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Full Length Article

Hemato-biochemical profile of meat cattle submitted to different types of pre-loading handling and transport times

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ABSTRACT

Pre-loading handling and conditions of transport are related to welfare, disease risk and product quality of production animals. These steps continue to be one of the major animal management problems in Brazil. This study evaluated the effects of different types of pre-loading handling and road transport times on the haematological and biochemical traits of cattle. Eighteen male cattle were submitted to three travel times (24, 48 and 72 h) in a truck soon after load using different types of pre-loading handling: traditional (rough handling), training (gentle handling) and use of flags to movement cattle. Haematological traits, blood biochemical measures as well as blood and faecal cortisol were analysed in order to assess animal welfare and physiological status. The traditional management showed to be more stressful, also had animals with a greater number of neutrophils and lower numbers of lymphocytes than handling with flags, showing that animals submitted to more stressful situations can have compromised immune system. Serum aspartate aminotransferase concentrations were within the reference levels and when taken together with increased creatine kinase patterns observed indicate muscle damage in traditional management. Decrease in glucose concentrations over time from traditional management to flag management was observed, while fructosamine was increased in traditional management with 72 h of travel. When taken together, all reported factors, immune, enzymatic, energetic and hormonal, indicate that the quality of pre-loading handling and time of transport were determinant for animal welfare, its homeostatic balance and sanitary conditions.

1. Introduction

Pre-loading handling, transport and the time that the cattle travel to their final destination or slaughterhouse, represent critical stages in the production chain. These are some of the main causes of stress with negative repercussions on animal welfare, including the health of the cattle [1]. The option of gentle rather than aggressive management has a direct effect on homeostasis, leading to adaptive responses mediated by physiological processes aimed at avoiding the onset of stressful processes [2]. These responses act as indicators of animal welfare and are targeted for stress analysis [3].

In countries where beef cattle is raised extensively, the animal has the liberty to make choices, but also do not have much contact with human being. This can become a problem during pre-loading handling of these individuals, who can become reactive, stressed and dangerous, injuring themselves and others and bringing hazard not only to the

Abbreviations: ALB, albumin; AST, aspartate aminotransferase; CK, creatine kinase; Cort-FZ, faecal cortisol; Cort-SG, blood cortisol; ERT, erythrocyte; ALP, alkaline phosphatase; FLAG, flag handling; FrAm, fructosamine; GLI, glucose; Hb, haemoglobin; LEU, leukocytes; LINF, lymphocytes; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; NEU, neutrophils; PCV, packed cell volume; PLAQ, platelets; TPP, total plasma protein; TRAD, traditional management (rough handling); TRAIN, training management (gentle handling)

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handlers, but also for the whole group [4]. These animals are more susceptible to stressful situations, and it can be reflected in higher cortisol levels, decrease in feed intake, body weight gains, immune function and in agitation during simple routine husbandry procedures or even during transport [5]. Animals undergoing stressful conditions have their hypothalamic-pituitaryadrenal axis stimulated, culminating with the release of catecholamines, glucocorticoids and other hormones that may alter the blood biochemical and cellular composition, energy metabolism, and immune responsiveness [6] and therefore have a strong effect on meat characteristics [7].

Pre-loading handling, transportation and unloading are necessary and crucial steps in the production chain of cattle destined for slaughter and, therefore, the best conditions to reduce stress and promote the maintenance of good welfare of the animals must be observed, contributing to the quality of the final product. This study aimed to investigate the effects of different types of pre-loading handling and transport times on cellular and biochemical traits in the blood of beef cattle.

2. Material and methods

All animal use in this project have been approved by the appropriate ethics committee and have therefore been performed in accordance with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. The present study was approved by the Ethics Committee on Animal Use of the University of Brasília (CEUA-UnB), case n° 152862/2014.

The experiment was carried out in Planaltina-DF, Brazil (15.67° S, 47.58° W and 1240 m altitude) on Federal District roads, during the dry season, from May to July, with temperatures between 13 and 28 °C.

Eighteen male Nellore cattle, not castrated, aged between one and one and a half years were submitted to different handling and transportation times. The animals presented an average weight at the beginning of the experiment of 229.22 ± 5.32 kg. Three different pre-loading treatments were applied to the animals: 1) rough handling, called traditional management (TRAD), which consisted of the use of the methods commonly practised on beef cattle farms: rod with metallic stinger, whistles and shouts. 2) The training (gentle handling) management (TRAIN) consisted of the movement and loading of the cattle only with quiet voice commands and movement of the farmhand with respect to the animal's safety zone and stimulation to the movement of the animal group leader (this method was developed and executed in all of its extension by our scientific group members). This was obtained after training all animals with different commands four hours a day for a week. 3) Flag handling (FLAG) management consisted of the movement and loading of animals using a flag attached to a staff that was agitated to the extent necessary to control and move the animals. Between the application of one type of pre-loading handling and the next, the animals remained resting on grass pasture for a week. Prior to the beginning of the studies, the animals remained in the handling corral for 72 h. Tifton hay *ad libitum* and concentrated ration twice a day were offered. Prior to transportation, all animals were deprived of water and food for one to three hours before being loaded.

