INDICATORS OF PHYSIOLOGICAL STRESS IN BROILER CHICKENS DURING ROAD TRANSPORTATION

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Abstract

Differential leucocyte counts and plasma activities of the muscle enzyme, creatine kinase, have been determined in blood samples obtained from broiler chickens, immediately prior to and following road transportation from farm to processing plant for slaughter. These parameters are proposed as indicators of physiological stress based on previous findings.

Heterophil:lymphocyte ratios and plasma creatine kinase activities increased and eosinophil counts were decreased during the journey in birds tranported in both July and October when the curtain sides of the vehicles were open or closed respectively.

These findings are consistent with the presence of physiological stress during road transportation. The thermal microenvironments to which birds are exposed in transit are thought to represent one of the sources of this 'transportation stress'.

Keywords: *animal welfare, creatine kinase, haematology, physiological stress, transport stress*

Animal welfare implications

In order to optimize the welfare of animals during transportation from their site of production to that of slaughter, it is necessary to determine the animals physiological and behavioural responses to the journey, in relation to their biological needs, and to fully characterize the microenvironments to which the animals are exposed.

The present study has utilized measurements of physiological parameters previously demonstrated to respond to stressors, probably by activation of the hypothalamo-adenohypophyseal-adrenocortical axis, to assess the presence of physiological stress in broiler chickens following road transportation. Evaluation of 'stress' by the magnitude and form of such responses, particularly when coupled with simultaneous measurements of behavioural responses and environmental conditions, may form the basis for recommendations for improvements in the design of transport containers, vehicles and practices.

Introduction

The welfare of poultry during their transportation by road from sites of production to slaughter at processing plants is a matter of concern (FAWC 1990) and is a constituent topic of impending legislation (EC 1989). Immediately prior to and during transportation birds may be exposed to a wide range of potential stressors. These include, catching, handling, loading, motion, acceleration, impact, thermal demands imposed by the transport microclimate, fasting, withdrawal of water, restriction of behaviour, social disruption and noise. The adverse effects of these factors upon the bird may range from mild distress and aversion to injury and death. It has been reported that 40 per cent of the mortalities in 'dead on arrival' broilers are a consequence of stress (Bayliss & Hinton 1990) and that mortality increases with journey length (Warriss *et al* 1990). Whilst, in the UK, the duration of many journeys from farm to unloading at the factory may be approximately 3 h or less, on occasions birds may be confined in the vehicle for up to 12 h (Warriss *et al* 1990). It is thus imperative that techniques are developed to determine the physiological and behavioural consequences of transporting poultry under commercial conditions. A number of 'indices of stress' in poultry have been proposed (Stephens 1980, Hill 1983, Freeman 1987). Potential sources of stress in transit and possible methods for the assessment of the corresponding bird responses have been reviewed (Hails 1978, Swarbrick 1986, Duncan 1989, Kettlewell 1989, Broom 1990, Nicol & Scott 1990, Knowles & Broom 1990). In the present study the degree of physiological stress imposed upon broiler chickens during standard journeys under commercial conditions has been assessed by measuring the differential leucocyte counts and the plasma creatine kinase activities in blood samples obtained before departure from the farm and upon arrival at the processing plant. As well as determination of heterophil-lymphocyte ratios, which are influenced by corticosteroid concentrations (Jones *et al* 1988, Gray *et al* 1989, Gross 1990) and are accepted indicators of stress in poultry (Satterlee *et al* 1989, Maxwell *et al* 1990a, Gross & Siegel 1983, McFarlane & Curtis 1989), the effects of transportation upon other classes of leucocyte have been examined. This is considered appropriate because stressful stimuli have been demonstrated to alter the numbers of circulating monocytes, eosinophils and basophils, sometimes in the absence of changes in heterophil-lymphocyte ratios (Maxwell & Burns 1982, Gray *et al* 1989, Maxwell *et al* 1990b).

Creatine kinase is an important enzyme in skeletal muscle and is released into the plasma of birds in response to exercise, disease, stress and laying (Hollands *et al* 1980, Ostrowski-Meissner 1981, Tripp & Schmitz 1982). Indeed it has been suggested that elevated plasma creatine kinase activities are indicative of muscle damage (Lumeij *et al* 1988a,b) and one previous report indicated that transportation of poultry may increase the release of this enzyme from the muscle (Scholtyssek & Ehinger 1976). The present study was designed to investigate the use of plasma creatine kinase activities and differential leucocyte profiles as possible indices of physiological stress in transported broilers. In addition the microenvironments to which the birds were exposed in transit were characterized in terms of temperature and relative humidity for each journey.

