**NOTE** *Physiology*

# Acute Phase Protein Response during Road Transportation and Lairage at a Slaughterhouse in Feedlot Beef Cattle

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ABSTRACT. The effects of transport for 5 hr by road and lairage for 48 hr on acute phase protein in Limousine feedlot beef cattle (n=10, 14–16 months old, body weight 620 ± 70 kg) were examined. Blood was collected at before loading, immediately after unloading and 12, 24 and 48 hr after the initiation of transportation. Serum was collected for assay of haptoglobin (Hp) and serum amyloid A (SAA), plasma was obtained for assay fibrinogen (Fb) and the white blood cell count (WBC) was determined in whole blood at each time point. A significant effect of experimental conditions on Hp, SAA and WBC was observed. In particular, this effect was found 24 hr after transport for Hp and SAA and after 48 hr for WBC. Application of a linear regression model showed a high correlation between WBC and Hp and SAA. Lairage in a slaughterhouse represents a stressful condition that can compromise animal welfare and meat quality. Monitoring of SAA with the highest sensitivity and specificity could be a useful marker of welfare condition in this period.

KEY WORDS: acute phase protein, cattle, lairage, transport.

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Road transportation of food animals is an inevitable husbandry practice that animals encounter in the livestock industry. It represents a critical phase in animal production and utilization and is often considered one of the main causes of stress, often associated with different forms of injuries sustained by the animals, and raises considerable interest both in economic and animal welfare terms. Also, transported animals are often exposed to a variety of physical and psychological stimuli that disrupt their homeostasis and metabolism and consequently increase the activity of enzymes and hormones [1].

Studies have been carried out to determine the optimum stocking density, the maximum duration of transportation, the timing of rest stops and which components of the transport process are the most stressful to cattle [8]. The stress may be more or less severe depending on a number of physical factors, such as noise or vibrations; psychological and/or emotional factors, such as unfamiliar environment or social regrouping; and climatic factors, such as temperature and humidity [23]. This often results into increased morbidity and mortality, poor meat and skin quantity and consequently a substantial economic loss [17]. Another important factor not be underestimated is the farmers' behavior during loading, unloading and lairage at the slaughterhouse. Compared with on-farm slaughter, handling associated with the journey to the slaughterhouse is more stressful to calves [12].

The welfare of the animals during transport and their response to handling and farmers' behavior toward calves should be assessed using a range of behavioral, physiological and carcass quality measures [4, 15]. But use of these indicators presents a large number of application problems due to the high variability of stressors [6]. Acute phase proteins (APPs) play a potential role as an indicator of stress response in calves [18, 21]. They are a group of negative and positive proteins whose serum concentrations decrease or increase, respectively, in response to challenge [10, 19] and are often undetectable in unstressed cattle [3]. Increased blood concentrations of APPs have been demonstrated in calves after exposure to different stressors such as housing on slippery floors [2], abrupt weaning [13] and mixing animals [3]. The findings reported in the literature concerning the effect of transportation on APPs in cattle vary. In particular, Arthington *et al.* [3] observed a higher average haptoglobin concentration in nontrasported calves than in transported calves. Earley and O'Riordan [9] had found an unchanged concentration of haptoglobin following transportation, while the plasma fibrinogen concentrations were significantly reduced. Buckham Sporer *et al.* [5] observed a reduction of haptoglobin and fibrinogen posttransportation. No effect of slaughterhouse on APPs has been reported.

Therefore, the objective of this study was to investigate the effects of truck transportation and slaughterhouse and to determine a pattern of positive serum concentrations of APPs that may aid in the early identification of stress conditions in Limousine feedlot beef cattle.

