PROCESSING, PRODUCTS, AND FOOD SAFETY

The Effect of Feed Withdrawal and Crating Density in Transit on Metabolism and Meat Quality of Broilers at Slaughter Weight

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ABSTRACT Commercial broilers are exposed to a number of stressors prior to slaughter, including feed deprivation, crating density (high vs. low), and transportation. Hence, the individual and additive or overruling effects of these stressors on welfare and energy metabolism were examined. Live weight gain, rectal temperature, physiological responses, and meat quality of broilers were determined. The fasting of broilers before being transported resulted in a decrease of triglycerides, uric acid, and triiodothyronine concentrations, indicating a negative energy balance. Feed withdrawal was also associated with a reduction in body weight, and highest body weight losses were observed after being fasted for 13 h. For some parameters there was a combined effect of feed withdrawal and crating density, whereas for others the crating density overruled the effect of previous feed withdrawal: broilers that had no access to feed before being transported had higher thyroxine and lower lactate concentrations (only at high crating density) compared with their fed counterparts before the transport process, indicating the combined effect of both actions. The distinction due to the feeding pattern could no longer be observed for the plasma uric acid, nonesterified fatty acids, triglycerides, and triiodothyronine concentrations because it was overruled by the transport effect, especially if broilers were transported at high crating density. Plasma corticosterone concentrations increased as a consequence of the procedure of transportation and peaked if broilers were crated at high density. In our study, no significant effect of preslaughter stressors on meat quality, plasma creatine kinase activity, or lipid peroxidation levels were noticed. It can be concluded that transportation at high stocking densities should be avoided to reduce economic losses and stress to broilers. Plasma hormone as well as metabolites, rectal temperature, and heat shock protein 70 mRNA all indicated the high stress level of broilers. Furthermore, this effect often overruled the feed withdrawal and transport effect, indicating the importance of crating density.

Key words: broiler, feed withdrawal, crating density, metabolism, meat quality

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INTRODUCTION

The welfare of broiler chickens during transport is a matter for concern. Indeed, broilers are exposed to a wide variety of stressors including (un)loading (Knierim and Gocke, 2003), vehicle characteristics, transport duration (Cashman et al., 1989), and weather conditions (Mayes, 1980). Severe preslaughter stress has been reported to adversely affect welfare of the broilers and consequently their meat quality (Kannan et al., 1997, 1998). Interactions between these factors make it difficult to interpret and compare results of different studies. The physiological challenges presented by these factors are compounded by extended periods without access to food or water (Savenije et al., 2002), which is of particular economic importance; indeed, duration of feed deprivation and microclimate during transport determine the overall weight loss of the broilers (Elrom, 2000; Warriss et al., 2004).

Physiological responses to preslaughter processes have often been used to assess the efforts animals have to exert to cope with the associated stresses. Stress such as fatigue will increase the activities of enzymes such as creatine kinase in the blood, and fear and apprehension elevate the concentration of hormones like corticosterone (**CORT**; Freeman et al., 1984; Kannan and Mench, 1996). Broilers also respond to physiological stresses and to elevated temperatures by an increase in the synthesis of heat shock proteins (**HSP**) or stress proteins. Most of the HSP interact with other proteins in cells and alter their function, thus protecting the cell against harmful effects of stressors (Einat et al., 1996). The most important HSP of avian cells have molecular masses of approximately 90 kDA, 70 kDa (**HSP70**), and 27 kDa (Schlesinger, 1990). It has been shown that HSP70 expression increases following thermal stress or stimulation from a variety of other environmental stressors (Mahmoud et al., 2004b).

Effects of transport and feed withdrawal on metabolic parameters have been extensively investigated. Feed

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withdrawal affects several metabolic processes: it causes a shift from anabolism to catabolism, from lipogenesis to lipolysis, and a reduced metabolic rate (Buyse et al., 2002). In general, it is accepted that transport can negatively influence the welfare (Freeman et al., 1984; Nicol and Scott, 1990) and meat quality of poultry (Savenije et al., 2002).

Density of crated broilers plays a major role in the ability of the broiler to cope as a homeotherm animal with environmental changes during transport. High crating density results in a reduction of average costs of transport per animal. However, these densities must be balanced in view of animal welfare. Lower densities will allow more space for sitting and greater opportunity for the broilers to regulate their body temperatures by behavioral adaptations. On the other hand, too much space per animal may increase the risk of broilers being thrown around resulting in physical injuries. Furthermore, stocking density also depends on weather conditions, total live bird weight, and age of the birds to be transported (Elrom, 2000).

Despite these considerations there is little information on the effects of different crating densities during transport on indices of welfare and on meat quality. As broilers are feed-withdrawn before transport, the effect of being feed-deprived on these parameters could not be excluded. Therefore, the aim of this study was to incorporate the effect of feed withdrawal and to determine the separate and additive or overruling effects of crating density (high vs. low) during transport on animals fasted or fed ad libitum before transport. Live weight shrinkage, rectal temperature, physiological responses, and meat quality of broilers were determined.

