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Prior handling does not significantly reduce the stress response to pre-slaughter handling in broiler chickens

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Abstract

Two experiments were conducted to determine whether previously handled male broilers would show a decreased corticosterone (CORT) response to commercial-type handling at market age. In Experiment 1, broilers ($N = 36$) were either repeatedly handled (H) or non-handled (NH) from day 1 to 6 weeks of age. At 7 weeks, they were exposed to either a three-bird inverted (multiple inverted, MI), one-bird inverted (single inverted, SI) or upright (U) handling treatment for 2 min. Plasma CORT levels were determined at 1 h or 3 h after the handling treatments were imposed. Although CORT levels were higher (average 2 ng ml^{-1}) in the NH than in the H pre-treatment group in response to handling, this difference was not significant except for H birds handled in the SI position, resulting in a significant interaction effect ($P < 0.05$). CORT levels were lower in both groups after U as opposed to MI or SI handling ($P < 0.03$). In Experiment 2, broilers ($N = 216$) were subjected to one of three pre-treatments—repeated upright handling (RU), repeated inverted handling (RI), or no handling (NH)—every day until 6 weeks of age. At 7 weeks of age birds in each pre-treatment group were then exposed to either the MI, SI or U handling treatment for 2 min. Blood samples were collected at 0, 1, 3 and 4 h after handling. Again, U handling resulted in a lower ($P < 0.001$) CORT response than MI or SI handling across pre-treatments. CORT levels were higher ($P < 0.001$) at 0 h than at the other time periods. Pre-treatment had no significant effect on CORT levels, and there was no interaction among any of the factors. These results show that upright handling is less stressful to broilers than inverted handling, and that stress levels are highest immediately after handling. However, there is little

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evidence that the CORT response to handling at market age can be markedly or reliably reduced by prior handling during rearing.

Keywords: Chicken welfare; Handling; Corticosterone

1. Introduction

Commercial broiler chickens are exposed to a number of potential stressors prior to slaughter, including food deprivation, social disruption, handling, crating, transportation, shackling and stunning. Handling by humans may be one of the most serious factors affecting bird welfare and carcass quality. Rough handling can cause physical injuries to the birds, including bruises, broken bones and dislocations. Violent wing flapping during commercial inverted handling of broilers may contribute to the occurrence of red wingtips (Gregory et al., 1989). These conditions not only affect welfare but also result in economic losses due to carcass downgrading. The fear caused due to human approach and handling (Duncan and Filshie, 1980; Duncan, 1981) can further affect the welfare of the birds.

Attempts to reduce fear and stress associated with handling in poultry have been the subject of a number of recent studies. These have emphasized the use of mechanical harvesters, gentle handling by trained personnel, or continuous association with humans during the broilers' growth, to reduce handling stress.

A variety of prototype mechanical harvesters are in experimental use. Existing mechanized procedures for handling poultry are extensively reviewed by Kettlewell and Turner (1985) and Scott (1993). In an investigation of one of these prototype harvesters, Duncan et al. (1986) found that machine harvesting or gentle handling were less stressful for the birds than typical commercial manual harvesting, as measured by heart rate and duration of tonic immobility. However, there are some problems associated with the use of mechanical harvesters under commercial conditions. Poultry houses in many countries are not designed for the use of mobile mechanical harvesters (Kettlewell and Turner, 1985). Even when mechanical harvesting is used, some manual assistance is still necessary in order to move the birds from the corners of the house towards the harvester. Furthermore, the capital investment on such machines, combined with the expenditure necessary to redesign existing poultry houses, makes it difficult to popularize mechanical harvesters, at least among smaller-scale producers. The development of improved manual handling methods is thus still of importance.

Previous studies have shown that the handling method used is important in influencing the level of injury, fear reactions, and the elicitation of physiological stress responses. It is known that the personnel engaged in catching can influence the degree of injury that the birds sustain during capture (Knowles, 1994). Inverted handling increases the duration of tonic immobility in both layers and broilers (Jones, 1992), and also produces elevated plasma corticosterone (CORT) levels in spent hens (Broom et al., 1986; Broom and Knowles, 1989) and broilers (Kannan and Mench, 1996) as compared to upright handling.

