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SHORT COMMUNICATION

Effects of selling through an auction market on physiological variables in beef calves

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ABSTRACT. In Chile, selling cattle through auction markets is common, and involves various stressful events. The objective of this study was to determine the effects of selling weaned beef calves through an auction market on indicators of physiological stress. Considering that transportation is invariably linked to any commercialization procedure, we compared the effects of exposing calves to transport and sale through an auction market versus transporting them directly between farms. Twenty calves of 175 (+/-37.5) kg live weight were transported from their origin farm to an auction market (2.5 h journey), where 10 calves were unloaded and exposed to regular handling within the market (unloading, sorting, regrouping, penning, sale yard showing) and thereafter loaded and transported back to the farm (TM). The remaining 10 calves were not unloaded at the market and were immediately transported back to the farm, simulating a direct sale (TD). Data collection (blood samples, body temperature, and live weight) was performed at the farm in both groups before loading (initial, 0 h), at the end of the process, after unloading TM calves back on the farm (after unloading, 12 h), and during recovery at 24, 48, and 72 h after the initial sampling. We used mixed-effects models to compare treatments and samplings. No differences were found between treatments in terms of live weight loss, maximum eye temperature, plasma β-hydroxybutyrate, and serum cortisol concentrations. The rectal temperature was higher ($p < 0.05$) in TM after treatment and remained higher ($p > 0.05$) in the samples than in TD. Calves that passed through the market showed higher ($p < 0.05$) plasma CK activity after unloading (12 h) than did those that did not. The mean glucose concentration increased ($p < 0.05$) at 12h and remained higher than the initial value (0 h) in both the calf groups. This was a preliminary study, and according to the results, experiments should be repeated to test more animals under different situations and premises.

Keywords: cattle, transport, fasting, marketing, stress indicators, welfare.

INTRODUCTION

The marketing process is stressful for calves because they are taken away from their environment and often weaned immediately before loading, transported, and unloaded in a new environment. In addition, during marketing, cattle are deprived of food and water, usually from the moment they are herded before being transported, until they arrive at their destination. Selling animals through auctions also increases their handling and exposes them to a greater number of interactions with humans (Weeks *et al.*, 2002; Gregory *et al.*, 2009).

As in many other Latin American countries, selling animals through livestock auction markets is still common in Chile, particularly for calves sold for backgrounding purposes (Gallo & Tadich, 2008). In 2020, 900,185 cattle were auctioned in Chilean markets, 33% of which were calves (INE, 2021). Studies conducted in Chilean auction markets using behavioral indicators have shown that calf welfare is negatively affected, mostly associated with poor handling by market staff (De Vries, 2011; Bravo *et al.*, 2019, 2020).

The objective of this study was to determine the effects of selling weaned beef calves through an auction market on physiological variables indicators of stress. The hypothesis states that the mean values of physiological indicators of stress would be higher in calves passing through the auction market than in those sold directly.

MATERIALS AND METHODS

Animals and study design

Twenty Angus calves (black and red) of both sexes (15 females and 5 males), weaned a month before the experiment, with an approximate age of 7 (± 1) months, weighing 175 kg (± 37.5), and clinically healthy were chosen for the study. All calves were born and raised on the same farm where the experiments were conducted. They were kept on pasture during the day, with *ad libitum* access to water. During the night, calves were placed in a barn and received 1 kg/head of sugar beet pulp pellets (87% dry matter and 15% protein) and hay (*Lolium perenne*) *ad libitum*. The experiment was conducted in southern Chile during winter (2-10°C and 85-95% humidity during the experimental period).

The calves were divided according to sex and weight into two homogeneous groups. They were loaded and transported in the same truck to an auction market for 2.5 hours.