MATERIALS AND METHODS

Animals and Housing

A total of eighty-eight 1-d-old Ross broilers supplied by Avibel, Zoersel (Belgium), were used in this experiment. The birds were randomly assigned in litter-floored pens and raised in an environmentally controlled room at standard conditions of temperature and light from 1 d of age until slaughter. The birds were all fed a commercial starter diet (0 to 14 d) followed by a commercial grower diet (14 to 42 d; see Buyse et al., 2001, for diet composition), and water was provided ad libitum.

Experimental Design

Seven different treatments were given to the broilers each consisting of 2 periods (period 1 and period 2). The first period lasted for 10 h, and broilers were fed ad libitum or fasted while water remained constantly available. The second period took only 3 h, and in this period some groups experienced stress in agreement with the practical situation. The stress application started with grasping a bird by the leg and inverting the bird. After catching 4 to 6 birds in this way, the catcher carried them for 10 m to a crate. The crates were loaded, and thereafter the birds were transported for approximately 1.5 h. The broilers were crated at a different crating density: half of them were crated at a high stocking density (0.0350 m^2/broiler) and the other half at low stocking density (0.0575 m^2/broiler). Broilers were not transported to a slaughterhouse but returned to the farm. After transport the birds remained in the crates for 1 h, which can be compared with the time at lairage. During these stress situations there was no access to feed and water. If the broilers did not receive a stress period (the broilers remained in the stable without being caught, crated, or transported), some of them were deprived of feed, whereas others still had access to feed and water.

Treatment 1:	Feed deprived	Transport:High
Treatment 2:	Feed deprived	Transport:Low
Treatment 3:	Fed ad libitum	Transport:High
Treatment 4:	Fed ad libitum	Transport:Low
Treatment 5:	Feed deprived	
Treatment 6:	Fed ad libitum	
Treatment 7:	Fed ad libitum	Feed deprived

Figure 1. Scheme of the different treatments where high and low refer to stocking density.

The treatment given to the broilers consisted of a combination of the 2 periods, so total duration of each treatment was about 13 h. For treatments 1 to 4, 16 broilers were involved, whereas for treatment 5 to 7 only 8 broilers were tested (Figure 1, Table 1).

The experiment was carried out over 2 d: on d 2 the experiment was repeated with other broilers. The experimental applications were done at slaughter age: broilers were 42 d of age the first day of the experiment and 43 d of age on the second day. Approval for carrying out the experiment was obtained from the Ethical Commission of the Katholieke Universiteit of Leuven.

Measurements

Before the treatments were applied, all the broilers were weighed and their rectal temperature was measured. Deep-body temperatures were obtained using a digital thermometer (accuracy ± 0.1°C) inserted 5 cm into the rectum. Immediately after the treatments, which lasted for about 13 h, broilers were blood sampled, and their weight and rectal temperature were measured again. Birds were taken out of the pen or crates, depending on the treatment, and blood was collected by puncturing the vena ulnaris with a heparinized syringe and transferred into iced tubes. Immediately after euthanasia, liver samples were collected and frozen in liquid nitrogen and stored at −80°C until further analysis.

The pH of the right pectoralis major (**PM**) muscle was measured at 20 min (**pHi**) and 24 h (ultimate; **pHu**); water-holding capacity (**WHC**) was also determined. Finally, drip losses were determined at the left PM muscle.

Table 1. Summary of the different treatments

Treatment	Period 1 (10 h): Fasted or fed	Period 2 (3 h): being transported (stress application[1]) or not	Stocking density[2]	Total feed withdrawal time (h)	Number of birds (n)
1	Fasted	Stress application	H	13	16
2	Fasted	Stress application	L	13	16
3	Fed	Stress application	H	3	16
4	Fed	Stress application	L	3	16
5	Fasted	No stress application[3]	—	13	8
6	Fed	No stress application	—	0	8
7	Fed	No stress application[3]	—	3	8

[1]The application started with grasping a bird by the leg and inverting the bird. After catching 4 to 6 birds in this way, the catcher carried them for 10 m to a crate. The crates were loaded, and thereafter the birds were transported for approximately 1.5 h. Broilers were not transported to a slaughterhouse but returned to the farm. After transport the birds remained in the crates for approximately 1 h. During these activities there was no access to feed and water.

[2]H = high stocking density; L = low stocking density.

[3]Broilers were not subjected to a stress application but were feed deprived during this period (3 h).

Analysis of Plasma Samples

After centrifugation for 10 min at 1,500 rpm, plasma was stored frozen pending analysis for metabolites and hormones. Corticosterone, triiodothyronine (**T_3**), thyroxine (**T_4**), glucose, lactate, uric acid, triglycerides (**TG**), and nonesterified fatty acid (**NEFA**) concentrations were determined. Plasma CORT concentration was measured using a sensitive and highly specific radioimmunoassay kit (IDS Inc., Boldon, UK), with a sensitivity of 0.39 ng/mL, and low cross-reactions with aldosterone (0.20%), cortisol (0.40%), and deoxycorticosterone (3.30%). The intraassay variability was 3.9%. Plasma T_3 and T_4 concentrations were measured by radioimmunoassay as described by Darras et al. (1992). Briefly, T_3 and T_4 measurements were performed using commercial available antisera purchased from Byk-Belga (Brussels, Belgium) in combination with a specific tracer (Amersham International, Slough, UK). The plasma concentrations had an intraassay variability of 4.5 and 5.4% for T_3 and T_4, respectively.

