# Feed Withdrawal of Broilers Before Transport Changes Plasma Hormone and Metabolite Concentrations

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**ABSTRACT** Two experiments were completed to observe the combined effects of feed withdrawal and the catching and transport process on stress and energy metabolism. During one experiment 192 male broilers (46 d of age) were used, and in the other we used 240 male broilers (49 d of age). The experiments consisted of 2 interventions: feed intervention and transport intervention. The feed intervention took 10 h, in which broilers had full access to feed or feed was withdrawn and, thereafter, had a transport intervention that took 3 h, in which broilers were caught, crated, loaded, transported, and then had to wait in the crates for 1 h or remained in the pens. After the transport intervention, blood samples were taken to determine plasma corticosterone, triiodothyronine, thyroxine, glucose, lactate, uric acid, nonesterified fatty acid, and triglyceride concentrations. Changes in BW were also assessed.

Broilers from which feed was withdrawn before the transport intervention showed higher thyroxine concentration and lower triiodothyronine, triglyceride, glucose, and lactate concentrations compared with broilers that had access to feed before the transport intervention. These findings indicate a negative energy balance and stress. Broilers that were transported after feed withdrawal had BW losses of approximately 0.42% per hour, which is approximately 0.30% per hour more than those that had full access to feed. To continue feeding broilers until catching resulted in higher BW at the slaughterhouse and less stress, as shown by a negative energy balance and might improve meat quality.

(*Key words*: broiler, feed withdrawal, transport, stress, metabolite)

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## INTRODUCTION

Before broilers are slaughtered, they are exposed to an array of events on the last day of life. These events include feed withdrawal, catching, and placement into crates or containers. Subsequently, broilers are transported to the processing plant, and finally they have to wait in the lairage of the processing plant before being slaughtered.

Various studies show that catching, placement into crates or containers, and transport cause severe stress to broilers (Knowles and Broom, 1990; Von Borrell, 2001). This is shown by an increase in plasma corticosterone (CORT) that occurs during catching, crating (Kannan and Mench, 1996), and transport (Freeman et al., 1984). Moreover, catching prolongs tonic immobility (Jones, 1992), which is further prolonged by increased transport times (Cashman et al., 1989). Furthermore, during lairage the body temperature of broilers increases and the liver glycogen stores become depleted, indicating significant periods of negative energy balance (Warriss et al., 1999). However, results of studies on the effects of transport on plasma glucose levels are in conflict with each other. Halliday et al. (1977) and Freeman et al. (1984) observed a reduction in plasma glucose concentrations after transport, whereas Warriss et al. (1993) and Savenije et al. (2002) claimed that no significant differences are observed in plasma glucose and lactate concentrations of transported broilers.

Feed is normally withdrawn for several hours before catching in order to reduce the danger of carcass contamination. Total feed withdrawal times of 8 to 10 h prior to slaughter are recommended (Wabeck, 1972), although in practice longer periods sometimes occur. In the Netherlands, feed is withdrawn from broilers for at least 5 h before catching starts, as recommended by the Dutch


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**Abbreviation Key:** CORT = corticosterone; NEFA = nonesterified fatty acid; $T_3$ = triiodothyronine; $T_4$ = thyroxine; TG = triglyceride.

Product Boards for Livestock, Meat, and Eggs (PVE, 1992). In addition, the loading time for one transport vehicle with a capacity of approximately 6,500 broilers is about 55 min. The catching and loading process for an average house with 20,000 broilers will take about 3 h but can take up to 9 h for a house of 60,000 birds. Transportation takes 134 min on average but can take up to 210 min, and finally birds have to wait in the lairage at the processing plant for 150 min on average, but this can increase to 955 min (Nijdam et al., 2004). Therefore, total feed withdrawal time will take about 12 h and 45 min; however, in a worst-case scenario, feed withdrawal can be 33 h and 30 min. Consequently, feed withdrawal causes BW losses between 0.22 and 0.56% / h (Veerkamp, 1978, 1986; Chen et al., 1983; Rasmussen and Mast, 1987; Lyon et al., 1991; Knowles et al., 1995; Warriss et al., 2004) and in reductions in carcass yields (Wabeck, 1972).

