, glucose, lactate, TI duration) were subjected to a 1-way ANOVA and subsequently to a Tukey honestly significant difference test (Zar, 1999) for multiple comparisons to assess the statistical significance of differences between all possible pairs of groups. Data with heterogeneous variances (corticosterone, aspartate aminotransferase, heterophils, lymphocytes, H:L ratio, TI induction) were subjected to a Kruskal-Wallis ANOVA and subsequently to a nonparametric Tukey-type multiple comparisons test with ranked sums to assess the differences between all possible pairs of groups (Zar, 1999). To assess correlations in the experiment, Spearman rank correlation coefficients were calculated between shackling duration and monitored stress indices.

RESULTS

Table 1 shows that shackling treatment resulted in an elevation of corticosterone plasma concentration, and this

Table 2. Mean (±SEM) heterophil counts, lymphocyte counts, and heterophil:lymphocyte (H:L) ratios by shackling treatment

Shackling treatment	n	Heterophil count (G/L)[1]	Lymphocyte count (G/L)[1]	H:L ratio
Nonshackled	20	1.67 ± 0.25[b]	9.63 ± 1.35	0.17 ± 0.02[b]
T_{30}	20	2.28 ± 0.63[b]	10.31 ± 2.29	0.24 ± 0.03[b]
T_{60}	20	2.49 ± 0.38[a,b]	10.77 ± 1.19	0.26 ± 0.04[b]
T_{120}	20	7.93 ± 0.60[a]	9.68 ± 0.95	0.88 ± 0.10[a]

[a,b]Means in the same column with no common superscript differ significantly ($P < 0.05$).

[1]G = giga; G/L = 10^9/L.

Table 3. Mean (±SEM) duration of tonic immobility and number of attempts to induce tonic immobility by shackling treatment

Shackling treatment	n	Tonic immobility	
		Duration (s)	Induction (attempts)
Nonshackled	20	126.21 ± 42.56[b]	2.07 ± 0.22[a]
T_{30}	20	303.90 ± 66.82[a,b]	1.00 ± 0.00[b]
T_{60}	20	384.90 ± 77.27[a]	1.10 ± 0.10[b]
T_{120}	20	396.20 ± 71.41[a]	1.20 ± 0.13[b]

[a,b]Means in the same column with no common superscript differ significantly ($P < 0.05$).

was significant ($P < 0.05$) in T_{60} broilers and highly significant ($P < 0.01$) in T_{120} birds. Glucose level was significantly increased ($P < 0.05$) in both T_{60} and T_{120} broilers when compared with nonshackled control; T_{30} birds did not show any significant changes. There was a significant increase ($P < 0.05$) in lactate concentrations in T_{30} birds and a highly significant increase ($P < 0.01$) in both T_{60} and T_{120} birds when compared with the control group. The other biochemical indices monitored did not show any significant changes in shackled broilers compared with the control group.

Table 2 indicates that T_{120} broilers exhibited a highly significant increase ($P < 0.01$) in heterophil counts and H:L ratio when compared with the nonshackled control, whereas T_{30} and T_{60} broilers did not show any significant changes in these parameters. Heterophil count was significantly increased ($P < 0.05$) in T_{120} broilers when compared with T_{30} broilers, and H:L ratio was significantly elevated ($P < 0.01$) in T_{120} broilers when compared with T_{30} and T_{60} birds. Duration of shackling period did not significantly affect lymphocyte counts in any of the monitored test groups.

There was a significant increase ($P < 0.05$) in duration of TI in T_{60} and T_{120} broilers when compared with the nonshackled control, whereas T_{30} birds did not show any significant changes in duration of TI (Table 3). As measured by number of attempts to induce TI, shackling treatment irrespective of its duration caused a highly significant decrease ($P < 0.01$) in all test groups (T_{30}, T_{60}, T_{120}) when compared with the control group.

Table 4 indicates that duration of shackling period was highly significantly ($P < 0.001$) and positively correlated with corticosterone, glucose and lactate level, TI duration, and H:L ratio. The number of inductions was highly significantly ($P < 0.001$) and negatively correlated with duration of the shackling period.