Cattle were submitted to one type of pre-loading management and, subsequently, travelled 24, 48 or 72 h in a latin square scheme. All animals were submitted to all treatments. Animals were rested in the corral with *ad libitum* water, hay and mineral salt, for the same duration as the previous transport. The journeys travelled were of approximately 200 km/day. Immediately prior to loading and subsequent to unloading, blood and faeces samples were taken from all individuals. Samples were obtained from puncture of the ventral coccygeal vein using vacuum tubes (Vacutainer®) with EDTA and without anticoagulant. After collection, the tubes were packed in a thermal box for further analysis. Packed Cell Volume (PCV) – obtained by the use of a microcentrifuge and erythrocyte count (ERT), leukocytes count (LEU), platelets count (PLAQ) and haemoglobin concentration (Hb) were performed in automatic counter (ABCVet-ABX®, Montpellier, France). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (HCM), and mean corpuscular haemoglobin concentration (MCHC) were determined by calculation. The differential leukocyte count was performed manually by a trained technician using an optical microscope and the total plasma protein (TPP) was measured using a refractometer.

For biochemical analyses, the serum was obtained from EDTA-free blood centrifuged at 2000 rpm for 5 min. Aspartate aminotransferase (AST), albumin (ALB), alkaline phosphatase (ALP), glucose (GLI), and creatine kinase (CK) were quantified using specific kits (LABTEST®, Lagoa Santa, MG, Brazil) by spectrophotometry in a semi-automatic biochemical analyser (Cobas C111, Roche®). Faeces were submitted to a cortisol extraction protocol as recommended by Palme and Möstl [8]. Extracted faeces and serum samples were then analysed for ELISA using commercial kits (Biochem®) and spectrophotometry (Biotek EL800 – Winooski, VT, USA).

Statistical analyses evaluated the effects of fixed factors (handling type, travel time, time of samples collection, loading and unloading) on variable factors (haematological, biochemical and cortisol parameters). The SAS program (v9.3, Cary, North Carolina, USA) was used for analysis of variance (PROC MIXED) with subsequent comparison of means by the Tukey test ($P < 0.05$) and for the data with non-parametric distribution PROC GLIMMIX used with subsequent application of the Dunn test. In addition, principal component analysis (PROC PRINCOMP) in order to investigate possibly correlation between traits measured.

3. Results

Haematological parameters measured are presented on Table 1. Higher levels of stress (TRAD and 72 h of transportation) presented higher ($P < 0.001$) PCV values (Table 1). There was a tendency for lower values of PCV with pre-loading handling with flags. The MCHC ($P < 0.05$) and Hb ($P < 0.001$) showed similar behaviour to PCV, with higher values for traditional handling at all transport times and with the trend of values decreasing for the management with flags, with higher Hb at the end of the 72 h of transport. There was a difference between load and unload (Fig. 1) for PCV ($P = 0.03$), erithrocytes ($P = 0.01$), MCHC (P = 0.02) and fructosamine ($P < 0.0001$). TRAD treatment showed higher number of NEU (4859.6) when compared ($P < 0.001$) with TRAIN (3807.3) and FLAG (2713.5). Lower lymphocytes (LINF) ($P < 0.001$) were seen in for TRAD (7580.9) when compared with FLAG (9008.7).

Biochemical parameters of cattle submitted to different types of pre-loading handling and transport time are on Table 2. When assessing biochemical measures, such as AST ($P < 0.05$) and CK, interactions were observed between the types of pre-loading handling and travel time (Table 2).

AST was shown for all cases within the reference values without showing a fixed pattern throughout the experiment. Particular cases such as unloading at 24 h of transport, after traditional handling (88.9 U/L) had the highest value and loading for 72 h of transport, in the flag handling (65.9 U/L) were the extreme and most highly different values. CK presented a different behaviour to AST, with higher concentrations when the animals were more stressed, such as when the animals were submitted to traditional handling at unloading after 48 h of transport (707.9 U/L) or when the animals remained longer (72 h) in the truck (TRAD – 671.6; TRAIN – 451.8 and FLAG – 705.8 U/L) (Table 2), with a tendency towards a decrease in concentrations, the highest very with traditional handling, intermediate values in training handling and lower values in flag handling (Fig. 2). All values found were higher than the reference values, which indicates that the movement of cattle in the corral or during transportation was reason enough to change the blood values of CK.

The amount of GLI present in the blood had a progressive decrease