Feed withdrawal affects a lot of metabolic processes. Feed deprivation causes a shift from anabolism to catabolism, from lipogenesis to lipolysis, and a reduced metabolic rate. A study by Murray and Rosenberg (1953) revealed that the plasma glucose concentration of fasted chickens declined rapidly until a new equilibrium was reached and became stabilized after 3 h of fasting for at least 16 h. Furthermore, the amount of nonesterified fatty acid (NEFA) is increased at feed withdrawal (Langslow et al., 1970; Van der Wal et al., 1999) and circulating triiodothyronine ($T_3$) concentrations are decreased (Buyse et al., 2000). Furthermore, liver glycogen will become depleted to negligible amounts after feed withdrawal of 6 h (Warriss et al., 1988). Moreover, feed withdrawal induces behavioral and physiological responses, indicating that broilers probably suffer from stress (Freeman, 1980). An increase in CORT concentrations has been shown in broilers after a feed and water withdrawal of 24 h (Knowles et al., 1995). Also in growing broiler breeders, feed restriction leads to plasma CORT increases (De Jong et al., 2003). Withdrawal of feed in broilers before catching is likely to increase CORT as well.

Despite the general acceptance of the need for feed and water deprivation, the consequences of feed withdrawal in combination with catching and transport for stress levels and energy metabolism of broilers have not been investigated. Therefore, we experimentally examined the combined effects of feed withdrawal and a transport intervention (including catching, crating, transport of approximately 1.5 h, and lairage of approximately 1 h) on stress and energy metabolism. For this purpose, plasma concentrations of CORT, $T_3$, thyroxine ($T_4$), glucose, lactate, uric acid, NEFA, and triglyceride (TG) and changes in BW were assessed.

## MATERIALS AND METHODS

### Birds and Housing

Two experiments were performed with commercial Ross 308 male broilers.[3]

For the first experiment, in spring 2002, 192 male broilers (38 d of age) were obtained from a commercial broiler farm. At the Farm Animal Health Research Farm of the Utrecht University, The Netherlands, these broilers were kept in 24 floor pens (8 birds/pen) of approximately 1 $m^2$ on wood shavings. The lighting schedule was 24 h of light per day. Water and a grower diet (21% crude protein and 2,940 kcal of ME/kg) were provided ad libitum. Due to respiratory distress, the birds were treated with Baytril[4] in a dose of 20 mg/kg from d 41 up to and including d 43. The experimental interventions were done at 46 d of age, when the broilers had a mean BW of 2,770 g.

For the second experiment, in autumn 2003, 240 Ross 308 male broilers of 21 d of age were obtained from the same commercial broiler farm. Broilers were kept in the same 24 floor pens (10 birds/pen) under the same conditions as in the first experiment. Due to respiratory distress the birds were treated with Baytril in a dose of 20 mg/kg from d 24 up to and including d 27. The experimental interventions were done at an age of 49 d, when broilers had mean BW of 3,930 g.

Approval for carrying out both experiments was obtained from the Animal Experimental Committee of the Veterinary Faculty of Utrecht University, The Netherlands.

### Experimental Design

Experiments 1 and 2 included 4 and 5 treatments, respectively. For experiment 1 the treatments were as follows:

Treatment (TR) 1: feed was available and the broilers were not transported.

TR 2: feed withdrawal during the whole experiment (about 13 h).

TR 3: feed withdrawal for 10 h, and the broilers were caught and transported.

TR 4: feed was available until the moment that the broilers were caught and transported.

Experiment 2 consisted of an extra treatment to give more insight in the consequences of transport stress:

TR 5: feed was available during the feed procedure but removed during the transport intervention.

The transport intervention was performed on 96 broilers (12 pens) for experiment 1 and 120 broilers (12 pens) for experiment 2. The intervention 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 with a surface of 0.53 $m^2$. Birds of one pen were placed together into one crate. The crates were loaded into a ventilated van (approximately 0.5 h). Thereafter the birds were transported for approximately 1.5 h. After transport the birds remained in the crates for approximately 1 h. During these activities there was no access to feed and water. The transport intervention had a total duration of 3 h and 20 min for experiment 1 and 3 h for experiment 2. The birds that were not transported remained in the pens.