Plasma metabolite concentrations of glucose (IL Test kit, No. 182508-00), lactate (Procedure 826-UV, Sigma Diagnostics, Steinheim, Germany), uric acid (IL Test kit, No. 181685-00), TG (IL Test kit, No 181610-60), NEFA (WAKO NEFA C test kit, an enzymatic colorimetric test), and creatine kinase (**CK**; IL Test kit, No. 181605-90) were determined spectrophotometrically using a commercial kit developed for an automated apparatus, the Monarch 2000 system (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium). Hematocrit values, expressed as percentage blood packed cell volume, were also measured in all samples after centrifugation of the blood in a micro hematocrit centrifuge for 7 min (Haemofuge A, Heraeus Sepatech, Usingen, Germany). Plasma lipid peroxidation was estimated by spectrophotometric determination of thiobarbituric acid reactive substances (**TBARS**), as described by Lin et al. (2004). In brief, 100 μL of plasma was mixed with 0.3 mL of 10% phosphotungstic acid and 2.0 mL of 0.17 *M* H_2SO_4 and centrifuged (1,200 × *g*, 10 min). After standing at room temperature for 5 min, the obtained sediment was mixed with 2.0 mL of 0.17 *M* H_2SO_4 and 0.3 mL of 10% phosphotungstic acid and centrifuged again. The sediment was suspended in 2.0 mL of distilled water and mixed with 0.5 mL of 0.34% thiobarbituric acid (50% acetic acid solution) and 25 μL of 0.5 *M* butylated hydroxytoluene (Sigma). For 60 min, the mixture was heated at 95°C in a water bath. After cooling, the chromogen was extracted with 2.0 mL of a mixture of n-butanol and pyridine (1:15, vol/vol). After centrifugation at 1,400 × *g* for 10 min, the organic layer was extracted and read spectrophotometrically at 532 nm against a reaction blank. To correct for the background, absorbance values at 572 nm were subtracted. The TBARS were expressed as nanomoles of malondialdehyde per milliliter of plasma.

All measurements for each variable were run in the same assay to avoid interassay variability.

Meat Quality

Immediately after euthanasia, the left breast muscles were weighed and placed in a plastic self-sealing bag and freely suspended via a hook-shaped cable through both the bag and muscle on a rack in a chilling room maintained at 4°C. Muscle contact with the inside surface of the bag was kept to a minimum. Measurements of drip loss were done 48 h after the muscle was excised from the carcass. Fillets were reweighed to determine drip losses, drip losses were expressed as a percentage of the initial sample weight.

The pH of the PM muscle was measured at 20 min (pHi) and 24 h (pHu) postmortem with a pH meter with a xerolyt pH electrode (Schott CG 818 pH meter with a xerolyt pH electrode, Schott-Gerate GmbH, Hofheim, Germany). For 10 s the electrode was inserted approximately 2.5 cm below the surface of the anterior portion of the muscle.

At 24 h postmortem WHC was measured with a filter paper absorption method as described in Nijdam et al. (2005). Briefly, an incision was made in the PM muscle. A filter paper with a diameter of 45 mm (Schleicher and Schuel 589/3 rundfilter blauband (Schleicher and Schuel,

Dassel, Germany) was placed into the incision during 30 s, after which the increase in weight of the paper was measured.

HSP 70

The HSP70 was only determined on liver samples of broilers subjected to treatment (**Tr**) 1, 3, 5, and 6. This was done because results of hormones and metabolites indicated a clear feeding and density effect.

The mRNA was extracted from liver samples with Tri-Reagent (Sigma-Aldrich, Saint Louis, MO) in accordance with the manufacturer's instructions. The amount of extracted mRNA was quantified by measuring the absorbance at 260 nm with a UV-spectrophotometer. Standard protocol for the reverse transcriptase step was followed (Sambrook and Russell, 2001).

Real-time PCR was performed using the ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). In brief, final amounts used in the 20-μL reaction mix were 4 μg of mRNA (*HSP70* or β-actine), 20 μ*M* of each primer (forward primer 5'-AACCGCACCACACCCAGCTATG -3', reverse primer 5'-CTGGAGTCGTTGAAGTAAGCG -3'), and 10 μL of Sybrgreen and H_2O up to 20 μL. Thermal cycling conditions were as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The expression levels of each group of animals were normalized to β-actin levels and displayed as the fold change relative to the experimental no treatment group, which was fed ad libitum (Tr 6) without transport application.

Statistical Analyses

The statistical analyses were performed in the SAS-PC System (SAS Institute, Cary, NC, 2000). The PROC FREQ and PROC MEANS were used for the descriptive analyses. The assumption of normality of the outcomes was assessed applying stem-and-leaf plots and normal probability plots. The distribution of the plasma CORT concentrations was skewed, and therefore a logarithmic transformation was applied. Day, treatment, and the interaction term day × treatment were analyzed using a generalized linear model performed by PROC GLM on plasma concentrations, rectal temperature, and BW. The day effect was not significant, and therefore the results for both days are presented together. Significant differences between treatments were separated using least squares means procedures of SAS. All statements of significance are based on a probability level of 0.05.