Each group was placed in a separate pen with a similar space allowance within the truck (approximately 1 m^2 per 270 kg). One group was unloaded at the market and exposed to all regular handlings within the market (TM), including classifying, penning, auctioning, re-penning, and loading, to be transported back to the farm of origin. The other group was not unloaded at the market and was immediately transported back to the farm of origin, simulating a direct sale between farms (TD). At the farm, the TD group was unloaded and maintained in a pen without food or water until the TM group arrived to preserve the same fasting time in both groups. The total fasting time was 12 h, including 5 h of transportation to and from the auction market.

Data collection

Blood samples were taken from all calves five times during the experiment, while restrained in a chute at the farm, by coccygeal venipuncture using a Vacutainer® (needle 20G x 1"). Initial sampling (0h) was performed before loading the calves for transport in both groups. The next sampling also took place for both groups simultaneously, coinciding with the arrival and unloading of the calves that passed through the auction market 12 h after the initial sampling. This sampling (12 h) included the possible effects of 5 h of total transportation time (to the auction market and back to the farm in both groups), plus fasting for 7 h and exposure to several handling procedures during marketing in TM, including one extra loading and unloading. Further sampling was performed during the recovery period of both groups of calves at 24, 48, and 72 h after the initial sampling. Body temperature was measured, and the live weight of the calves was recorded at each sampling time.

Blood variables

Three collection tubes were used during blood sampling: a tube without additives was used for measurements of cortisol serum concentrations, and a tube with EDTA was used to measure creatine kinase activity (CK) and the concentration of β-hydroxybutyrate (β-HB). A third collection tube containing sodium fluoride (NaF) was used for glucose measurements. All blood samples were immediately placed on ice and transported to the Veterinary Clinical Pathology Laboratory of Universidad Austral de Chile. Frozen serum samples were sent to PetLab Laboratory, located in Santiago, Chile, and cortisol concentrations were determined using chemiluminescent immunoassay (CLIA). Plasma glucose concentration was determined using the GOD-PAP test. The plasma concentrations of β-HB were determined by an enzymatic technique using 3-hydroxybutyrate dehydrogenase, and plasma CK activity was measured using the IFCC and ECCLS kinetic methods.

Body temperature

The Maximum Eye Temperature was obtained by capturing infrared images of the left eye at approximately 0.5 m distance (90 ° angle from the individual) using a thermal camera (FLIR i5, FLIR Systems, Wilsonville, OR, USA). The camera was calibrated with an emissivity of 0.95, according to the information provided by the manufacturer. Image analysis was performed using FLIR Tools 3.1 (FLIR Systems, Wilsonville, OR, USA). The atmospheric temperature and relative humidity were included in the calculations. The rectal temperature was measured after blood samples were collected using a digital thermometer.

Live weight

All calves were individually weighed at all samplings, after all other measurements were obtained, using a mechanical cattle scale (Romana, 0.5–1000 kg). To control for weight differences between animals, the percentage of weight variation in relation to the initial weight before loading was estimated for each animal weighed during each sampling period.

Data analysis

For descriptive analyses, variables were shown as mean and standard error (SE) using Microsoft Excel 2016. To assess the effect of direct sale (TD) or sale through an auction market (TM) on the dependent variables, mixed-effect models were built, including treatment and sampling time as fixed effects and calf as a random effect. Data were analysed using the "lme4" statistical package and multiple comparisons were explored using a Tukey's adjustment included in the "lsmeans" function in R Studio software version 3.2.2; $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

To the best of our knowledge, this is the first study to measure physiological variables related to stress in calves in a real commercial situation after transporting and passing through an auction market. To date, studies have mainly reported descriptive conditions in the markets related to behavioral indicators of animals, handling by stockpersons, and infrastructure (Weeks *et al*., 2002; Gregory *et al*., 2009; Romero *et al*., 2017; Bravo *et al*., 2019). In this study, both groups had equal transport and fasting times. However, the potential stress associated with regular handling during the marketing process is present only in calves that pass through the market.