[3]Aviagen, Newbridge, Scotland.
[4]Bayer, Mydrecht, The Netherlands.

In experiment 1 six pens per treatment were involved, in experiment 2 for TR 2, TR 3, and TR 4 six pens were involved, and for TR 1 and TR 5 three pens were involved.

### Measurements

In both experiments the broilers were weighed before the start of the different treatments. In experiment 1 the broilers were weighed again just before euthanasia by electrocution. The period between the first weighing and the second weighing of the birds was 16 h and 40 min. In experiment 2 the second weighing was immediately after the end of the treatments, and the period between the first and second weighing was 13 h and 20 min.

Blood sampling started immediately after the end of the treatments. Birds were taken out of the pen or crates, depending on the treatment, and blood was collected by puncturing the vena ulnaris. About 4 mL of blood was taken and stored on ice in tubes containing sodium fluoride.

### Analysis of Plasma Samples

Blood samples were kept on ice until plasma was separated by centrifugation for 10 min at 1,500 rpm. Plasma samples were stored at –20°C until assayed. CORT, $T_3$, $T_4$, glucose, lactate, uric acid, TG, and NEFA concentrations were determined. Plasma CORT, $T_3$, and $T_4$ concentrations were measured using a sensitive and highly specific radioimmunoassay kit[5] with a sensitivity of 0.39 ng/mL and cross-reactions with aldosterone (0.20%), cortisol (0.40%), and deoxycorticosterone (3.30%). Samples were added in duplicate to check intraassay variability. Plasma CORT, $T_3$, and $T_4$ concentrations had intraassay variabilities of 3.9, 4.5, and 5.4% respectively. Plasma metabolite concentrations of glucose, lactate, uric acid, TG, and NEFA were determined using a commercial kit validated for chicken plasma[6] modified for use in the Monarch Chemistry System.[7] All measurements for each variable were run in the same assay to avoid interassay variability.

### Statistical Analyses

The statistical analyses were performed in the SAS-PC System (SAS Institute, 2000). PROC FREQ and PROC MEANS were used for 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.

Broiler was taken as the statistical unit, but pen was included as a random effect in the model to account for dependency between birds in the same pen (SAS Institute, 2000). Therefore standard errors and probabilities were calculated using the type III MS for pen as an error term. Experiment, treatment, and the interaction term experiment × treatment were analyzed using a generalized linear model performed by PROC GLM on plasma concentrations and BW. The experiment effect was highly significant ($P = 0.0002$), and therefore the results are presented separately for both experiments. Significant differences between treatments were separated using least squares means procedures of the SAS software (SAS Institute, 2000). All statements of significance were based on a probability level of 0.05.

## RESULTS

### Plasma Concentrations and Hormones

In experiment 1, no significant differences in CORT were found; however, in experiment 2 average plasma CORT concentrations of broilers that were transported (TR 3 and 4) were significantly higher than those of broilers exposed to other treatments (Figure 1A). For plasma $T_3$ concentration both experiments gave similar results. Treatments with long feed withdrawal times (TR 2 and 3) induced decreased $T_3$ values. For TR 4, experiment 2 gave significantly lower values as compared with TR 5. Both treatments had a similar feed withdrawal period, but TR 4 birds were also transported (Figure 1B). In experiment 1 significantly higher plasma $T_4$ levels were found for TR 2 and 3, which included feed withdrawal (Figure 1C). For Tr2, experiment 2 gave increased $T_4$ levels compared with TR 1, 3, and 4. Plasma $T_4$ levels in TR 5 were significantly higher than in TR 4.

### Plasma Concentrations and Metabolites

Plasma uric acid concentrations in experiment 1 were significantly decreased for broilers on feed withdrawal in TR 2. Broilers that were on feed withdrawn and transported (TR 3) had higher uric acid values than TR 2 birds. In experiment 2, TR 3 and 4 had significantly higher concentrations than treatments that did not include transport (TR 1, 2, and 5; Figure 2A). In both experiments the concentrations of TG found in TR 2, 3, 4, and 5 were significantly lower than the concentration of TG in TR 1. Feed withdrawal during the whole experiment (TR 2) resulted in the greatest decrease (Figure 2B).