The study was carried out on 10 clinically healthy Limousine feedlot beef cattle between 14 and 16 months of age and with a mean body weight of 620 ± 70 kg. All animals were transported at the same time and covered a distance of about 450 km within 5 hr with an average speed of 80 km/hr,

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involving a combination of road surfaces ranging from small country lanes (5 km) to secondary roads (50 km) to motorways (395 km). Transport took place during spring, with an outside temperature of 18–20°C and 50–60% relative humidity. The truck was ventilated. The loading space of the truck gave a space allowance of about 1.62 $m^2$/head. At unloading the animals were lead by halter directly to tie stalls (1.4 m wide and 2.7 m. long) and tethered using a 70-cm chain. In the slaughterhouse, the cattle were kept with other cattle from the same farm, and no cattle from other farms were housed there. The environmental temperature in the stalls was between 18 and 25°C, and the relative humidity was 70–75%. The animals were fed hay and straw to guarantee rumination. Blood samples were obtained before transport (before loading), immediately after unloading and 12, 24 and 48 hr after transport. Blood samples were collected from each animal by jugular venipuncture and stored in three different types of vacutainer tubes: with no additive, containing 3.8 per cent sodium citrate (1 part citrate and 9 parts blood) and containing EDTA. Rectal temperatures and respiratory and heart rates were recorded before each blood sampling.

Determination of haptoglobin and serum amyloid A (SAA) concentrations was performed an enzyme-linked immunosorbent assay using ELISA kits (Tridelta Development, Ltd., Wicklow, Ireland). The concentration of fibrinogen was assessed in blood samples containing citrated sodium, after centrifugation, using a coagulometer (Clot 2S; SEAC). The white blood cell (WBC) count was assessed in blood samples containing EDTA using a multiparametric automatic analyzer (HecoVet; SEAC).

All the results were expressed as means ± SD. Data were normally distributed ($P$<0.05, Kolmogorov-Smirnov test). One-way repeated measures ANOVA was used to determine statistically significant effects of experimental conditions (before transport, after transport and 12, 24 and 48 hr after transport). A $P$ value < 0.05 was considered statistically significant. Bonferroni's multiple comparison test was applied for post hoc comparison. The data were analyzed using the STATISTICA 8 software (StatSoft, Inc.).

For individual values of WBC and APPs, a linear regression model (y=a+bx) was applied in order to determine the correlation degree between the studied parameters, and the correlation coefficient (r) was determined.

All animals remained clinically healthy and had normal rectal temperatures during the experimental period. The application of 1-way repeated measures ANOVA showed a significant effect of experimental conditions on Hp ($F_{(4,36)}$ = 53.14, $P$<0.0001), SAA ($F_{(4,36)}$=0229.40, $P$<0.0001) and WBC ($F_{(4,36)}$=5.79, $P$<0.001; Fig. 1). In particular, Hp showed a statistically significant increase after 24 hr with respect to before the start of transport and after 48 hr with respect to the other time points; SAA showed a statistically significant increase after 24 and 48 hr with respect to the