Table 4. Spearman rank correlation coefficients between shackling duration (s) and monitored stress indices

Parameter	n	Correlation coefficient	Significance (P)
Corticosterone	80	0.8145	<0.001
Aspartate aminotransferase	80	0.1213	>0.05
Triglycerides	80	–0.1732	>0.05
Glucose	80	0.5563	<0.001
Lactate	80	0.5552	<0.001
Tonic immobility duration	80	0.5066	<0.001
Tonic immobility induction	80	–0.4754	<0.001
Heterophil:lymphocyte ratio	80	0.6945	<0.001

DISCUSSION

Shackling duration had a significant influence on stress indicators that we monitored in this study, particularly on plasma corticosterone concentrations, H:L ratio, TI, and plasma concentrations of glucose and lactate. Whereas shackling duration of 30 s only resulted in changes in the lactate level and number of attempts to induce TI, the 120-s shackling period manifested itself by changes in all the above-mentioned parameters. The most distinct changes associated with the shackling duration occurred in plasma corticosterone concentrations and H:L ratio, which are considered the major indicators of stress in birds (Gross and Siegel, 1983; McFarlane and Curtis, 1989; Maxwell, 1993). In our experiment, we observed an insignificant elevation of corticosterone plasma concentration due to the 30-s shackling period, whereas due to the 60-s shackling period, the corticosterone increased 4 times compared with the nonshackled control, and shackling for 120 s resulted in a 9-fold increase in plasma corticosterone. Consistent with our findings are the results published by Kannan et al. (1997), who also found that shackling elevated the concentration of plasma corticosterone depending on shackling duration. Similarly, plasma levels of both glucose and lactate monitored in our experiment showed a significant increase with the extension of the shackling period. A similar pattern of the dynamics of corticosterone, glucose, and lactate levels, which increased due to the preslaughter processing of broilers, was also reported by Nijdam et al. (2005). The abovementioned dynamics of changes in the monitored parameters indicated a high level of stress in broiler chickens during long-lasting shackling at the slaughter line unless they were stunned immediately after shackling.

In addition, the H:L ratio was increased from 0.17 to 0.88 due to a 2-min shackling period in our experiment. Shorter shackling periods do not result in any major changes in the H:L ratio. According to Siegel and Gross (2000), who stated that H:L ratios ranging from 0.6 to 1.2 indicate higher levels of stress, we can deduce that the shackling of broilers for a 2-min period is a very stressful procedure.

A highly significant positive correlation between duration of shackling period and TI duration was found in our experiment, which indicated an increased level of fear in shackled broilers that grew with the extension of the shackling period. Similarly, Zulkifli et al. (2000) observed a prolonged TI duration in response of broiler chicks to hanging in an inverted position and claimed augmented fearfulness. Furthermore, Jones (1989, 1992), Scott et al. (1998), and many others also report increased TI duration in association with rough handling in domestic poultry and related these changes to the fear level of birds. In addition, even after 30-s shackling of broilers, the number of attempts to induce TI was decreased in

our experiment, whereas the majority of other monitored indices did not exhibit any significant changes in response to this shackling period. As measured by number of attempts to induce TI, duration of shackling period was highly significantly and negatively correlated with the susceptibility to TI in our experiment, which corresponds to the findings of Jones (1992). However, Zulkifli et al. (2000), from their experiment in broilers, indicated no significant effect of handling in inverted position on susceptibility to TI.

According to the results of our study, the act of shackling is a considerable traumatic procedure for broilers in preslaughter handling, and its stress effect is markedly dependent on duration of shackling period that the broiler chickens experience. Kannan et al. (1997), who also studied the welfare and meat quality effects of shackling, suggested a minimization of stress and meat quality changes in poultry by reducing time lapse between shackling and stunning or killing to a maximum of 2 min. However, our results show that the shackling period of 60 s induces a major stress response in broilers, which may have adverse effects on meat quality. Both our study and the suggestions by Gregory and Bell (1987), who recommend the minimum shackling time of chickens of 12 s (to ensure sufficient relaxation which allows efficient stunning), show that the optimum shackling period should range from 12 to 60 s.

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