Calves that passed through the market showed higher plasma CK activity after unloading (12 h) than those that did not ($p < 0.05$; Table 1). CK activity has been shown to increase in steers transported (3 or 16 h) as well as in steers confined to a pen for the same time (Tadich *et al*., 2003). Bravo *et al.* (2018) also reported increases in CK concentrations in calves transported for 3 h. Increases are related to exercise and/or muscle damage caused by fighting, mounting, and bruising (Knowles *et al*., 2014). Therefore, the higher CK activity in TM calves could be due to handling and movement within the market premises, likely mixing and interacting with other cattle, and hitting themselves with the infrastructure (Weeks *et al*., 2002; Gregory *et al*., 2009;

Romero *et al*., 2017; Bravo *et al*., 2019). All mean CK activity values were higher than reference values (Wittwer, 2012). Interestingly, the mean CK activities in calves transported directly to the farm were significantly lower ($p < 0.05$) at 48 and 72 h than those initially (0 h). This could indicate that CK values were already high at the start of the experiment, probably because of herding and sampling, which has also been found in other experiments with calves of similar age kept under extensive conditions and sampled before loading for transport (Werner *et al*., 2013).

When comparing plasma glucose concentrations at different sampling times, an increase was found between 0 and 12 h in both groups of calves ($p < 0.05$), without differences between treatments (Table 1). In some cases, the concentrations were even higher than the normal reference values for the specie (2.5-4.1 mmol/L; Wittwer, 2021). The before-loading concentrations (0 h) recovered 72 h after loading in both groups, with non-significant interactions between groups and sampling times. Increases in blood glucose concentrations have been previously reported in calves and steers after transport and fasting owing to confinement (Tadich *et al*., 2003; Earley *et al*., 2006; Werner *et al*., 2013; Bravo *et al*., 2018). The increase in the glucose concentration observed in the present study may be related to the primary response to stress (Knowles *et al*., 2014).

Mean cortisol concentrations did not differ between

Table 1.
Means and standard errors (±SE) of the blood variables related to stress in only transported (TD) and transported and marketed (TM) calves at different sampling times (Initial, After Unloading (AU), and during recovery at 24, 48, and 72 h after the initial sampling).

Sampling times	CK (U/L)			Glucose (mmol/L)			β-HB (mmol/L)			Cortisol µg/dL		
	TD	TM	p	TD	TM	p	TD	TM	p	TD	TM	p
Initial 0h	302±31^{a}	245±12^{a}	ns	3.6±0.1^{a}	3.4±0.1^{a}	ns	0.28 ±0.04a,b	0.19±0.02a	ns	1.5±0.2^{a,b}	1.4±0.2^{a,b}	ns
AU 12h	298±37^{a}	442±54^{b}	*	4.3±0.2^{b}	4.1±0.1^{b,e}	ns	0.19±0.02a	0.27±0.04a	ns	2.0±0.4^{a,b}	2.4±0.3^{b,c}	ns
Recov 24h	220±17^{a,b}	288±25^{a}	ns	4.4±0.2^{b}	4.4±0.1^{b,c}	ns	0.36±0.05b	0.22±0.03a	ns	2.4±0.5^{b,c}	1.6±0.3^{a,b}	ns
Recov 48h	169±29^{b}	164±08^{c}	ns	4.1±0.1^{b,c}	3.9±0.1^{d,e}	ns	0.18±0.02a	0.20±0.02a	ns	1.4±0.2^{a,b}	1.6±0.2^{a,b}	ns
Recov 72h	146±11^{b}	163±20^{c}	ns	3.9±0.1^{a,c}	3.6±0.1^{a,d}	ns	0.19±0.02a	0.18±0.03a	ns	1.3±0.3^{a,d}	1.1±0.1^{a,d}	ns

Different letters indicate statistically significant differences ($p < 0.05$) among the sampling times within each group. (*) indicates statistical differences ($p < 0.05$), whereas (ns) represents non-significant differences between the TD and TM groups. CK = creatin phosphokinase; β-HB, beta-hydroxybutyrate.

