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COMMUNICATION

A note on the effects of pre-slaughter operations of llamas (*Lama glama*) on the concentrations of some blood constituents related to stress and carcass quality

Una nota sobre el efecto de las operaciones presacrificio en llamas (*Lama glama*) sobre las concentraciones de algunos componentes sanguíneos relacionados al estrés y la calidad de la canal

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RESUMEN

El objetivo de este trabajo fue evaluar el efecto de las operaciones presacrificio sobre indicadores fisiológicos de estrés (constituyentes sanguíneos) y de calidad de la canal (pH y contusiones) en llamas bajo condiciones comerciales. Treinta llamas criadas a pastoreo, de 18-24 meses de edad y peso vivo 54,2±6,4 kg fueron transportadas durante 3 h en un vehículo a matadero. Se tomaron muestras de sangre a las llamas en predio una hora antes de cargar, después de descargar, después del reposo en matadero (18 h) y durante la exsanguinación. Se encontró un aumento significativo (P < 0,05) de cortisol después del transporte (16,9 ng/mL), como también de actividad de CK después del reposo (528,4 UI/L); la concentración de β-hidroxibutirato se mantuvo en los muestreos. Se registraron 19 contusiones en 9 de las 30 canales de llamas. Las lesiones superficiales, pequeñas y de color rojo brillante, fueron las más frecuentes. La región del lomo presentó más contusiones (57,89%), seguida del tórax (21,05%). Las lesiones de forma irregular fueron más frecuentes (81,25%), seguidas de circulares (18,75%). Se concluyó que las operaciones presacrificio de llamas bajo las condiciones comerciales del estudio produjeron cambios fisiológicos similares a los observados en otras especies, que caen dentro de límites aceptables para su bienestar; sin embargo, el estrés y los efectos adversos en las canales, como las contusiones, podrían minimizarse mediante diseño de estructuras apropiadas para el manejo, aplicación de las recomendaciones de OIE para el bienestar durante el sacrificio y capacitación del personal que maneja las llamas a lo largo de la cadena cárnica en Bolivia.

Palabras clave: operaciones presacrificio, constituyentes sanguíneos, contusiones, *Lama glama.*

SUMMARY

The objective of the current study was to assess the effects of pre-slaughter operations in llamas on physiological (concentrations of some blood constituents) and carcass quality (pH and bruises) indicators under commercial conditions. Thirty llamas raised on pasture, 18-24 months old and average live weight of 54.2±6.4 kg were transported on a single 3 h journey in one batch to the slaughterhouse. Blood samples were taken on farm one hour before loading, after unloading, after lairage (18 h) and during exsanguination to measure the concentrations of various stress-related variables. Mean values for the blood variables showed a significant rise (P < 0.05) in the concentration of cortisol immediately after transport (16.9 ng/mL), as well as CK activity after lairage (528.4 UI/L); β-hydroxybutyrate concentration showed similar mean values for the different sampling times. A total of 19 bruises were found on 9 of the carcasses. The backs (loin) of carcasses had more bruises (57.89%), followed by thorax (21.05%). Irregularly shaped bruises were the most frequent (81.25%), followed by circular bruises (18.75%). It was concluded that pre-slaughter handling of llamas under these commercial conditions produced physiological changes similar to those in other species, which fall within acceptable limits for their welfare; however stress could be reduced and adverse effects like bruises could be minimized by designing proper facilities, by following OIE recommendations on the welfare of animals during stunning and by training of personnel handling llamas all along the Bolivian meat chain.

Key words: road transport, pre-slaughter operations, blood constituents, bruising, *Lama glama.*

INTRODUCTION

According to Cristofanelli *et al* (2005), of all the South American camelids the llama has potential merits as a source of meat in the Andean highlands, producing carcasses that are significantly larger than those from alpacas. The population of llamas in Bolivia is 2,486,169, representing over 60% of the world population (INE 2008). Although conventional llama production systems are based on native grazing feeding and llamas are adapted to areas where coarse forage is available, Mamani-Linares and Gallo (2013[a]) showed that supplementation of young llamas grazing native pasture with concentrate can lead to greater live weight, greater carcass weight, greater fat deposits and improved the carcass characteristics, supporting the idea that it is a good alternative in the production of llama meat, especially during the dry season when there is poor pasture availability. Moreover, llama meat can be considered a healthy food as it is characterised by a relatively low cholesterol (39.04 mg/100g) and intramuscular fat content (1.56%), and a high ratio of

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protein to fat, with an adequate amino acid composition (Mamani-Linares and Gallo 2013[b]). While there is substantial study on the effects of handling and transportation of cattle, horse, pig, sheep, goat and various wildlife species (Grandin 1997, Tadich *et al* 2009, Miranda-de la Lama *et al* 2012), no work has been carried out to assess the effects of pre-slaughter operations in llamas.