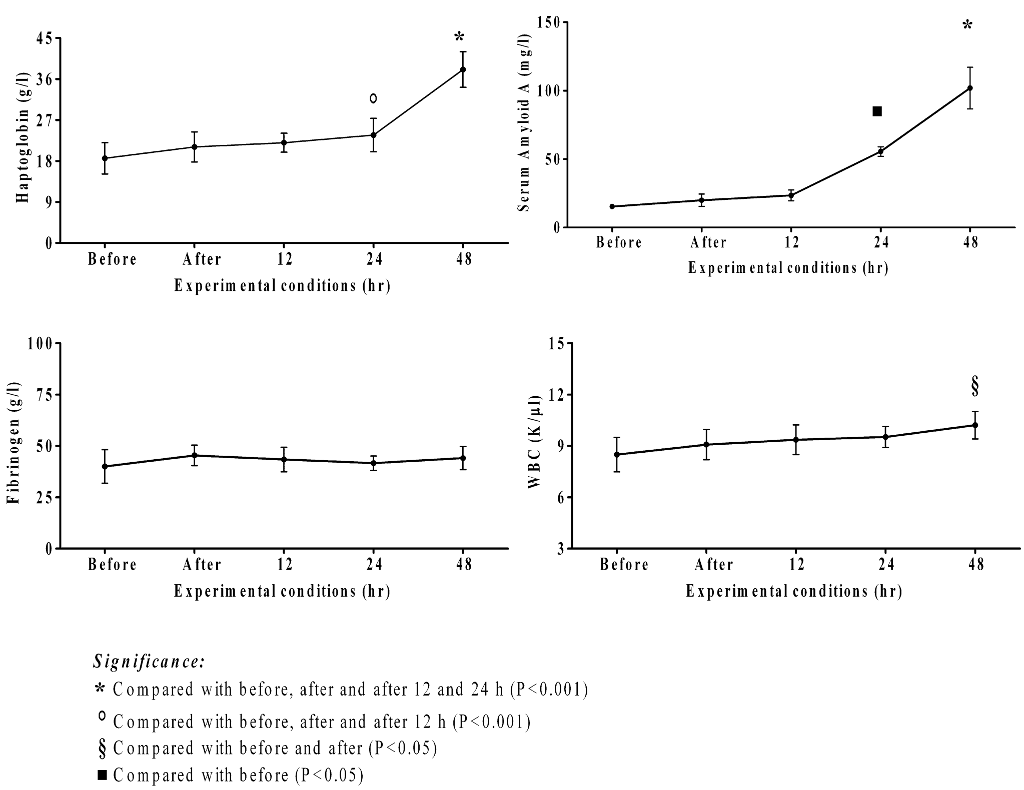


Fig. 1. Mean patterns ± standard deviation of acute phase proteins and white blood cells (WBC), with their statistical significance, observed during the experimental conditions.

other time points; WBC statistically increased after 48 hr with respect to before and after transport. Application of the linear regression model showed a correlation between individual values of WBC and Hp (r=0.66–0.87) and SAA (r=0.67–0.98).

Before using APP as objective and nonspecific markers of animal health, an important point to consider is the possible influence of environmental factors, handling and other types of stress in the absence of disease [20].

Transport of live vertebrate animals is regulated by strong existing legislation (Council Regulation EC No. 1/2005) to ensure that they have appropriate conditions that meet their needs. The key determinant of animal welfare is the way in which transport is conducted. Our results showed no statistically significant effect of experimental conditions after transport with respect to before transport. The stress response during loading and the initial stages of transport may be minimized by careful handling, good design of facilities and appropriate stocking densities and driving techniques [11].

At the slaughterhouse, the official veterinarian is to carry out an antemortem inspection of all animals before slaughter in accordance with Council Regulation EC No. 854/antemortem 2004. The antemortem inspection must in particular determine whether there is any sign that welfare has been compromised or of any condition which might adversely affect human or animal health. During lairage, contact between healthy and sick animals, promiscuity of different age and size animals, scarcity of water, inadequate ventilation and temperature can lead to contamination by biological agents, thirst, dehydration, agitation, anxiety, trauma, heat or cold stress. The direct effects of the stress factors (heat, anxiety, suffering, etc.), as well as the indirect effects, can be responsible for prepathological consequences that reduce animal welfare [22].

Our results showed a statistically significant increase in Hp 24 hr after transport with respect to before the start of transport and of SAA with respect to the previous data point, and 48 hr after transport, both parameters reached statistically significant higher values. Lomborg *et al.* [16] attributed the 48 hr after transport increase of Hp and SAA to an influence of complex stress: transportation, tie stall housing, slippery floors and social isolation.

Since it is likely that rates of synthesis or catabolism of virtually all plasma proteins are affected to some extent during the acute phase response, the precise definition of what constitutes an acute phase protein is somewhat uncertain. The rapidity of rise generally differs as well, and generally appears to vary with the magnitude of the response, starting from a serum level often undetectable in physiological conditions [3]. In particular, SAA begin to rise very rapidly soon after injury [14]. If we consider the rapid rise of APPs after stimulus, it is possible to exclude the influence of loading, 5 hr of road transport and unloading on APPs in cattle; whereas, lairage can be considered a stressful condition. The SAA response is generally stronger than that of Hp in the sense that the fold increase from the baseline levels is higher and occurs within 4 hr [20]. Other proteins often are observed to increase markedly within the first 48 hr after the triggering event and often have a rapid decline due to their very short half-lives [7]. Both Hp and SAA have been observed to have bacteriostatic and immunomodulatory effects [7], and their increases during the experimental period were correlated with the increase in WBC.

Until now, transport has been considered the major factor inducing stress influencing meat quality. Therefore, we can claim that lairage prior to antemortem inspection and slaughter represents a stressful condition in livestock that can compromise animal welfare and meat quality. In this period, monitoring of APPs, and in particular, monitoring of SAA with the highest sensitivity and specificity, could be a useful marker of welfare condition in lairage.

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