RESULTS

Relative Body Weight Losses and Rectal Temperature

The effects of feed deprivation, transport, and crating density on relative BW gain and rectal temperature are shown in Table 2. Relative BW gain was significantly lower in feed-deprived chickens ($P < 0.01$; Tr 1, 2, and 5) compared with their fed counterparts. The transport process resulted in a significant decrease of the relative BW gain if broilers were fed before being transported ($P < 0.01$; Tr 3 and 4 vs. Tr 7).

No differences in rectal temperature could be observed between fasted and fed broilers after transport or between both groups without being transported; however, high density significantly increased deep-body temperature in the fasted as well as in the fed broilers ($P < 0.01$). The extent of elevation in body temperature was similar for both feeding regimes.

Plasma Concentrations of Hormones

The transport or fasting process had no significant effect on plasma CORT concentrations, except if broilers were transported at high crating density (Figure 2A). All those birds exhibited a significant increase in plasma CORT concentrations ($P < 0.01$). Fasting of broilers had a significant effect on plasma T_3 concentrations ($P < 0.01$) when broilers were not transported (Figure 2B). Feed-deprived animals had significantly lower plasma T_3 concentrations (Tr 5 vs. Tr 6). If the period of feed deprivation was followed by a stressful action no further significant reduction of T_3 was observed. The stocking density in the crates had a significant effect on T_3 values in fed birds (Tr 3 vs. Tr 4): high density (Tr 3) resulted in lower concentrations compared with low stocking density (Tr 4). Broilers fasted for 13 h had similar plasma T_4 concentrations compared with the ones fed ad libitum (Tr 5 vs. Tr 6; Figure 2C). If broilers were transported at high density a significant difference in plasma T_4 concentrations could be observed between fasted and fed animals (Tr 1 vs. Tr 3). The same could be observed for broilers being transported at low crating density (Tr 2 vs. Tr 4).

Plasma Concentrations of Metabolites

No significant effect was seen in this experiment on plasma CK (Figure 3A) and plasma concentration of TBARS (data not shown) and glucose concentrations (Figure 3B), neither due to feed withdrawal nor to stress. If broilers were feed deprived (Tr 5) lower concentrations of uric acid were found compared with the fed broilers (Tr 6). Because of the experience of transport stress, levels of uric acid were significantly augmented, especially if stocking density was high (Tr 1 and Tr 3; Figure 3C). The transporting process at high stocking density augmented the plasma NEFA concentrations significantly. So, Tr 1 and Tr 3 resulted in significantly higher NEFA concentrations than the other treatments (Tr 2, 4, 5, 6, and 7; Figure 3D). Significantly higher concentrations of TG were found in Tr 6 compared with Tr 1 and Tr 5, indicating a significant effect of feed withdrawal (Figure 3E), and the experience of stress on lactate concentrations was absent (Figure 3F). Broilers of Tr 1 had the lowest concentrations. No

Table 2. Mean relative BW losses ± SEM and change in rectal temperature ± SEM (°C) for the different treatments (Tr)[1]

	Transport				No transport		
	Feed withdrawn		Fed until transport				
Parameter	High (Tr 1)	Low (Tr 2)	High (Tr 3)	Low (Tr 4)	Feed withdrawn (Tr 5)	Fed (Tr 6)	Fed followed by fasting (Tr 7)
Relative BW gain	$-0.054^{a} \pm 0.004$	$-0.047^{a} \pm 0.003$	$-0.019^{b} \pm 0.003$	$-0.0090^{bc} \pm 0.002$	$-0.040^{a} \pm 0.004$	$0.0040^{c} \pm 0.006$	$0.010^{d} \pm 0.005$
Δ rectal temp. (°C)	$1.040^{a} \pm 0.13$	$0.41^{b} \pm 0.09$	$1.020^{a} \pm 0.16$	$0.54^{b} \pm 0.15$	$0.45^{b} \pm 0.11$	$0.65^{b} \pm 0.12$	$0.43^{b} \pm 0.18$

[a–d]Values with different letters within row are significantly different ($P < 0.05$).

[1]n = 16 for Tr 1 to 4 and n = 8 for Tr 5 to 7.

significant differences were found between hematocrit values (data not shown).

Meat Quality

Feed withdrawal, transport, and stocking density did not significantly affect the pHu in the PM. However, pHi of broilers being fasted for 13 h (Tr 5) was significantly higher compared with the pHi of broilers being fasted for 13 h and subjected to a transport process (Tr 1 and Tr 2). This was not observed for their fed counterparts: there are no significant differences between Tr 7 and Tr 3, 4 (Table 3). Data on WHC and drip losses per treatment group are also shown in Table 3. Drip losses did not significantly differ between treatment groups. The WHC, expressed as the mean fluid weight in filter paper (g), was highest for broilers being fasted (Tr 5) or fasted and transported at high crating density (Tr 1).