Methods and materials

Preliminary laboratory studies

A preliminary study was conducted to assess the possible effects of the blood sampling procedure and withdrawal of food and water upon the physiological variables to be measured under commerical conditions. Ten female broiler birds, of the same strain employed in the later studies, were selected at 39 d of age and placed in pairs in cages in a controlled climate chamber maintained at 22°C (± 10%) and were allowed access to food and water *ad libitum*. A lighting cycle of 23 h light : 1 h dark was employed. At 42 d of age (approximately 2kg body-weight) food and water were withdrawn and 3 h later an initial blood sample was obtained. A second blood sample was taken after a further 3.5 h. The samples were treated and analysed as described for those obtained under commercial conditions. The durations of the component periods of this experiment were based upon previously determined values typical of commercial practice for the journeys studied.

Investigations under commercial conditions

Studies were performed upon mixed-sex broiler birds (approximately 2 kg body-weight) reared under commercial conditions until 42 d of age, when they were collected for transportation to the processing plant. In order to minimize the effects of variations associated with husbandry and transport practices, a standard journey of uniform length and duration from a single farm to one factory was selected. All birds were manually caught between 0800 and 1100 h following a minimum of 3 h food deprivation and at least 1 h after withdrawal of water. Birds were placed in plastic drawers (1.3m x 0.7m x 0.25m) whose sides were perforated by vertical slats (10 mm wide : 55 mm centre spacing). The number of birds per drawer ranged from 21 - 22 (summer) to 21 - 23 (winter). The drawers were placed in a modular system and were loaded onto the curtain-sided vehicle which consisted of a lorry and trailer. The same vehicle and driver were used on every run. Six specific drawer locations were identified as being representative of the possible distribution of the microenvironments within the vehicle (3 on the lorry, 3 on the trailer). The same 6 locations were employed on every journey. Two journeys were studied on consecutive days in the months of July, 1990 (summer configuration - curtain sides open) and October, 1990 (winter configuration - curtain sides closed). In each month the weather conditions were similar on the consecutive days of the trial.

Journeys were timed by an observer travelling with the vehicle from departure from the farm to the point of unloading at the processing plant. The journey time included a mandatory 'break'. The average speed was calculated from the total distance and the actual time in transit. Confirmation of the average speed could be obtained from the cab tachograph. The observer also produced a record of journey events which might influence the on-board environment or the birds' responses.

Blood samples (2 ml) were obtained by venepuncture (brachial vein) from 5 birds in each of the 6 crates immediately after catching but before loading on each day. A second sample was obtained immediately upon arrival at the factory. The samples were divided into two aliquots. One was placed in heparin for later determination of plasma enzyme activity and the other aliquot was treated with EDTA for subsequent haematological analyses. Samples were stored in a chilled container until return to the laboratory for processing. Plasmas were obtained from the heparinized samples by centrifugation at 1500 g for 10 min and stored at -20°C pending analysis.

Blood films were air dried (unfixed) and stained in concentrated May-Grunwald stain for 6 min, 1:1 May-Grunwald stain - distilled water for 1.5 min and 1:9 Geisma stain for 15 min (Robertson & Maxwell 1990). To determine differential leucocyte counts a minimum of 100 cells per film were examined by light microscopy. All blood counts were performed by the same investigator. The results are presented as the percentage of each cell occurring in each film. The heterophil-lymphocyte ratios were examined by dividing the number of heterophils by the number of lymphocytes (Gross & Siegel 1983).

Plasma creatine kinase (CK) activities were determined by spectro photometry using a commercially available kit (Wako CK-NAC; Alpha Laboratories UK) modified for use with an autoplate reader (Titertek 2 - Autoflow Laboratories UK) and measuring the rate of generation of nicrotinamide adenine dinucleotide phosphate (NADPH) by absorbance at 340 mm. Plasma dilutions (1:2), incubation times (5 min) and temperature (30°C) were optimized in previous experiments. The sensitivity of the assay procedure was 5 IU l^{-1}.