Sale and slaughter are the most common reasons for transporting llamas in Bolivia and transport time varies depending on the distance from farm to slaughterhouse. Transportation is an inevitable husbandry practice which is often considered as one of the main causes of stress, raising interest regarding animal welfare standpoints and economic losses, as it reduces carcass weight, increases bruises and negatively affects muscle pH (Gallo *et al* 2000, 2003). During transport, animals are exposed to a variety of potential stressors such as handling by humans, gathering, loading and unloading, motion of the vehicle, noise, vibrations, centrifugal forces, heat or cold, poor road conditions and lack of water and feed (Hartung 2003). These stressors may lead to some changes in blood constituents such as plasma concentration of glucose, cortisol, creatine kinase (CK), lactate dehydrogenase and packed cell volume (Knowles and Warriss 2007). Bruises can occur at any point of the meat chain, due to inappropriate handling of the animal during the pre-slaughter period. Bruises are indicative of violence and pain suffered by the animals which is related to poor welfare conditions during pre-slaughter period (Strappini *et al* 2009). Moreover, bruises in bovine carcasses affect the quality of the carcass and the meat. Another reason for downgrading carcasses is the presence of dark, firm and dry meat. Presence of bruises is also significantly associated with increased carcass pH values (Strappini *et al* 2010). The OIE (2012) has issued standards for animal welfare during terrestrial transport and during slaughter that all country members should follow; therefore it is important to produce baseline information for llamas in this regard. The objective of the current study was to assess the effects of pre-slaughter operations in llamas on physiological (concentrations of some blood constituents) and carcass quality (pH and bruises) indicators under commercial conditions.

MATERIAL AND METHODS

ANIMALS AND PRE-SLAUGHTER CONDITIONS

A total of 30 entire male llamas of the Kh'ara genotype were used. The animals were 18-24 months old, fed on native grass pasture, originated from one producer in the Bolivian Altiplano and were ready for slaughter. The study was carried out between September and November 2011. The day before transport to slaughter, the llamas had been gathered from the fields and led to a pen for overnight keeping (usual procedure), where they had access to barley hay.

The animals were loaded into an open roof small truck, with wooden flooring and no bedding, with a single compartment, using the usual procedures. First, the truck was parked against a natural embankment; then llamas were driven out of the corral (figure 1a) towards the back of the truck using a "human fence" which was gradually closed forcing the animals to be loaded onto the truck (figure 1b). The transport involved using a combination of road types ranging from small country lanes (10 km stone road) to secondary roads (170 km asphalt) on a single 3 h journey at a stocking density of approximately 110 kg/m^2 (0.55 m^2 per animal, figure 1c), over a distance of about 180 km with an average speed of 60 km/h. On arrival at the slaughterhouse the llamas were unloaded by again parking the truck against a natural embankment close to the lairage pens and letting them "jump" out of the truck after opening the hind door (no unloading ramp was available, figure 1d); then they were driven to the lairage pen using again a human fence. The llamas remained in lairage for 18 h approximately (figure 1e), and were then taken one by one to the slaughter point and slaughtered using the puntilla (figure 1f).

BLOOD SAMPLING AND ANALYSES

Blood samples were collected directly into 10 mL vacuum tubes (Vacutainer, Beckton Dickinson) via jugular venipuncture at 4 sampling times: one hour before loading of the llamas, immediately after unloading, after lairage and during exsanguination (at bleeding). Llamas were restrained manually in the pen by one person who placed one hand on the anterior-medial part of the neck and the other one behind the leg, pressing the llama with his body and knee, as shown in figure 1e. Blood samples were left to coagulate for about 12 h at room temperature; then serum was separated and frozen for further analysis. Serum cortisol concentration was determined by radioimmunoassay (RIA), using a commercial kit (Cortisol Coat-A-Count, DPC, USA); CK activity was measured by the UV-kinetic method at 340 nm and 37 °C (Art. 12015 HUMAN) using a Kobas Mira Plus spectrophotometer (HITACHI 4020, Roche®); βOHB values were determined using an enzymatic technique based on 3-hydroxybutyrate-dehydrogenase enzyme in a spectrophotometer (HITACHI 4020, Roche®).