Heat Shock Proteins

The HSP70 expression was monitored for 4 different treatments (Tr 1, Tr 3, Tr 5, and Tr 6) in liver. Relative quantification was used to describe the change in expression of mRNA HSP70 relative to the reference group Tr 6, which is the untreated group. So mRNA expression of HSP70 is an increase or decrease of the expression relative to the control treatment. For example, mRNA HSP70 expression was increased 1.75-fold due to Tr 3 (Figure 4). The HSP70 mRNA expression between Tr 1 and Tr 3 and between Tr 5 and Tr 6 did not differ. In general, expression in Tr 1 and Tr 3 were highest, and the lowest expression was found in Tr 5 and Tr 6, although these differences were not significantly verifiable at the 5% level ($P = 0.07$; Figure 4).

DISCUSSION

As expected, broilers that were fasted for 13 h had the highest relative weight loss. No significant effects due to the transport application or stocking density on body weight losses were found. However, transporting feed-deprived broilers at high density resulted in a body weight loss of 5%, which reflects a substantial economic loss to the producer and processor.

Feed deprivation (Tr 5) of broilers resulted in a smaller increase in rectal temperature compared with broilers being fed (Tr 6). After transport this distinction due to the feeding pattern could no longer be observed as it was overruled by the effect of crating density. The most pronounced increase in rectal temperature was related with high crating density for feed deprived as well as for fed broilers. This may be the result of exposure to high temperatures as heat stress may occur if broilers are subjected to high environmental temperatures due to high crating densities. Turner and Kettlewell (1983) noted that the density in the crates during transport is about twice as that in the growing house. The high density in the crates makes it very difficult for the broilers to dissipate their heat (Metheringham and Hubrecht, 1996). The increase in rectal temperature due to heat stress is in agreement with studies of Berong and Wasburn (1998), Warriss et al. (1999), and Altan et al. (2000).

As a consequence of the procedure of transportation at high densities plasma CORT concentrations increased. This suggests that stress was maximum at high crating density during transport irrespective of whether the animals were fed or feed deprived. The induction of physiological stress by transportation has been reported in a number of studies (Freeman et al., 1984; Kannan and Mench, 1996). Feed deprivation is also known to elevate plasma CORT concentrations in poultry (Knowles et al., 1995; De Jong et al., 2003). However, in the present study, no effect of feed withdrawal could be observed. Duration of feed withdrawal might have been too short to induce a significant increase in CORT concentration.

In accordance with other studies (Decuypere and Kühn, 1984; Buyse et al., 2000), feed deprivation reduced plasma T_3 concentrations when broilers were not transported. The decrease of plasma T_3 is linked to an increase in hepatic inner ring deiodination type III activity. In this way metabolic rate is reduced and body reserves are saved (Kühn et al., 1996; Darras et al., 2000). It is likely that the higher, although not significant at the 5% level, plasma T_4 concentrations of broilers being fasted without transport are not caused by a peripheral effect. In other words, the higher plasma T_4 concentrations during fasting are not caused by a decreased conversion of T_4 to T_3 (Geris et al., 1999). The main reason for this increase is likely a central effect and is related to a reduced negative feedback of the decreased plasma T_3 concentrations on hypothalamic thyrotropin-releasing hormone and thyrotropin (thyroid stimulating hormone, **TSH**), resulting in an increased T_4 level.

Plasma T_3 concentrations decreased if broilers were transported at high crating density, but this decrease was