treatments across time (Table 1) and were mostly within the normal range (0.3-2.0 µg/dL; DCPAH, 2015), except for after unloading in TM calves and after 24 h recovery in TD calves, which were slightly above the normal range, reaching a mean of 2.4 µg/dL. These results agree with those of Bravo *et al*. (2018), who found non-significant increases in cortisol concentrations in calves due to 3 h transport and 24 h fasting afterwards in a pen, simulating commercial conditions. However, a higher concentration of cortisol was found in TM calves after unloading (12 h) than in animals in the same group at 72 h ($p < 0.05$), and a higher cortisol concentration at 24 h than at 72 h was found in the TD group ($p < 0.05$), indicating that there was an increase in cortisol due to transport and fasting, which tended to decrease during recovery.

The mean concentrations of β-HB were within the normal range (Wittwer, 2012) and did not differ between the treatments at any sampling time ($p > 0.05$; Table 1). Increases in β-HB concentrations indicate that animals use their body reserves due to fasting and/or exercise (Broom, 2003; Knowles *et al*., 2014). According to our results, fasting calves for 12 h, which included a total transport time of 5 h (total duration of the journeys to the market and back), plus 7 h of feed and water deprivation in the market (TM) or in a corral on the farm (TD), was not sufficient to make them use their body reserves. After 24 h of fasting (including 3 h

of transport), Bravo *et al.* (2018) found a significant increase in β-HB in calves, although the mean values were still within the normal ranges (0.1-0.6 mmol/L). It needs to be considered that the most common situation within markets is that animals remain in the premises for longer times (Bravo *et al*., 2019, 2020) than it was the case in the present study.

The average live weight of the calves was reduced by 6.5% between 0 and 12 h, without statistical differences between the TD and TM groups ($p > 0.05$, Table 2). This live weight loss is similar to the 6.8% reported earlier in calves by Bravo *et al.* (2018) after 3h of transportation and a total of 24 h of fasting in a pen without passing through a market. Live weight loss is probably the most significant economic effect associated with marketing procedures since animals are traded based on this variable (Bravo *et al*., 2019, 2020). In our study, live weight was not significantly affected by passing through the market; even when both groups were transported under the same conditions and exposed to the same fasting time, TM calves showed proportionately greater weight loss than TD calves at the final sampling (12 h) (7.5 vs. 5.7%). The initial weight before loading (0 h) recovered at 48 h in both treatments and a tendency to gain weight was observed at 72 h. It is likely that the differences in weight loss were not significant due to the short time spent in the market, the small sample size of calves, and their high weight dispersion. Therefore, experiments with more animals and less weight dispersion should be conducted, as well as replicate studies in different markets under various conditions.

Rectal temperature mean values throughout the study remained within normal rectal temperature ranges, from 38.6 to 39.4°C (University of Glasgow, 2013), but after unloading (12 h) values were higher in TM calves than in TD calves, showing that passing through the auction market increased rectal temperature ($p < 0.05$, Table 2). This result agrees with the increase in body temperature due to stress and physical exercise during marketing (King, 2004; Oka *et al*., 2001). In addition, the mean rectal temperature in TD calves was higher at 72 h than after unloading ($p < 0.05$), and in the TM group, the rectal temperature was also higher after 72 h than the initial value ($p < 0.05$). Considering that there were no calves with fever or other clinical signs of disease, this could reflect that repeated handling during daily sampling also caused some stress in the animals. An increase in tympanic temperature and maximum eye temperature associated with handling and transportation has been previously reported in beef calves transported for 3 h and fasted for 24 h without feed or water (Bravo *et al*., 2018); however, in the present study, maximum eye temperatures did not vary between treatments or sampling times (Table 2).