Environment

Each of the modular drawers in the 6 locations from which birds were selected for blood sampling, was instrumented in order to monitor the transport microenvironment. Temperature and relative humidity were measured using Vaisala HMP 31 UTA probes (Grant Instruments, Cambridge, UK), 2 per drawer, consisting of a thermistor head and a capacitive humidity sensor. The temperature sensor resolution was 0.1°C (accuracy ± 0.3%) and the relative humidity resolution was 1 per cent (accuracy ± 5%). All sensors were precalibrated and matched. The probes were connected to Grant Squirrel 1201 data loggers with 42K bytes of available memory. Probes were protected from direct contact with the birds by wire grid screens. Loggers were housed in waterproof containers and all connections were protected by rubber seals. Ambient temperature and relative humidity were monitored using an additional probe mounted rear-facing in a double concentric tube screen on the roof of the vehicle. This arrangement avoided spurious temperature measurement caused by heating of the probe by solar gain or by direct wetting of the probe in transit. When the vehicle was stationary the probe was ventilated by a small electric fan. Recordings of environmental data were made once per minute throughout the journey. The data were transferred from the data loggers to a portable computer (Epson PX4+ with PF10 disk drive) and were later transferred to a mainframe computer for more complex analysis. Relative humidities were transformed to water

vapour densities to facilitate comparisons of water vapour contents at different dry bulb temperatures. It is the gradient of water vapour density between a wetted surface (eg the skin or respiratory tract of a bird) and the air which determines the rate of evaporative heat loss.

Statistical analysis

Where appropriate, data are presented as the mean value ± one standard deviation of the mean. The statistical significance of differences was determined by analysis of variance or paired Students t-test.

Results

The results of the preliminary investigation to assess the effects of food and water deprivation and repeated blood sampling at intervals similar to those employed in transported broilers upon differential leucocyte counts and plasma CK activities are presented in Table 1. No significant difference between the pre- and post-'journey' values were observed in any of the leucocyte classes or in plasma enzyme activity.

Table 1 Differential leucocyte counts (%) and plasma creatine kinase activities (IU l^{-1}) in birds caged in pairs and deprived of food and water for periods corresponding to those experienced by transported broilers and blood sampled on two occasions at intervals corresponding to those in actual 'journeys'. Values are presented as means ± one standard deviation of the mean (n = 10).

	Heterophils	Eosinophils	Basophils	Lymphocytes	Monocytes	Heterophil/ Lymphocyte ratio	Plasma creatine kinase activity (IU/l^{-1})
Pre-'journey'	30.4±7.4	2.3±1.4	4.0±3.3	57.2±9.5	7.7±3.8	0.54±0.24	456.7±193.8
Post-'journey'	31.3±10.2	1.8±1.5	4.3±2.2	55.0±9.9	6.1±4.2	0.62±0.33	546.9±187.2

Details of broiler transport journeys in July and October, 1990 are presented in Tables 2 and 3. Journey times were relatively constant ranging from 209 to 218 min. Actual travel times were between 175 and 180 min and thus average speed varied little over the 4 journeys studied (Table 2). Ambient environmental conditions were similar within each two day period in July and October and thus the data are presented as the average values and ranges for each month (Table 3). There were, however, as might have been predicted, marked seasonal differences in external conditions. The range of temperatures and mean values were substantially higher in July than in October. The range of water vapour densities observed was wider in the summer month and the mean value slightly higher.

Table 2 Journey times and actual time in transit (travel time) with calculated average speed for each day studied.

Date	Journey time (min)	Travel time (min)	Average speed (km/h)
23/7	214	178	68.6
24/7	218	180	67.8
10/10	209	175	69.8
11/10	216	177	69.0

Table 3 Ambient environmental conditions during the journeys for each of the two day periods studied in each month. Values are based upon between 209 and 218 measurements per journey and are presented as ranges and means ± one standard deviation of the mean.

	July	October
Temperature range (°C)	15.4 - 23.5	6.5 - 12.9
Mean temperature (°C)	19.3 ± 1.1	8.8 ± 0.6
Vapour density range (g/m^3)	7.6 - 13.2	7.3 - 10.00
Mean vapour density (g/m^3)	9.4 ± 0.7	8.1 ± 0.3

The temperatures and humidities to which the birds were exposed in the transport microenvironment are presented in Table 4 and illustrate the effect of the differences in summer and winter vehicle configurations (side curtains off or on and closed). The 'on-board' temperature ranges and maxima (>30°C) were similar in each of the two months (Table 4) despite marked differences in ambient temperature (Table 3) and vapour densities were greatly elevated on the vehicle only in October, despite the relative constancy of external humidities in the two months.

The presence of a side curtain thus allowed the establishment of significant mean temperature and water vapour density gradients between crate and ambient conditions during the October runs of 13.4°C and 4.0 gm^{-3} compared to 3.9°C and 0.4 gm^{-3} in July in the open summer configuration.