Plasma NEFA concentrations in experiment 1 were significantly higher for TR 2, 3, and 4 birds than for fed birds (TR 1). In experiment 2 roughly similar results were obtained. TR 2, 3, and 4 showed significantly higher plasma NEFA concentrations than TR 1 and 5. Feed withdrawal and transport (TR 3) induced the highest values (Figure 2C). Plasma glucose concentrations found in experiments 1 and 2 for birds on feed withdrawal in TR 2 and TR 3 were significantly decreased. TR 4 gave significantly higher glucose values than TR 5 (Figure 2D). In both experiments, birds of TR 2 and 3 had the lowest lactate concentrations (Figure 2E).

[5]IDS, Inc., Boldon, UK.
[6]Procedure 826-UV, Sigma Diagnostics, Steinheim, Germany.
[7]Monarch Chemistry System, Instrumentation. Laboratories, Zaventem, Belgium.

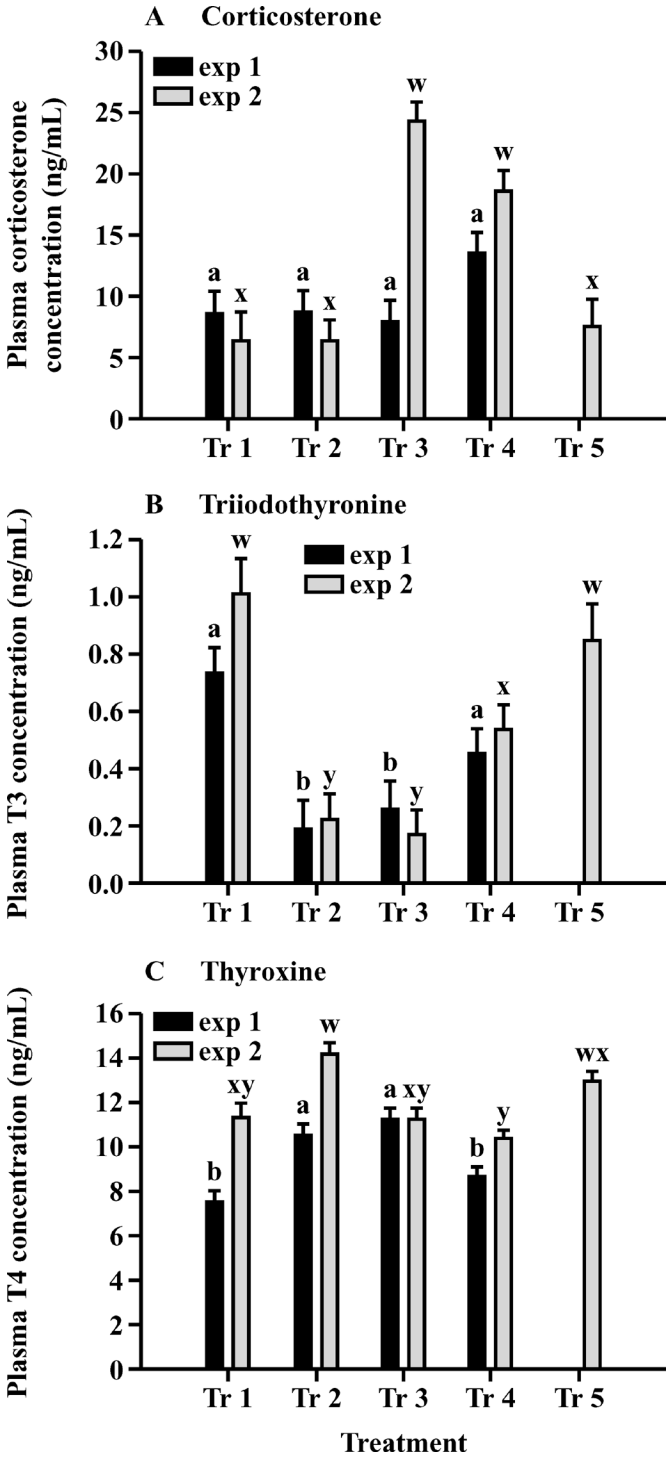


**FIGURE 1.** Mean concentration of plasma cortiocosterone (A), triiodothyrine (B), and thyroxine (C) for experiments 1 and 2 per treatment. Tr 1 = feed was available and the broilers were not transported; Tr 2 = feed withdrawal during the whole experiment; Tr 3 = feed withdrawal for 10 h, and the broilers were caught and transported; Tr 4 = feed was available until the moment that the broilers were caught and transported; and Tr 5 = feed was available during the feed procedure but removed during the transport intervention. Bars show standard errors of the mean. $^{a-c}$Values with different letters are significantly different for experiment 1 ($P < 0.05$). $^{w-y}$Values with different letters are significantly different for experiment 2 ($P < 0.05$).

### *BW*

Table 1 shows the growth (in %/h) per experiment and treatment. TR 3 led to a decrease in BW of 0.47 ± 0.02%/h for experiment 1 and 0.35 ± 0.02%/h for experiment 2. TR 2 produced a mean decrease in BW of 0.30%/h. The transport procedure obviously led to an extra decrease in BW of approximately 0.05 to 0.17%/h. When the broilers had access to feed until the moment of transport (TR 4) the mean decrease in final BW was 0.12%/h. At the start of experiments 1 and 2, BW did not differ significantly for any treatment group.