It is possible that repeated handling during the growing period could be used to habituate birds to the handling process, and thus decrease stress and fear. Regular gentle handling of broilers results in the birds becoming habituated to human beings, as measured by the tonic immobility response (Jones and Waddington, 1992). The duration of induced tonic immobility is also shorter in chicks whose main visual stimulation during growth is a human caretaker (Eddy and Gallup, 1994). Hemsworth et al. (1994) found that regularly handled broilers show a lower CORT response to restraint and less withdrawal to human approach than non-handled broilers. Nicol (1992), however, reported that the most fearful birds after 1 h of transportation were those that had been exposed to environmental enrichment and repeated gentle handling during development. The potential for using prior handling to improve the welfare of broilers during catching is thus uncertain.

The present studies were undertaken to determine whether repeated handling of broilers during the rearing period has any influence on CORT responses when birds are subjected to various methods of pre-slaughter handling at 7 weeks of age. The effect of different methods of repeated handling (repeated inverted and repeated upright) on CORT levels was also examined.

2. Materials and methods

2.1. Animals

Two experiments were conducted to study the influence of prior handling on CORT levels in broilers measured after imposing handling treatments at market age. Male Arbor Acres broilers were used in both experiments. Experiment 1 was started in February and completed in April; Experiment 2 was started in September and completed in November. The birds were housed in floor pens bedded with wood shavings. Each pen contained one automatic bell waterer and one tube feeder. Commercial starter, grower or finisher ration and water were available ad libitum throughout the studies. As the broiler house was not environmentally controlled, natural light and temperature inside the house was supplemented with heat lamps to maintain warmth in the pens.

2.2. Corticosterone analysis

Blood samples (2 ml) were collected from the brachial vein using a 23 gauge needle and dispensed into tubes containing one drop of 11% ethylenediaminetetraacetic acid (EDTA). The time taken for blood collection (from the time the birds were picked up for sampling to the time the blood was drawn) did not exceed 45 s. The tubes were kept on ice until the plasma was separated. Plasma was stored at $-20°C$ until assayed.

Plasma CORT concentrations were measured by radioimmunoassay using the RSL I_{125} (ICN Biomedicals, Costa Mesa, CA, USA) Corticosterone Kit (liquid phase), which was validated for parallelism and recovery for chickens in our laboratory. The sensitivity of the assay was 0.13 ng ml^{-1}. Within- and between-assay coefficients of variation were 6.2% and 9.8%, respectively.

2.3. Experiment 1

Fourteen birds were housed in each of four pens, with a floor space of 0.29 m^2 and a feeder space of 13.8 cm per bird. Nine birds from each pen were used, giving a total of 36 birds in the study.

The following pre-treatments were imposed every day from the day the chicks were received until 6 weeks of age:

(1) Repeated handling (H). Birds in two of the pens were handled every day from the day they were housed until they were 6 weeks of age. The experimenter (G.K.) entered each pen in the morning and spent about 5 min there. During the first 3 weeks, groups of four or five chicks were scooped up into both hands and gently returned to the litter after a few seconds. After 3 weeks of age as the birds grew bigger, the handling procedure was changed such that one bird was randomly selected and held for a few seconds using both hands, allowing the bird to stand still on the floor. Not all birds were handled every day in order to avoid chasing the birds and frightening the flock. Nevertheless, the experimenter spent some time moving closer to every bird by stretching his arm in its direction.

(2) Non-handled birds (NH). Birds in two pens were ignored apart from routine maintenance such as feeding and watering. These chores were carried out by the experimenter 2–3 times per week as quickly and with as little disturbance to the birds as possible.

At 7 weeks of age, treatments were applied to birds in all pens. H and NH birds were randomly allotted to one of the following three handling treatments:

(1) Multiple inverted (MI). Three birds were held by the legs in one hand in an inverted position (Fig. 1(A)).