The data describing the effects of transportation under these conditions upon the differential leucocyte counts are presented in Table 5.

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Table 4 **Environmental conditions within transport drawers during the journeys for each of the two day periods studied in each month. Values are based on between 209 and 218 measurements per journey and are presented as the range and means ± one standard deviation of the mean.**

	July	October
Temperature range (°C)	19.1 - 30.4	15.4 - 30.8
Mean temperature (°C)	23.2 ± 1.03	22.2 ± 2.7
Vapour density range (g/m^3)	7.4 - 17.0	7.6 - 26.5
Mean vapour density (g/m^3)	9.8 ± 0.91	13.0 ± 2.7

Table 5 **Differential leucocyte counts (%) and heterophil-lymphocyte ratios (H/L) in birds from each of the two day periods studied in each month. Values are presented as the mean ± one standard deviation of the mean (n=60).**

	July	October
Heterophils		
pre - transport	29.1 ± 12.3	32.1 ± 9.3
post - transport	46.1 ± 13.8	48.3 ± 10.6
Eosinophils		
pre - transport	2.3 ± 2.3	2.8 ± 2.4
post - transport	1.1 ± 1.6	1.3 ± 2.3
Basophils		
pre - transport	6.2 ± 3.7	6.7 ± 3.7
post - transport	4.2 ± 3.1	5.8 ± 3.7
Lymphocytes		
pre - transport	56.2 ± 14.0	50.7 ± 11.0
post - transport	39.6 ± 12.8	36.6 ± 10.2
Monocytes		
pre - transport	6.1 ± 3.7	7.7 ± 4.1
post- transport	9.2 ± 4.2	8.1 ± 3.9
Heterophil-lymphocyte ratios		
pre - transport	0.72 ± 0.57	0.74 ± 0.39
post - transport	1.56 ± 0.87	1.51 ± 0.77

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Comparison of pre-journey values indicated that relative lymphocyte numbers were a little lower in October than in July (10%; P<0.02) and monocytes were slightly elevated in the later month (21%; P<0.05). In general, however, the leucocyte profiles were very similar during each of the two periods examined. Transportation produced highly significant and common patterns of change in eosinophil, heterophil and lymphocyte counts in the two months and thus in heterophil-lymphocyte ratios. Heterophils were increased by 58 per cent (P<0.001) and 50 per cent (P<0.001) following transportation in July and October respectively. Concomitant decreases in lymphocyte counts of 29 per cent (P<0.001) and 28 per cent (P<0.001) were observed. The heterophil-lymphocyte ratios were therefore increased by 117 per cent (P<0.001) in July and by 104 per cent (P<0.001) in October. Eosinophil counts were decreased in each of the two months by 52 per cent (P<0.05) in July and 54 per cent (P<0.01) in October. In addition to these responses basophil count was decreased by transportation in July (32%; P<0.005) and relative monocyte numbers were elevated by 51 per cent (P<0.001).

The pre- and post-transportation activities of plasma creatine kinase are presented in Table 6. There was no significant difference between the pre-transport values in the two months. Transportation produced highly significant increases in plasma CK activity in both July (72.6%; P<0.001) and in October (97.4%; P<0.001).

Table 6 Plasma creatine kinase activity (IU/l^{-1}) in blood samples obtained pre- and post-transport in each of the two day periods in each month. Values are presented as the mean ± one standard deviation of the mean (n = 60).

	July	October
Pre-transport	507.4 ± 181.4	481.1 ± 179.5
Post-transport	875.8 ± 331.9	949.6 ± 277.1

Discussion

The primary objectives of the current study were to characterize physiological stress responses which may occur during the commercial transportation of broiler chickens and to relate these observations to some aspects of the transportation environment. By limiting the number of variables which might affect these parameters and by optimal standardization of the journey it is possible to reduce the variability in the biological response and thereby identify changes in the indices of physiological stress caused by the journey and the transport conditions. Before determining the effects of commercial transportation it was necessary to eliminate any possible influence of serial blood sampling and withdrawal of food and water, for the appropriate period, upon the chosen physiological indices of stress. The preliminary experiment clearly confirmed that these procedures did not alter the leucocyte profiles or plasma CK activities.