CARCASS INDICATORS

The live weights of the animals were taken on the farm before loading. The animals were slaughtered following the usual commercial procedures in Bolivia; the hot carcasses were weighed and the external fat finishing score visually appraised (5 = extremely abundant, 4 = abundant, 3 = medium 2 = slight, 1 = scarce). The muscle pH (1 and 24 h post-mortem) was measured in the *Longissimus thoracis* muscle at the level of the 1st lumbar vertebrae, using a portable Hanna HI99163 pH meter, and following manufacturer instructions (HANNA Instruments®, USA).

Figure 1. Pre-slaughter handling of llamas. (a) On farm, before the first blood sampling; (b) Loading; (c) Transport; (d) Unloading; (e) During lairage (showing also handling for blood sampling); (f) Slaughter/stunning using puntilla.

Manejo antemortem de las llamas: (a) en el predio, antes del primer muestreo de sangre; (b) carga; (c) transporte; (d) descarga; (e) en corrales de reposo en matadero (incluido el manejo durante el muestreo de sangre); (f) insensibilización/sacrificio usando puntilla española.

For each carcass, the presence of bruises (yes or no) was recorded. If bruises were present, the number of bruises per carcass and per anatomical site was assessed. Each bruise present on the llama carcasses was evaluated by registering its anatomical site, size, shape and colour adapting the protocol used by Strappini (2010) for cattle. The anatomical site of the bruise was recorded dividing the carcass into eight sites: leg, abdomen, thorax, shoulder, loin, neck, ischial tuberosity, coxal tuberosity (figure 2). The size of each bruise was assessed on the basis of its diameter according to the ACBSS: small: ≥ 2 to ≤ 8 cm; medium: 8 to ≤ 16 cm; large: > 16 cm. The severity of the bruises was scored by the observer according to the Chilean carcass grading classification (INN 2002) as grade 1 (only subcutaneous tissue affected); grade 2 (as grade 1, but with muscle tissue affected); grade 3 (as grades 1 and 2, plus presence of fracture).

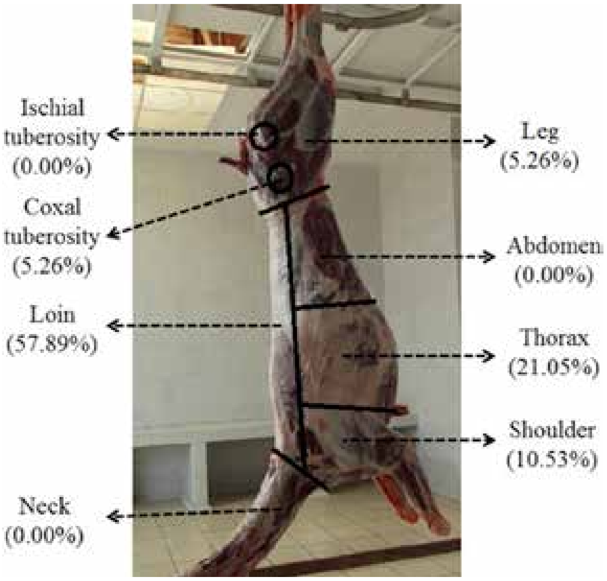

Figure 2. Distribution of bruises according to anatomical location in llama carcasses.

Distribución de los hematomas según su ubicación anatómica en las canales de las llamas.

ANALYTICAL METHODS

Descriptive statistics was used, presenting weights at slaughter and carcass pH data as the mean±standard deviations, characteristics and anatomical location of bruises in percentage. The mean values of the blood constituents at each sampling time were analysed by means of an analysis of variance (ANOVA) using a GLM model. When significant differences were detected with the ANOVA analysis, the differences between the mean values of each sampling time were analysed by Tukey's test. For all the analyses the Statistix version 8.0 for Windows was used (Statistix 8, Copyright© 1985-2003, Analytical Software, USA) and a $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