(2) Single inverted (SI). One bird was held by the legs in one hand in an inverted position (Fig. 1(B)).

(3) Upright handling (U). Each bird was gently held close to the body of the handler in an upright position (Fig. 1(C)).

For all treatments the handler walked a distance of about 10 m outside the pens and held the birds for a total period of 2 min. After the handling treatment, birds were marked with colored dyes on the wings to identify them by treatment and returned to their respective pens. Blood samples were collected at 1 h and 3 h after handling, and no bird was sampled more than once. These two time periods were selected because birds from these two groups were found to have the lowest and the highest plasma CORT concentrations, respectively, after handling in a previous study (Kannan and Mench, 1996).

2.4. Experiment 2

This experiment was designed to increase replication of the handling treatment in Experiment 1. The frequency of blood sampling was increased to study the trend in CORT responses over a period of 4 h after handling. The effect of different methods of repeated handling (repeated upright and repeated inverted) was also studied. Repeated inverted handling was included as a pre-treatment because broilers are normally caught

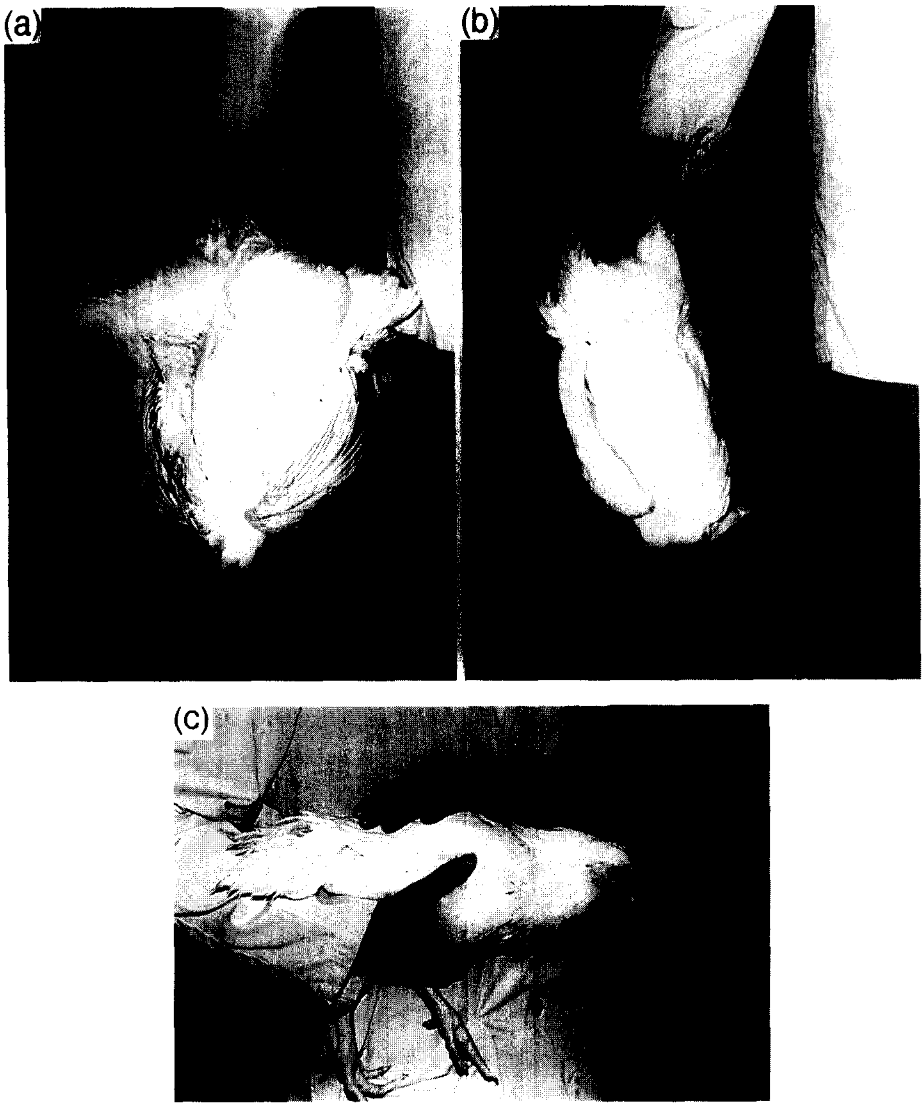