Differential responses in leucocyte classes to a variety of stressors have been demonstrated in a number of domestic animals (Stephens 1980). In birds heterophil-lymphocyte ratios have proved to be a particularly useful indicator of stress (Gross & Siegel 1983). The mechanism may be mediated by corticosterone (Jones *et al* 1988, Gray *et al* 1989, Gross 1990) producing demargination of cells bound to the capillary endothelium as suggested in other species (Phillips *et al* 1989, Bly et al 1990). It has also been demonstrated, however, that simulation of stress by injection of ACTH may additionally produce eosinopenia (Maxwell & Burns 1982) or monocytosis, eosinophilia and basophilia (Gray *et al* 1989). Most recently Maxwell *et al* (1990b) have reported a basophilia in response to stress caused by food restriction.

The data presented herein indicate that the pre-journey leucocyte profiles, as well as the subsequent responses to transportation, were very similar in the birds studied in each of the two periods. In each case increases in heterophil counts of at least 50 per cent, accompanied by approximately 30 per cent reductions in lymphocyte counts, resulted in a doubling of heterophil-lymphocyte ratio at the end of the journey. Another consistent response was the large reduction (>50%) in eosinophil count. These findings are consistent with physiological stress producing activation of the hypothalamo-adenohypophyseal adrenocortical axis and are in agreement with those of Freeman *et al* (1984) and Duncan (1989) who demonstrated increased plasma corticosterone concentrations in broiler birds subjected to small-scale, short-duration journeys.

The presence of this physiological stress may correlate with the results of behavioural analysis of poultry following transportation (Cashman *et al* 1989, Duncan 1989, Mills & Nicol 1990, Nicol & Scott 1990). These studies report increased fearfulness in birds, as assessed by prolonged tonic immobility, at the end of journeys.

The great similarity in the leucocyte responses observed in the two separate periods in July and October may be attributable to the efforts to minimize the number of uncontrolled variables. Constancy of journey durations (Table 2) and the route and location of the crates studied upon the vehicle may be factors. Another striking feature, however, is the relative constancy of the average internal thermal environment of the vehicle during the two seasons (Table 4) despite major differences in ambient conditions (Table 3). The presence of the curtains on the sides of the vehicle in the winter configuration reduces the removal of heat and water vapour by air movement and may also reduce direct convective cooling, of at least some of the birds.

Evaporative cooling is limited by increases in water vapour density (Smith 1972, Richards 1976) and maximal increases in respiratory frequencies may be achieved at temperatures as low as 27°C in saturated air (Kettlewell & Moran 1990). The maximum temperatures and vapour densities (>30°C and 17-26.5 gm^{-3}) observed in the crates during the journeys described herein would impose a substantial demand upon thermoregulatory mechanisms of birds in some parts of the vehicle and result in increased deep body temperatures (Mitchell *et al* 1990). The spatial and temporal distribution of such thermal loads within the vehicle will be the topic of a future publication.

In October a mean crate temperature of only 1°C lower than the corresponding July value, accompanied by an elevated vapour density, may indeed have imposed a greater thermal load upon the birds despite a much cooler external environment. High water vapour densities may have a profound influence on respiratory and transcutaneous evaporative heat loss (Mitchell *et al* 1990), particularly in a closed vehicle where dissipation of heat and water vapour may be minimal and behavioural thermoregulation may be compromised by high bird densities within the crates. In this context the observed significant increases in plasma CK activity (slightly greater in October) may reflect, at least in part, a response to hyperthermia. Plasma CK is elevated in a number of species following road transportation (Codazza *et al* 1974, Boss & McMurray 1979) including poultry (Scholtyssek & Ehinger 1976), although the changes in this latter study were much lower than those reported here. Plasma CK may be regarded as a specific and sensitive indicator of muscle cell damage (Lumeij *et al* 1988a, b) but the mechanism of its release during transportation awaits elucidation. A possible origin is overt muscle injury caused by handling, impacts in transit and unbalancing during static exercise against acceleration forces imposed by vehicle motion. Many broiler birds exhibit bruising on arrival at the processing plant (Scholtyssek & Ehinger 1976, Knowles & Broom 1990). Hyperthermia, however, also causes increases in plasma CK in chickens (Ostrowski-Meissner 1981) as well as in other species (Magazanik *et al* 1981).

It is thus clear that transportation of broiler birds under the commercial conditions defined in this study precipitates physiological stress. This is indicated by the effects upon differential leucocyte counts including heterophil-lymphocyte ratio. Concomitant elevations of plasma CK activity may reflect overt muscle damage in transit or changes in integrity of the muscle cell membrane brought about by unidentified stress mediators.

The thermal microenvironments to which birds are exposed in transit are thought to represent one of the sources of this 'transportation stress'.

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