In a study under commercial conditions such as ours, it was difficult to standardize the different factors that could increase stress and affect the welfare of calves sold in commercial situations. Many factors could have influenced the results; therefore, few differences were found between

Table 2.
Means and standard errors (±SE) of the blood variables related to stress in only transported (TD) and transported and marketed (TM) calves at different sampling times (Initial, After Unloading (AU), and during recovery at 24, 48, and 72 h after the initial sampling).

Sampling times	Live weight (kg)			Rectal temp (°C)			Maximum Eye Temp (°C)		
	TD	TM	p	TD	TM	p	TD	TM	p
Initial 0h	176±12	173±12	ns	38.5±0.1^{a,b}	38.5±0.1^{a,b}	ns	34.4±0.3^{a}	34.3±0.3^{a}	ns
AU 12h	166±11^{a}	160±11^{a}	ns	38.4±0.1^{b,c}	38.9±0.1^{a,c}	*	34.3±0.2^{a}	34.4±0.3^{a}	ns
Recov 24h	171±11^{b}	167±12^{b}	ns	38.4±0.1^{a,b}	38.6±0.1^{a,c}	ns	33.7±0.3^{a}	33.3±0.3^{a}	ns
Recov 48h	177±12^{c}	172±12^{c}	ns	38.8±0.1^{a,b}	38.9±0.1^{a,c}	ns	34.0±0.2^{a}	34.2±0.2^{a}	ns
Recov 72h	181±12^{c}	175±13^{c}	ns	38.8±0.1^{a,d}	39.1±0.1^{c,d}	ns	33.7±0.4^{a}	34.2±0.3^{a}	ns

Different letters indicate statistically significant differences ($p < 0.05$) among the sampling times within each group. (*) indicates statistical differences ($p < 0.05$), whereas (ns) represents non-significant differences between the TD and TD groups. Letters next to the live weight values represent statistical analyses comparing the variation from the initial sampling time to other measurements in both groups. CK = creatin phosphokinase; β-HB, beta-hydroxybutyrate.

calves transported directly from farm to farm and those passing through the market. For instance, the time spent in the market by TM calves was less than the regular 12 hours previously described by Bravo *et al*. (2020). Moreover, the authors (although not measured) observed that handling by stockpersons was mostly gentle, with low stress and no rush, as there were few animals for sale. This is uncommon in Chilean livestock markets (Bravo *et al*., 2019; De Vries, 2011). This also suggests that it is possible that selling calves through the market does not significantly affect the physiological variables compared to a direct sale from farm to farm if transport and auctioning times are kept short and handling within the market is less stressful.

The limitations of this study are the small number of animals used and the absence of blood sampling of animals upon arrival at the market and after their stay on the market before loading again. Unfortunately, it is not allowed to perform any sampling or treatment within the premises; moreover, this would have meant extra handling and stress for the calves, a situation that usually does not occur within the market. Therefore, these should be considered preliminary results with a small number of calves, and experiments should be repeated with more animals in different markets and situations.

CONCLUSIONS

Contrary to expectations, we can conclude that under the conditions of our study, weaned beef calves that passed through the auction market exhibited higher final CK activity and rectal temperatures than those transported directly from farm to farm. However, these findings should be considered preliminary because of the small number of calves involved and the relatively short exposure time to the handling conditions of a livestock auction market (7 h), which are not representative of typical scenarios. Experiments should be repeated to test more animals under different conditions and handling conditions.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Ethics Statement

The Bioethics Committee "Use of animals in research" of the Universidad Austral de Chile, approved the present study (Approval N°325/2018).

Author Contributions

Conceptualization VB, CG; Data curation VB; Formal analysis VB, GAJ; Funding acquisition VB, CG; Investigation VB; Methodology VB, CG; Project administration VB, CG; Resources and software VB, CG, GAJ; Supervision CG; Validation and Visualization CG, GAJ; Writing – review and editing VB, GAJ, CG.

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