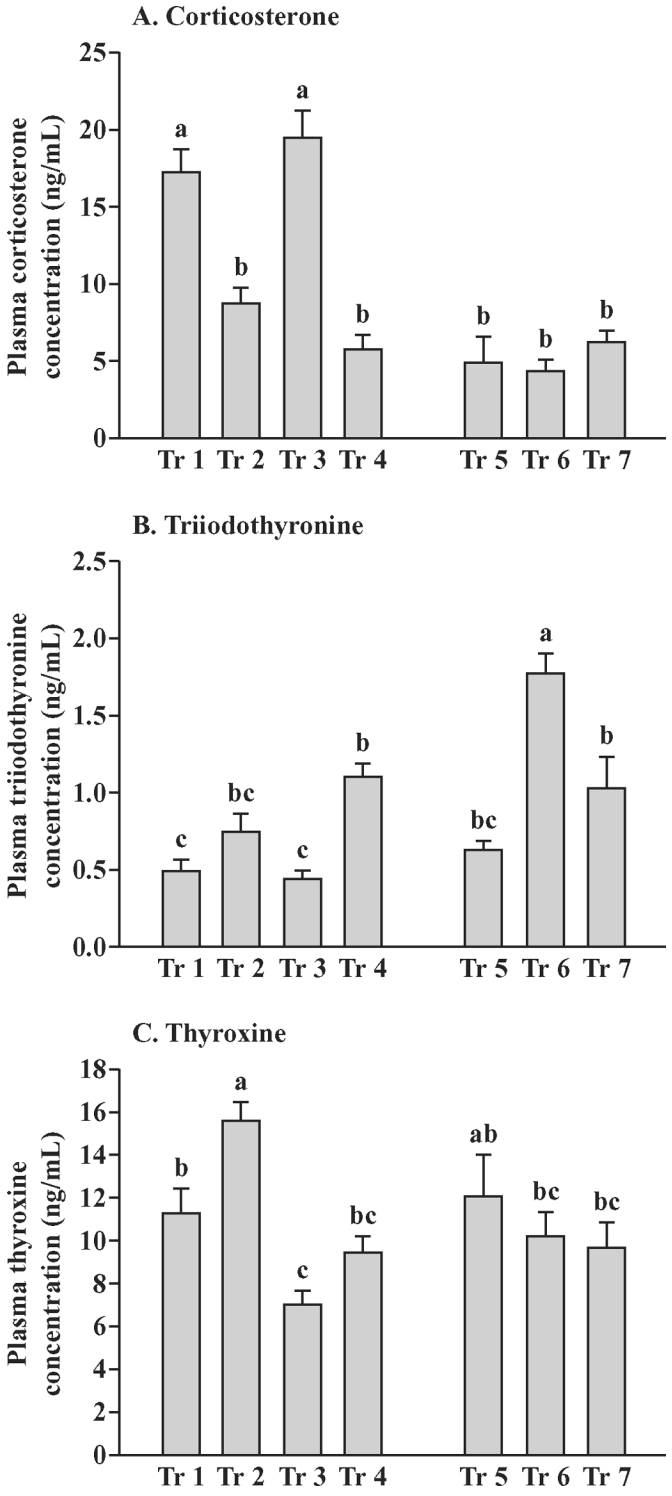

Figure 2. Mean concentrations of plasma corticosterone concentrations (A), triiodothyronine (B), and thyroxine (C) per treatment (Tr). Tr 1 = feed withdrawal and the broilers were transported at high stocking density; Tr 2 = feed withdrawal and the broilers were transported at low stocking density; Tr 3 = feed was available till the moment of transport, and the broilers were transported at high density; Tr 4 = feed was available till the moment of transport, and the broilers were transported at low density; Tr 5 = feed withdrawal during the whole experiment and no transport application; Tr 6 = feed was available and no transport application; Tr 7 = feed was available during the feed procedure but removed during the transport application, broilers were not transported. Bars show SEM. $^{a-c}$Values with different letters are significantly different ($P < 0.05$).

only significant if broilers were fed before being transported (Tr 3 vs. Tr 7). Probably, the feed-deprived broilers (Tr 5) were already experiencing hypothyroidism and a further decrease was not possible (Buyse et al., 2000). Furthermore, the effect of being fasted or fed before the transport process was no longer manifested if broilers were transported, indicating that the transport process dominated the influence of the feeding-fasting effect. Indeed, plasma T_3 concentrations of feed-deprived as well as ad libitum-fed broilers were reduced to similar concentrations after transport especially at high density. These results are due to the stress broilers experienced at high density as reflected in the high plasma CORT concentrations. Indeed, this decrease in plasma T_3 levels may result from an increase in hepatic outer ring deiodinase activity (Darras et al., 2000) or a decrease in secretory activity of the thyroid gland. In addition, broilers can experience heat stress at high crating density, and Bowen et al. (1984) and Sahin et al. (2001) reported reduced concentrations of plasma T_3 in heat-stressed chickens.

In contradiction to the plasma T_3 levels, the effect of being fasted or fed before the transport process on plasma T_4 concentrations remained present after transport application: fasted birds had higher concentrations compared with the fed ones. Furthermore, a significant difference between the high and low crating densities could be observed. High crating density significantly decreased plasma T_4 concentrations, and a significant difference could be observed between fasted and fed broilers transported at high and respectively low crating density. The significant decrease of T_4 due to the stress experienced by transport at high crating density probably results from a decrease in secretory activity of the thyroid gland (Helmreich et al., 2005), which is in accordance with the high plasma CORT concentrations for broilers transported at high crating density. In broilers, a close interaction is present between the thyroidal and adrenal axis (Helmreich et al., 2005), and the activity of 1 axis alters the activity of the other. Although not measured in the current study, previous work has indicated that acute stressors can cause a decrease in plasma TSH levels (Marti et al., 1996; Kondo et al., 1997). Furthermore, corticotropin releasing factor is known to decrease TSH secretion and hence thyroidal T_4 secretion (Geris et al., 1999). In addition, this central regulation was demonstrated because physiological stressors in addition to metabolic stressors (Van Haasteren et al., 1995) caused a decrease in hypothalamic thyrotropin-releasing hormone mRNA levels. Summarized, it can be stated that the fasting effect caused a decrease in T_3 levels and increase in T_4 levels, whereas the stress effect provoked a further decrease of T_3 but also of T_4.

Environmental stressors also influence energy metabolism in poultry. Only a small feeding effect could be observed for glucose and lactate concentrations in our experiment. The mild decrease of these concentrations is in accordance with previous studies (Warriss et al., 1993; van der Wal et al., 1999).