Fig. 1. Photographs showing the methods of imposition of handling treatments in the preliminary experiment and Experiment 1. (A) Multiple inverted handling. (B) Single inverted handling. (C) Upright handling.

and carried by the legs in an inverted manner prior to crating and transportation for slaughter. Day-old chicks were housed in 12 pens with 21 chicks in each pen. The floor space was 0.20 m^2 and the feeder space 9 cm per bird. Eighteen birds from each pen were used for this experiment, giving a total of 216 birds.

Pre-treatments were allotted randomly to pens. The following pre-treatments were imposed every day from the day the chicks were received until 6 weeks of age:

(1) Repeated upright handling (RU). The experimenter entered each of four pens and

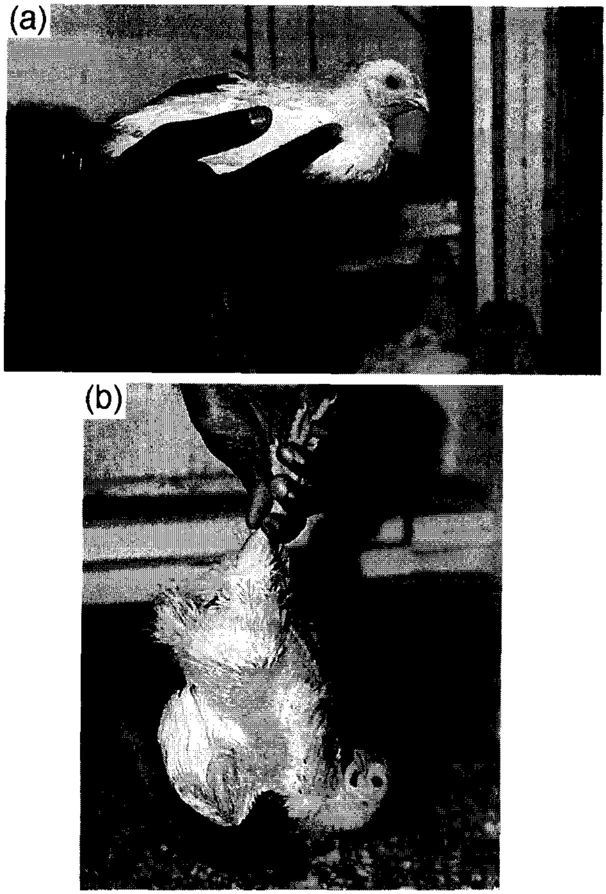

Fig. 2. Photographs showing the methods of imposition of pre-treatments in Experiment 1. (A) Repeated upright handling. (B) Repeated inverted handling.

picked up every bird individually and placed it back on the floor. Each bird was held gently in an upright position with both hands for a couple of seconds (Fig. 2(A)).

(2) Repeated inverted handling (RI). The experimenter entered each of four pens, picked up every bird individually and held it by the legs in an inverted position for a couple of seconds (Fig. 2(B)). The bird was turned upright before placing it on the floor.

(3) Non-handled birds (NH). Birds in four pens were ignored apart from routine maintenance, as in Experiment 1.