## DISCUSSION

Broilers that had no access to feed before being caught, loaded, and transported had higher $T_4$, and lower $T_3$, TG, glucose, and lactate concentrations compared with broilers that had access to feed before catching, loading, and transport, which indicated the combined effect of both actions. Besides that, the separate effect of feed withdrawal and transport intervention on hormones, except for CORT, and metabolites was in accordance with results obtained in previous studies (Buyse et al., 2000; Puvadolpirod and Thaxton, 2000).

Corticosterone had different patterns in both experiments. A striking difference was found for transport. The higher CORT levels found in the second experiment might express more severe physical stress. Roughly, the broilers used in each experiment were distinct with respect to disease history, medication history, feed intake, and final BW. Broilers in experiment 1 were subjected to transport stress a few days after respiratory infection occurred. Broilers in experiment 2, however, were subjected to transport stress 3 wk after respiratory infection had occurred. It is possible that respiratory infections of the broilers in experiment 1 caused a lower response to transport stress. In humans cortisol-binding globulin, which binds free cortisol and reduces its biological activity, is down-regulated by the acute phase response from respiratory infections. Down-regulation of cortisol-binding globulin results in negative adrenocorticotropin feedback. Although, recovery from respiratory infection may result in up-regulation of cortisol-binding globulin, resulting in a decrease of free cortisol in the plasma and, therefore, enhancement of adrenocorticotropin secretion (Garrel, 1996), it is unlikely that this phenomenon still occurs 3 wk after infection.

The significant increase of the CORT concentration due to transport in experiment 2 was in agreement with the results of Freeman et al. (1984), Kannan and Mench (1996), and Carlisle et al. (1998), but in the literature (Freeman et al., 1984; Kannan and Mench, 1996) it is not always clear whether the broilers were fed ad libitum, on restricted feeding, or feed was withdrawn before they were exposed to handling, crating, or transport. In this study feed withdrawal did not result in higher CORT values, which contradicts the findings of Knowles et al. (1995) and De Jong et al. (2003). Duration of feed withdrawal in our experi-