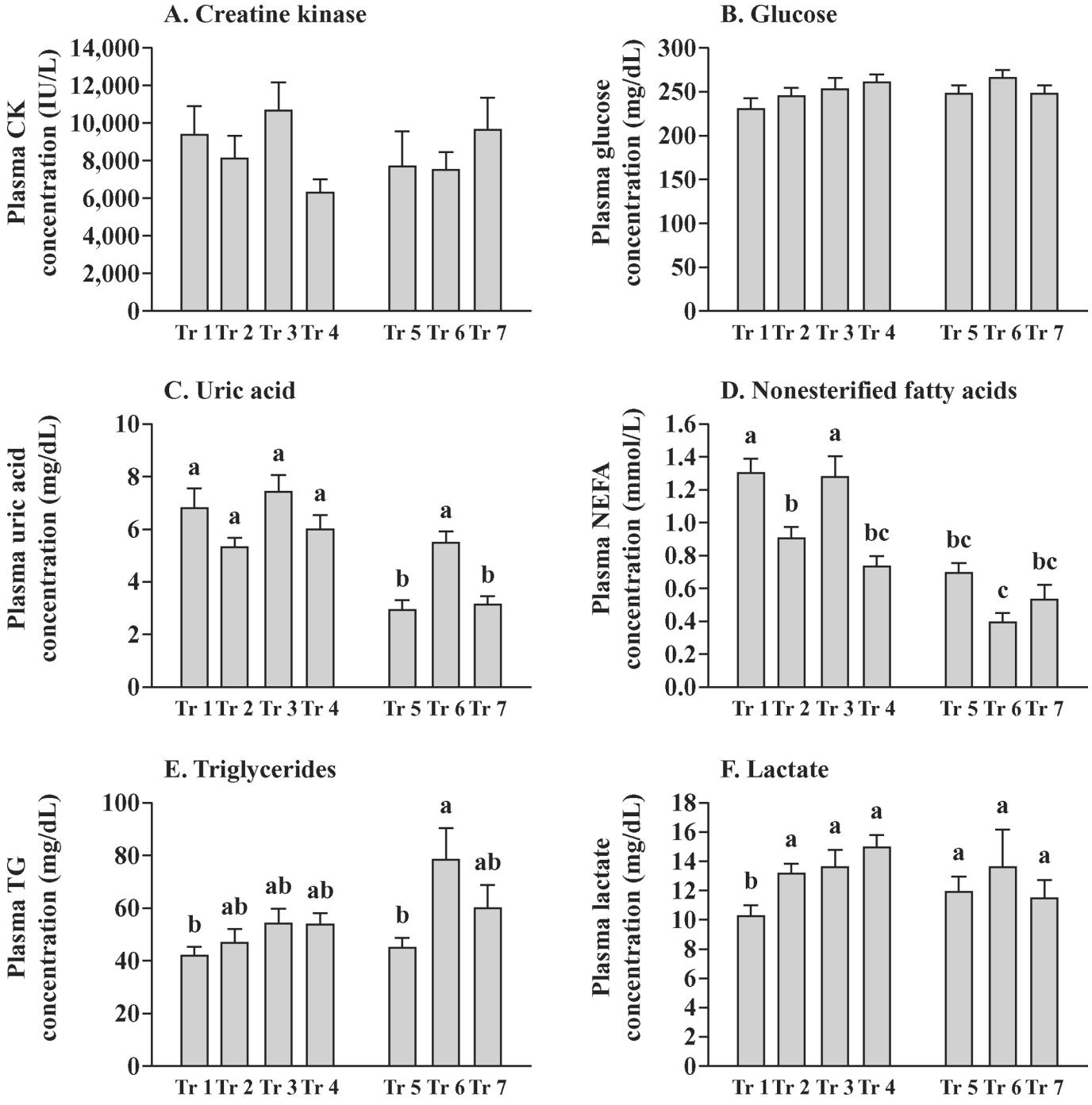

Figure 3. Mean concentrations of plasma creatine kinase (A), glucose (B), uric acid (C), nonesterified fatty acid (D), triglycerides (E), and lactate (F) concentrations per treatment (Tr). Tr 1 = feed withdrawal and the broilers were transported at high stocking density; Tr 2 = feed withdrawal and the broilers were transported at low stocking density; Tr 3 = feed was available till the moment of transport, and the broilers were transported at high density; Tr 4 = feed was available till the moment of transport, and the broilers were transported at low density; Tr 5 = feed withdrawal during the whole experiment and no transport application; Tr 6 = feed was available and no transport application; Tr 7 = feed was available during the feed procedure but removed during the transport application, broilers were not transported. Bars show SEM. $^{a-c}$Values with different letters are significantly different ($P < 0.05$).