At 7 weeks of age, handling treatments were imposed twice with a time interval of 2 days between treatments. The details of allotment of pre-treatment to pens, treatments to birds within pens and blood sampling times on the first sampling day are illustrated in Fig. 3. The imposition of handling treatments (MI, SI and U) was the same as that described in the preliminary experiment. Birds were blood sampled after either 1 h, 3 h or 4 h after being returned to their pens. The 0 h groups were blood sampled

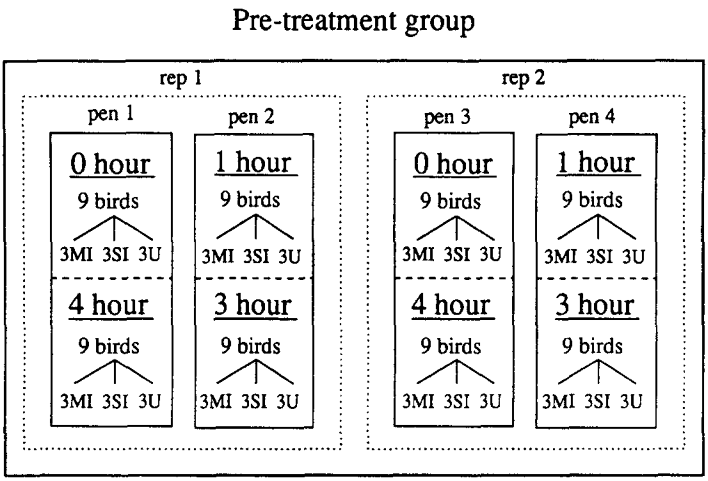

Fig. 3. Illustration of experimental design (Experiment 2) showing the details of allotment of a pre-treatment to pens, and treatments (multiple inverted, MI; single inverted, SI; upright handling, U) to birds within each pen. Each pre-treatment consisted of two replicates (reps 1 and 2). Two pens of birds were blood sampled at four different time periods (0 h, 1 h, 3 h, 4 h) and considered as one replicate for the purpose of generating error terms.

immediately after the handling treatments. No bird was sampled more than once on a test day. At each sampling period 18 birds each from RU, RI and NH groups were blood sampled, giving a total of 54 birds. The 18 birds sampled at each time period within a pre-treatment group were taken from two different pens (nine birds from each pen) in order to have pen replication.

To increase replication, imposition of handling treatments and blood sampling were repeated after a lapse of 2 days (day 2). We assumed that there would be minimal carryover effects of handling treatment on CORT levels after 2 days. On day 2 the handling treatments were randomly allotted to birds within each pre-treatment pen, irrespective of the handling treatment they underwent on day 1. Birds were blood sampled as previously described, but the pens within a replicate were interchanged for time sampling purposes. For example, on day 1 birds in pen 1 were sampled at 0 h or 4 h, and birds in pen 2 were sampled at 1 h or 3 h after handling (Fig. 3). On day 2, birds from pen 1 were blood sampled at 1 h or 3 h, while those from pen 2 were sampled at 0 h or 4 h.

2.5. Statistical analysis

The data were analyzed using the General Linear Models procedure in SAS[R] (Statistical Analysis Systems Institute Inc., 1987). The data from Experiment 1 were analyzed as a split-plot design with pre-treatments as whole-plot factors. Since time after handling (time) and pen effects were confounded in this experiment, it was not possible to perform a valid test for the main effects of pre-treatment and time. The split-plot factor was a comparison among the three handling treatments (treatment). The differences among the main effect means were tested using the least significant difference (LSD) test at a 5% level of probability.

A split-split plot design (Fig. 3) was used in Experiment 2, with pre-treatments (four pens each) as whole-plot factors, time after handling (time) as the split-plot factor and treatments as the split-split plot factors. Two of the pens within each pre-treatment were