BLOOD CONSTITUENTS

The mean concentrations obtained for each blood variable in the different sampling times are shown in table 1. Sampling time ($P < 0.01$) significantly affected cortisol concentration and activity of creatine kinase (CK, table 1). The cortisol showed a significant rise ($P < 0.05$) in its concentration immediately after transport (16.9 ng/mL). In agreement with the current result, Anderson *et al* (1999) and Zapata *et al* (2004) also reported a significant increase in plasma cortisol concentration due to transportation stress in guanacos (37.3 nmol L^{-1}), finding that values returned to baseline 2 h post-transport (25.4 nmol L^{-1}). Kannan *et al* (2000) found that cortisol values in goats decreased to the baseline level at 1 h after transportation, remained at that level during 18 h and increased again at 18 h probably due to feed deprivation stress. According to Tadich *et al* (2009) the increased cortisol concentration in lambs after transport is likely to be due to the stress of unloading, handling and bleeding procedure, more than to transport itself, especially considering that from the start of the unloading to the time of bleeding there was a 30 minute interval, as in this case also.

Plasma cortisol level is a reliable indicator of stress experienced by an animal (Gregory 1998). Broom *et al* (1996), who investigated the physiological effects of road transport on sheep, found that loading, penning and start of transport caused an increase in plasma cortisol concentration. They also observed a greater plasma cortisol level during the first 180 min of journey in transported sheep than that of non-transported counterparts. Hall *et al* (1998) observed the main increase in plasma cortisol level during the first 90 min of journey in sheep. Similarly Kannan *et al* (2003) found that the cortisol concentrations increased markedly within 1-h after the beginning of transportation in goats.

Loading and unloading are usually the most stressful stages of animal transport (Hall and Bradshaw 1998). The presence of well-designed loading and unloading ramps is critical for minimising stress and improving animal welfare (Grandin 2010). Therefore, methods of loading and unloading might be one of the reasons of high cortisol levels determined in the current study. In Bolivia, the lack of loading ramps on farms and llama slaughterhouses increases animal-handler interactions which could also lead to bruising.

CK is released into the blood in response to muscle damage, or when there is a vigorous exercise and has been used as indicator of trauma, high levels of physical

Table 1. Means and standard deviation (SD) of the blood concentrations of cortisol, β-hydroxybutyrate (β-OHB) and activity of creatine kinase (CK), at different sampling times during preslaughter handling of llamas.

Promedios y desviaciones estándar (SD) de las concentraciones sanguíneas de cortisol, β-hidroxibutirato (β-OHB) y actividad de creatin quinasa (CK) en las diferentes etapas de muestreo durante el manejo *antemortem* de las llamas.

Response variable	Before Transport	After Transport	Lairage	At Slaughter	*P*-value
Cortisol (ng/ml)	2.93±1.36^{c}	16.95±6.37^{a}	8.58±4.04^{b}	10.34±5.90^{b}	0.000
β-OHBA (mmol/L)	0.08±0.06	0.17±0.11	0.18±0.10	0.19±0.13	0.161
CK (UI/l)	135.79±98.31^{b}	277.58±249.85^{ab}	528.44±629.98^{a}	178.27±73.58^{b}	0.013

^{abc}Different letters in the row indicate significant differences according to Tukey's test($P < 0.05$).

activity or other damages during handling and transport in farm animals (Knowles and Warriss 2007). In the current study, there was a significant increase in CK levels after transport and even higher levels after lairage (table 1). This result could indicate the possible trauma during loading, journey, unloading or injury due to behavioural interaction between animals during the journey. According to Romero *et al* (2013) truck load density, stops during transport of cattle and lairage time at the plant increased the risk of bruises appearing on carcasses. Other authors have also observed an increase in CK level due to transportation in veal (Grigor *et al* 2004) and lambs (Tadich *et al* 2009). As previously demonstrated in sheep, the cortisol concentration tends to increase rapidly (reaction mediated by activity hormones), whereas CK concentrations increase at a slower rate during the pre-slaughter management (reaction mediated by activity enzymes) which is consistent with the findings of the present study.

Mean β-hydroxybutyrate concentration obtained in the present study, did not change significantly due to the 3 h transportation stress. However according to results of Saeb *et al* (2010) and our results, β-hydroxybutyrate concentration in camels is lower than reference ranges recorded for other ruminants (Ramin *et al* 2005). According to Chandrasena *et al* (1979), in dromedary camel the activity of the enzyme β-hydroxybutyrate dehydrogenase in both the rumen epithelium and the liver is low, and the rumen epithelium is devoid of papillae which would greatly reduce the surface area available for metabolic functions.