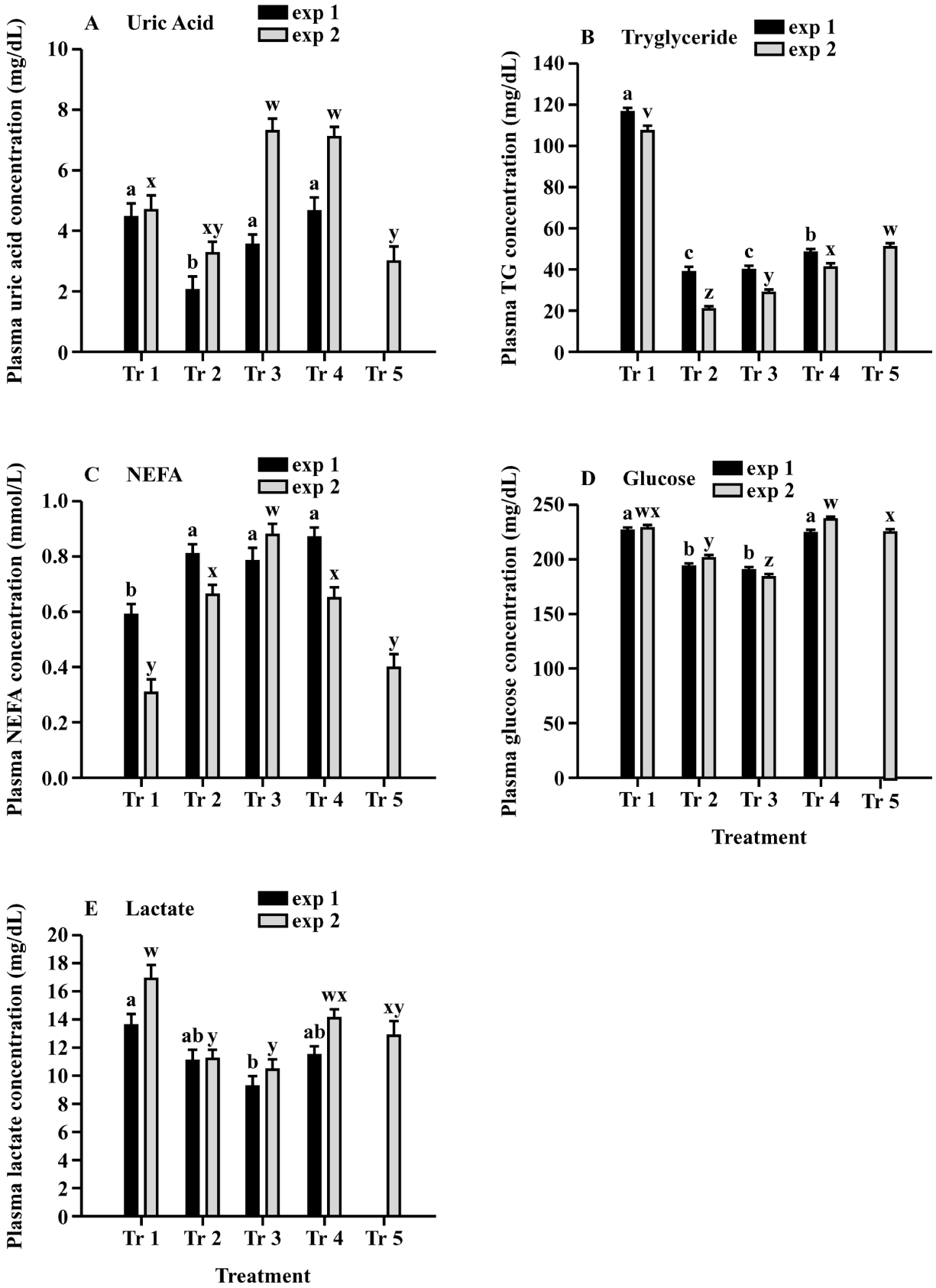


**FIGURE 2.** Mean concentration of plasma uric acid (A), triglyceride (B), nonesterified fatty acid (NEFA) (C), glucose (D), and lactate (E) for experiment 1 and 2 per treatment. Tr 1 = feed was available and the broilers were not transported; Tr 2 = feed withdrawal during the whole experiment; Tr 3 = feed withdrawal for 10 h, and the broilers were caught and transported; Tr 4 = feed was available until the moment that the broilers were caught and transported; and Tr 5 = feed was available during the feed procedure but removed during the transport intervention. Bars show standard errors of the mean. [a–c]Values with different letters are significantly different for experiment 1 ($P < 0.05$). [w–z]Values with different letters are significantly different for experiment 2 ($P < 0.05$).