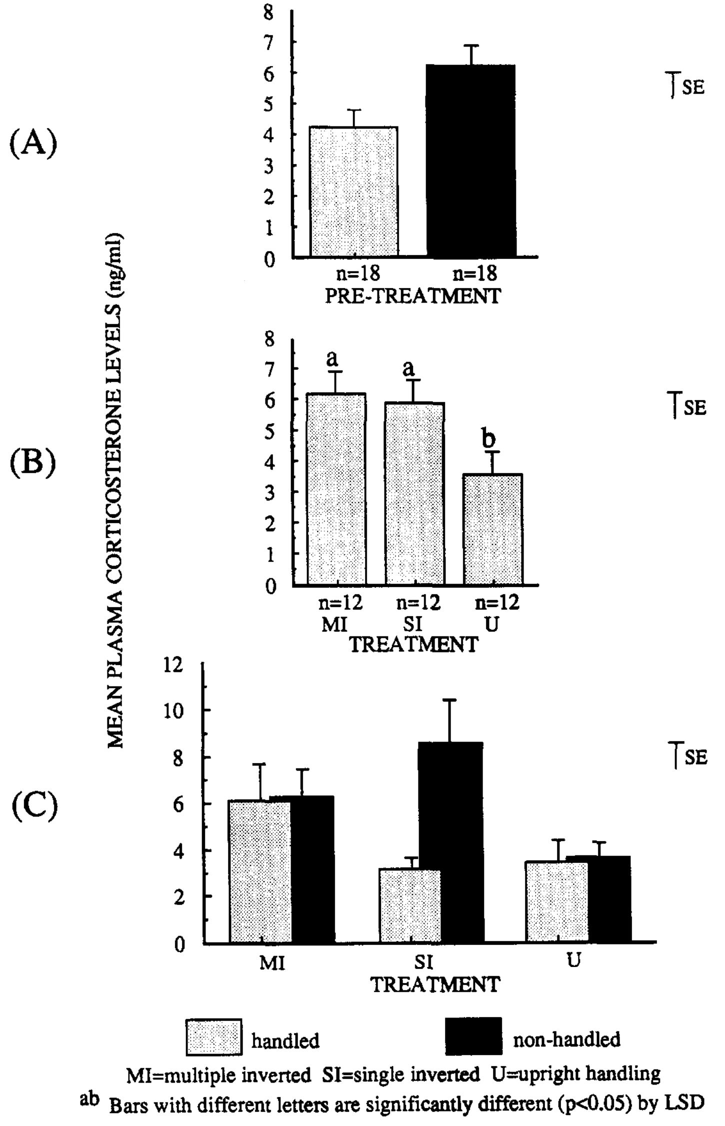

Fig. 4. (A) Bar charts showing: (A) the main effects of pre-treatments (handled and non-handled); (B) the main effects of treatments (multiple inverted, single inverted and upright handling); (C) pre-treatment × treatment interaction.