Plasma uric acid concentrations were lower in feed-deprived broilers compared with fed broilers before being transported. Because circulating uric acid is a measure for protein catabolism in birds (Sturkie, 2000), this indicated that the feed-deprived broilers had a reduced protein oxidation rate. Because no dietary protein was available, this is a protein sparing mechanism. Transportation of broilers resulted in a significant increase of plasma uric acid concentrations and overruled this feeding effect. Therefore, after transportation no significant differences between the feed-deprived and ad libitum-fed animals were observed anymore. As the hormone CORT helps in the adaptation of the animal to its new set of environmental conditions, gluconeogenesis is stimulated, in which

Table 3. Muscle pH of pectoralis major (PM) of broilers, mean fluid weight in filter paper (g), and drip loss (%) per treatment (Tr)[1]

	Transport				No transport		
	Feed withdrawn		Fed until transport		Feed withdrawn (Tr 5)	Fed (Tr 6)	Fed followed by fasting (Tr 7)
Parameter	High (Tr 1)	Low (Tr 2)	High (Tr 3)	Low (Tr 4)			
Muscle pH							
pHi	6.37^{b} ± 0.04	6.42^{b} ± 0.05	6.34^{b} ± 0.05	6.42^{b} ± 0.04	6.70^{a} ± 0.03	6.53^{ab} ± 0.04	6.36^{b} ± 0.03
pHu	5.75 ± 0.03	5.71 ± 0.04	5.75 ± 0.03	5.74 ± 0.05	5.78 ± 0.04	5.74 ± 0.06	5.70 ± 0.03
Mean fluid weight in filter paper (g)	0.073^{a} ± 0.005	0.061^{ab} ± 0.005	0.051^{b} ± 0.003	0.052^{b} ± 0.005	0.071^{a} ± 0.007	0.055^{ab} ± 0.005	0.057^{ab} ± 0.003
Drip loss (%)	0.27 ± 0.02	0.24 ± 0.03	0.24 ± 0.02	0.23 ± 0.02	0.26 ± 0.03	0.33 ± 0.10	0.25 ± 0.02

^{a,b}Values with different letters are significantly different ($P < 0.05$).

[1]pHi = muscle pH obtained within 20 min of slaughter; pHu = muscle pH obtained 24 h postslaughter; n = 16 for Tr 1 to 4 and n = 8 for Tr 5 to 7.

Figure 4. Analysis of heat shock protein 70 (HSP70) mRNA expression as the fold change relative to treatment (Tr) 6 per treatment. Tr 1 = feed withdrawal and the broilers were transported at high stocking density; Tr 3 = feed was available till the moment of transport, and the broilers were transported at high density; Tr 5 = feed withdrawal during the whole experiment and no transport application; Tr 6 = feed was available and no transport application.

amino acids are converted to glucose and therefore uric acid concentrations increase as a waste product (Siegel and Van Kampen, 1984; Malheiros et al., 2003).

A similar pattern was found for the plasma NEFA concentrations. Transportation, and especially at high crating density, resulted in an increased lipolysis and caused plasma concentrations of NEFA to increase (Freeman et al., 1984). There was only an effect of feeding/fasting on plasma TG levels, whereas no significant changes were observed after the broilers were exposed to stress. The lower concentrations of TG at high crating density can be explained by a reduced lipogenesis (Buyse et al., 2002).

This study indicated higher HSP70 mRNA expression if broilers were transported at high density regardless of their feeding status. These findings corroborate the results of Mahmoud et al. (2004b), who reported a significant increase in hepatic HSP70 mRNA expression after broilers were subjected to acute heat stress. A study of Mahmoud et al. (2004a) confirmed the hypothesis that HSP70 responds primarily to a thermal change. Dionello (1998) found a positive correlation between the change in rectal temperature and HSP70 mRNA expression in the liver of broilers submitted to heat stress, which is confirmed by our results. From these findings it can be concluded that broilers subjected to the high crating densities experienced heat stress.

Plasma CK levels reflect muscle tissue damage (Mitchell and Sandercock, 1995). Several authors have reported an increase in plasma CK activity after severe physical stress or exercise (Warriss et al., 1998). Hocking et al. (1994) showed that blood CK activity increased under thermal stress due to skeletal muscle damage. In the present study, no significant differences were observed between treatments for plasma CK activity. Mitchell et al. (1992), however, obtained significant increases in plasma CK activity after 2 to 3 h of transportation. Therefore, as

broilers were only transported for 1.5 h duration, this was probably too short to obtain effects of transportation on CK in our study.

Feed withdrawal, transport, and stocking density did not significantly influence pHu temperature of PS, or drip losses, whereas WHC was significantly influenced. Changes in muscle pH have been shown to affect WHC from meat. Poultry meat with low pHu is related with low WHC, which can result in increased drip losses (Froning et al., 1978). However, this argument is not applicable to explain the influence of treatments on WHC and pHu. The WHC, however, was lower in muscles from fasted broilers, and for Tr 5 this appeared to be more closely related with the rate of muscle pH immediately postslaughter (–0.92 pH units). This is consistent with previous finding of Warriss and Brown (1987) who proposed that the initial rate in pH decline was more important than pHu in determining meat water loss in muscle.

It can be concluded from our result that transportation at high stocking densities should be avoided to reduce economic losses and stress to broilers. Plasma hormone as well as metabolites, rectal temperature, and HSP70 mRNA all indicated the high stress level of broilers transported at high density. Furthermore, this effect often overruled the feed withdrawal and transport effect indicating the importance of crating density. Thus, attempts to reduce stress in broilers during transport could be done by focusing on achieving a set of recommended crating densities.

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