**TABLE 1. Mean percentages ± SEM of growth per hour by percentage of BW per treatment for experiments 1 and 2**

| | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| Treatment[1] | n[2] | Mean ± SEM | n[2] | Mean ± SEM |
| TR 1 | 48 | 0.04[a] ± 0.02 | 30 | 0.09[a] ± 0.02 |
| TR 2 | 48 | –0.37[c] ± 0.02 | 58 | –0.23[c] ± 0.02 |
| TR 3 | 48 | –0.47[d] ± 0.02 | 56 | –0.35[d] ± 0.02 |
| TR 4 | 47 | –0.19[b] ± 0.02 | 58 | –0.05[b] ± 0.02 |
| TR 5 | — | — | 29 | 0.01[ab] ± 0.02 |

[a–d]Means within a column and for each experiment without a common superscript differ significantly ($P < 0.05$).

[1]TR 1 = feed was available during the whole experiment; TR 2 = feed withdrawal during the whole experiment; TR 3 = feed withdrawal for 10 h, and the broilers were caught and transported; TR 4 = feed was available until the moment that the broilers were caught and transported; TR 5 = feed was available during the feed intervention but removed during the transport intervention.

[2]Number of observations per mean.

ment might have been too short to induce a significant increase in CORT concentration. Feed was withdrawn from broilers for 13 h only, whereas broilers in the experiment of Knowles et al. (1995) experienced feed withdrawal for 24 h, and the broiler breeders in the experiment of De Jong et al. (2003) were limited in feed consumption for 35 d.

Broilers subjected to transport intervention had lower concentrations of $T_3$; however, this decrease was only obvious in chickens that were fed before being caught and transported. If the period of feed deprivation was followed by transport intervention, no further reduction of $T_3$ was observed. The reason for this finding was probably that after feed was withdrawn for approximately 13 h, birds were already experiencing hypothyroidism, and so a further decrease of $T_3$ was not detectable (Buyse et al., 2000).

The source for the serum TG was dietary lipids or de novo synthesis from carbohydrates or amino acids (i.e., lipogenesis). Both sources that form TG are absent or are present only to a small extent if broilers are fasted. This explains the significantly lower concentrations of TG in these birds. There is an effect of feeding or fasting only; no significant changes were observed after the broilers were exposed to stress.

A possible explanation for the significantly lower glucose and lactate concentrations in broilers that had feed withdrawn before transport compared with the levels in broilers that had access to feed before transport may be that the transport intervention demands more energy obtained by oxidation of glucose than the additional neoglucogenetic effect of CORT increase by combining stress and feed withdrawal. Oxidation of glucose is possibly the preferred initial energy source. However, the high concentrations of NEFA also indicate increased lipolysis. Therefore, further research is needed to examine to what extent glucose and lipids are used to fulfill the energy needs of feed withdrawn and stressed broilers.

Feed withdrawal and transport led to decreased BW. Broilers that were transported after a feed withdrawal period of 10 h had weight losses of approximately 0.42% / h, which is approximately 0.30% / h more than for broilers that had full access to feed until the moment of transport. Other studies (Veerkamp, 1978; Chen et al., 1983; Veerkamp, 1986; Rasmussen and Mast, 1987; Lyon et al., 1991; Knowles et al., 1995; Warriss et al., 2004) showed losses in BW between 0.22 and 0.56% / h, which are in agreement with our results. However, the range in BW losses is great because in some studies broilers had feed withdrawn, and in others they were also transported before slaughter. At high ambient temperatures, BW losses will be greater (Chen et al., 1983). Moreover, in some studies birds had water withdrawn, and the total withdrawal times differed. A large amount of the BW reduction is due to the clearance of the gastrointestinal tract (Warriss et al., 2004). However, there will also be loss of edible parts due to dehydration (Kamus and Farr, 1981; Knowles et al., 1995) and losses of fat and protein (Knowles et al., 1995). These changes can influence meat quality by affecting muscle glycogen content at slaughtering and, therefore, reduce the rate of rigor as well as the ultimate pH (Fletcher, 2002).

In conclusion, to continue feeding the broilers until they are caught results in higher BW at the slaughterhouse and less stress and may improve the meat quality compared with broilers that have no access to feed before they are caught. Feed withdrawal of broilers before the transport procedure had a negative effect not only from an economical point of view but maybe also in light of animal welfare. Therefore, research to apply some energy-delivering supplement during the last day of broilers' lives to cope with stressors, such as catching and transport, would be worthwhile.

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