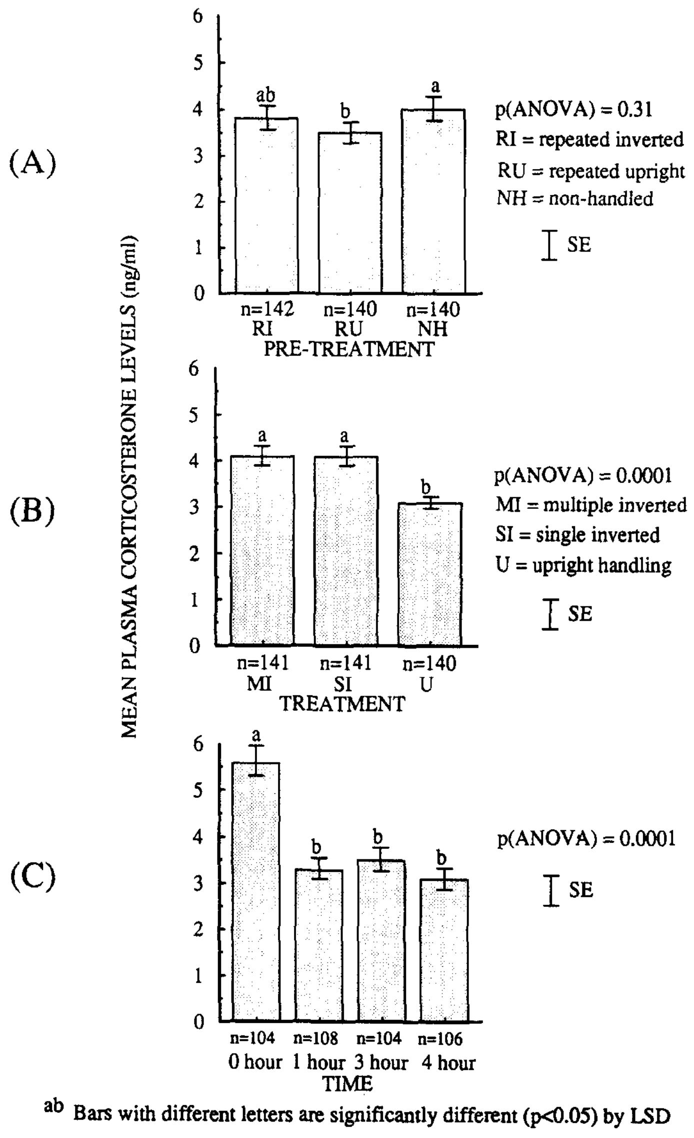

Fig. 5. Bar charts showing: (A) the main effects of pre-treatments (repeated inverted, repeated upright and non-handled); (B) the main effects of treatments (multiple inverted, single inverted and upright handling); (C) the main effects of time (0 h, 1 h, 3 h, 4 h).

grouped arbitrarily and considered as replicate (rep) 1 or 2 for the purpose of calculation of error terms. Therefore, the pre-treatment main effect was tested with the variance between reps within pre-treatment as the error term. The time main effect and pre-treatment × time interaction effect were tested with rep × pre-treatment × time as the error term. The sources of variation in the split-split plot (treatment, treatment × pre-treatment, treatment × time and treatment × pre-treatment × time) were tested with rep × pen ×

pre-treatment × treatment × time as the error term. The main effects means were separated using an LSD test. Examination of residuals for the CORT concentrations showed that a log transformation of the CORT values was necessary to satisfy the assumption of a normal distribution.

3. Results

3.1. Experiment 1

The H group showed a mean CORT response of 2 ng ml^{-1} less than that of the NH group, although this was not statistically significant (Fig. 4(A)). CORT levels were slightly higher at 3 h (5.5 ng ml^{-1}) than at 1 h (4.9 ng ml^{-1}). The treatment main effect was significant ($F = 3.94$; d.f. = 2,24; $P < 0.03$), and pairwise comparisons of the means by LSD showed that the U group had a significantly lower ($P < 0.05$; Fig. 4(B)) mean CORT level than the MI or SI groups. There was no significant difference between the MI and SI groups. There was also a significant pre-treatment × treatment interaction ($F = 4.31$; d.f. = 2,24; $P < 0.03$; Fig. 4(C)). The mean CORT level was higher in NH than in H birds subjected to the SI treatment. CORT levels in H birds subjected to the SI treatment were similar to the levels of birds in the U treatment group.

3.2. Experiment 2

CORT levels were influenced by treatment and time, but not by pre-treatment (Fig. 5). The mean CORT level of the NH group was higher than that of the RU group, while RI was intermediate (Fig. 5(A)), but, again, this was not significant ($F = 1.34$; d.f. = 2,9; $P < 0.31$). The U group had significantly ($F = 14.93$; d.f. = 2,72; $P < 0.001$) lower CORT levels than MI or SI (Fig. 5(B)). CORT levels were significantly ($F = 18.46$; d.f. = 3,27; $P < 0.001$) higher at 0 h than at the other time periods (Fig. 5(C)). There was no significant interaction among any of the factors.

4. Discussion

Regular handling or increased human contact has been reported to produce a number of beneficial effects in chickens. Feed efficiency and growth rate are improved in regularly handled broilers and layers (Thompson, 1976; Gross and Siegel, 1979; Jones and Hughes, 1981). Hughes and Black (1976) observed that flightiness decreased due to regular handling in young layers, although this effect diminished with age and disappeared at maturity. They also found that there was no difference in egg production between regularly handled and non-handled groups, but that egg production decreased when regular handling was imposed on birds that were not used to such treatment. Gross and Siegel (1979) found that Shaver Starcross male chickens that were handled regularly showed a greater antibody response and resistance to infection than non-handled birds. Besides improved performance, laying hens that experience additional human contact show a lower CORT response to handling than hens that have had minimal exposure to humans (Barnett et al., 1994).