The high concentrations of blood variables during exsanguination may be related to the fact that animals were slaughtered by puntilla. Although this method is still accepted for llama slaughter in Bolivia, as observed by Limon *et al* (2009), most animals showed rhythmic breathing movements at the flank following puntilla stabbing at the atlanto-occipital space, and also had a positive palpebral reflex. The use of the puntilla is not accepted by OIE (2012) animal welfare standards and it is recommended that it should be banned, as it does not properly stun animals.

CARCASS INDICATORS

Live weight at slaughter was 54.2±6.4 kg, which is lower than the 63.18 kg found by Cristofanelli *et al* (2004, 2005) for carcasses of 25-month old males, and probably reflects the younger age of our llamas (18 to 24 months). Carcasses weighed 26.1±4 kg and presented a light and scarce fat coverage (1.33 ±0.46 in a 1 to 5 point scale). The mean pH values found for llama meat were 6.84±0.16 at 1 h and 5.42±0.11 at 24 h *postmortem*, with no final pH values > 5.8, which could be considered normal (Cristofanelli *et al* 2004). Greater initial muscle glycogen content confers an increased capacity for postmortem glycolysis, or high "glycolytic potential," that in turn, extends pH decline. According to Van Saun (2006) and Cebra *et al* (2001), llamas and alpacas maintain higher blood glucose concentrations (mean: 7.0 mmol/l, range: 4.6-8.9 mmol/L) more similar to that of non-ruminants animals and also display an extreme hyperglycemic response (blood glucose concentrations > 11.1-16.6 mmol/L) to even minimal stress situations, a feature which could favor a decrease in pH in muscle of llamas.

Table 2. Characteristics of bruises found in the carcasses of the llamas.

Características de los hematomas encontrados en las canales de las llamas.

Characteristics of bruises	Class	%
Depth grades	0	69.35
	1	29.03
	2	1.61
Patterns	Circular	18.75
	Irregular	81.25
Color	Carcasses without bruising	69.35
	Bright Red	29.03
	Dark red	1.61
Size	Small	94.74
	Medium	5.26

Nine (30.0%) of the carcasses were bruised and 21 carcasses (70.0%) were recorded as non-bruised. A total of 19 bruises were found on 9 of the carcasses. Table 2 shows that superficial bruises affecting only subcutaneous tissues (grade 1) were the most frequently observed; bruises were mainly classified as small 1 (< 5 cm of diameter) and irregularly shaped. These results are similar to those of lamb carcasses as registered by Tarumán and Gallo (2008). According to Carter and Gallo (2008) in lambs transported for up to 12 h, there were only small bruises present in the carcasses, no greater than 5 cm in diameter. The lesions of small size could be attributed not only to transport, but also loading and unloading, as it was observed that llama were frequently pulled by the fleece or body parts when they refused to move, or to upload and download them manually from the truck. These human-animal interactions have also been observed in sheep (Tarumán and Gallo 2008) and can lead to bruising. During transport, many llamas were seen to lay down and others can trample on them, which can also lead to bruising. Although the handling of the llamas during blood sampling may also have led to some bruising (figure 1e), as they are immobilised by hand only, it was noted that none of them were aggressive during sampling; on the contrary they were very tame. However, most of them vocalised and refused to move at some stage during transfer from the lairage pen to the slaughter area. So, in many opportunities the handlers picked them up from the fleece or other body parts (tail, neck) in order to move them.

Fresh, bright red-coloured bruises were found more frequently on all the animals (29.3%) compared with dark red (1.6%). As a bright red colour is characteristic of fresh bruises (Grandin 2000), the observation of bruises of this colour mainly, confirms that lesions were recent (less than 24 hours) and were most likely produced during the immediate pre-slaughter period, probably during transit, at unloading, during lairage or during handling at the slaughterhouse. This has been also found in cattle (Strappini *et al* 2012) and lambs (Tarumán and Gallo 2008).

It can be concluded that pre-slaughter operations of llamas under the commercial conditions of this study produced physiological changes similar to those associated with a mild and transient stress response as in other species and which, we judge, fall within acceptable limits for their welfare. Considering that llamas are in general tame and have close contact with people, transport stress could be reduced by designing proper loading, unloading facilities and by adequate handling. Although bruises registered were small and superficial, they affected the most valuable cuts. It is highly recommended that OIE standards for the welfare of animals during stunning should be followed and that puntilla use in llamas should be banned. Training of personnel handling llamas at the slaughterhouses in Bolivia would help in improving welfare as well as minimising adverse effects on carcass quality.

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