Regular handling has been found to attenuate tonic immobility reactions in chicks (Jones and Waddington, 1992), and to decrease withdrawal responses to humans in broilers (Hemsworth et al., 1994). However, Nicol (1992) observed that enriched and handled broilers actually exhibited longer tonic immobility reactions after transportation than non-handled birds. The conflicting results seen in the literature regarding the effects of regular handling can probably be attributed to differences in various factors such as the frequency of handling, the age at which regular handling is applied and the method of handling.

Although not quantified, it was observed in our studies that there was a marked difference in the way the repeatedly handled and non-handled birds reacted to the experimenter's approach. After a couple of weeks of repeated handling, the birds appeared not to be bothered by the presence of the experimenter. The non-handled birds, on the other hand, were agitated, vocalized, and moved to the opposite wall when the pen door was opened, however gently, by the experimenter for maintenance purposes. Jones (1985) stated that regular handling may specifically reduce fear of humans through habituation and not by depressing general fearfulness. Although a direct comparison cannot be made between stress responses and fear reactions, it is considered that fear and stress are related (Faure, 1980; Duncan, 1981; Satterlee et al., 1991). Jones et al. (1994) suggested that avoiding fear may prevent excessive adrenocortical activity during stressful situations.

Nevertheless, our results suggest that, while the RU birds seemed to show decreased withdrawal responses to human approach, repeated handling had little influence on the CORT response to handling at market age. The overall difference in the mean plasma CORT levels between the NH group and the RU group was 2 ng ml^{-1} in Experiment 1 and about 0.6 ng ml^{-1} in Experiment 2, but neither difference was significant. In addition, although lower CORT levels were found in RU birds subjected to the SI treatment in Experiment 1, this was not replicated in Experiment 2. Repeated inverted handling during development also resulted in a lower mean plasma CORT response to handling than in NH birds, but the difference was very slight. In contrast to our results, Hemsworth et al. (1994) found that broiler chickens that were handled regularly showed a lower CORT response to upright handling than those that were not regularly handled. However, this difference was significant only after the birds had been handled for 12 min, a period considerably longer than they would typically be held under commercial conditions. It appears either that regular handling has less benefit when handling is of short duration, or that the sampling intervals we used were not sufficient to detect a difference between the pre-treatment groups. Another possibility is that the RU birds responded with increased CORT levels because the handlers they were exposed to at market age were unfamiliar to them. However, chicks habituated to humans do seem to generalize this response to unfamiliar individuals (Jones, 1994).

The effects of the different handling treatments (U, SI, MI) on plasma CORT levels were similar in Experiments 1 and 2, and are consistent with our previous findings (Kannan and Mench, 1996) showing that upright handling is less stressful to broilers than inverted handling. It is evident that hanging by the legs in an inverted position is more stressful irrespective of whether birds are held individually or in groups. The inverted handling treatments were imposed by holding both of the legs instead of only one leg, as is seen in commercial situations. Commercial handling is therefore probably much more stressful to broilers than the type of inverted handling used in our study.

In Experiment 2, the overall CORT levels immediately after handling were higher than at the other time periods tested. We found a similar pattern in a previous study for broilers that were exposed to the U treatment, although CORT levels in MI and SI handled birds in that study did not peak until 3 h after handling (Kannan and Mench, 1996). While the CORT response to handling is therefore generally greatest shortly after the handling episode, the time course of the response seems to depend on complex interactions between previous experience, the type of handling used, and perhaps other variables.

In conclusion, upright handling is less stressful to broilers than inverted handling. CORT levels were highest immediately after handling treatments and decreased within 1 h to a low level and stayed at that level throughout the time periods tested. There is little indication that the stress response to pre-slaughter handling can be markedly or consistently reduced by repeated handling during the rearing period.

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