
Effect of weaning and a 48 hours transport by road and ferry on some blood constituents in lambs

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Summary

The effect of weaning followed by long distance transport to slaughter on the concentrations of some blood constituents was studied in Corriedale lambs. Two commercial journeys of 48 h duration, including road and sea ferry crossing were followed; in each journey 500 lambs were transported in three deck trucks with a space allowance of 0.2 m²/lamb. Immediately previous to the transport the lambs were rounded up with their mothers from distant fields and weaned; in each journey 25 lambs were selected at random and a blood sample was obtained from each individual on the farm before loading, once unloaded after arrival at the slaughterhouse, after 10 h lairage and at exsanguination. The significance of the differences between means at different sampling points was determined using a repeated measure ANOVA or the Kruskal-Wallis test, when appropriate.

The handling procedures related to rounding up and weaning before transporting lambs for slaughter induced high initial plasma concentrations of cortisol, PCV, glucose, lactate, and activity of CK. All of these variables tended to decrease during transport for 48 hours immediately after, except cortisol, which increased. Transport of lambs without access to food and water for 48 hours immediately after rounding up and weaning increased the plasma concentrations of haptoglobin and β-HOB, reflecting the effects of the long term stress and fasting. Lairage for 10 hours decreased the concentrations of cortisol, glucose and lactate, but not PCV, haptoglobin or β-HOB. It was concluded that the commercial procedures of weaning and prolonged transport immediately after in lambs destined for slaughter are stressful and exhaust body reserves, and measures should be taken to improve welfare.

Key words: lambs, blood constituents, weaning, transport.
1. Introduction

The incorporation of Chile as a commercial partner of important world markets presents new technical and commercial challenges and the introduction of the animal welfare concept in farming has turned out to be a technical barrier for international commercialization of meat products (Bahamonde, 2005); hence animal welfare is an issue of government concern, commercial relevance and scientific research to sustain policy changes (Gallo, 2008). It is a common feature in Chile, and in several other countries in South America to transport cattle and sheep live from the main production centres to be slaughtered in the main consumption areas, either because there are not enough slaughterhouses accredited for export at regional level or because prices are higher at consumption centres (Gallo and Tadich, 2008). One of the longest journeys affects cattle and sheep produced in the Chilean Patagonia (Region of Aysen); these animals are frequently transported without water and food, by road and ferry, for distances up to 1700 km and durations up to 72 h (Aguayo and Gallo, 2005). Long transport journeys have been recognized as factors producing physiological and behavioural changes that can affect the welfare of the animals (Grandin, 1997; Broom, 2003) and in the case of animals sent for slaughter, can also affect the quantity and quality of the meat produced (Warriss, 1990; Gregory, 1998; Gallo et al, 2003, Gallo and Tadich 2005; Amtmann et al, 2006).

The effects of transport on animal welfare can be measured through physiological and behavioural indicators (Broom and Johnson 1993; Tarrant and Grandin 1993; Broom 2003). Different authors have used several blood constituents to determine stress during transport. Cortisol, despite its variability and short life, is still one of the most used indicators of stress; other blood constituents related to stress are packed cell volume (PCV), glucose, lactate, insulin, free fatty acids, plasma activity of creatine phosphokinase (CK, EC 2.7.3.2), β-hidroxibutirate (Shaw and Tume, 1992; Broom 2003). Lately, haptoglobin, a major acute phase protein has been used as an indicator of stress (Braumann and Gauldie, 1994; Arthington et al, 2003).

There are few studies related with the stress produced by commercial weaning (Sowinska et al, 2006) and long distance transport in lambs (Knowles et al 1993, 1996, 1998, Hall et al, 1999). Most studies cited have been done under controlled experimental conditions and using paved roads, conditions which are not comparable with commercial practice in Chile. On the other hand, under commercial rearing systems in Chile it is a common practice that lambs are weaned right before they are sent to the slaughterhouses, being this handling an additional stress to the transport. The
aim of this work was to study the effect of weaning followed by prolonged transport by road and ferry on the concentrations of some blood constituents in lambs.

2. Material and Methods

2.1 Description of the study design

Two commercial journeys lasting 48 hours by road and ferry were analysed; in each journey 500 Corriedale lambs of approximately 70 days of age and 29 ± 2.5 kg live weight were transported in a three deck lorry, which was divided in compartments carrying 12-14 lambs each, giving a space allowance of 0.2 m²/lamb. In each journey, 25 lambs were randomly selected (50 total), individualized and two blood samples, one with heparin and the other with NaF, were collected from the jugular vein at each of the following points: on the farm, after rounding up and weaning, before loading onto the lorries for transport (on farm); after approximately 48 h of terrestrial plus on ferry maritime transport, after unloading the lambs at the slaughterhouse (transport); after 10 h lairage (lairage) and during exsanguination (bleeding).

The day of transport, lambs and their mothers were rounded up from the grazing fields to the farm yards, starting at 6:00 AM in the morning and arriving to the farm yards around 12:00 AM. After arrival to the farm yards the lambs were weaned and weighed, a procedure that lasted around 4 hours. At this point the first blood sample was taken (on farm). Then all lambs were loaded into the lorry, allocating the sampled lambs randomly into the pens and decks, with the rest of the lambs. The first part of the journey, from the farm to the ferry, took 12 h on stone and asphalt roads (approximately 400 km); then the lorry was loaded onto a ferry for a sea journey of 26 h (approximately 494 km plus the time for loading and unloading the truck), afterwards driving again on asphalt roads for another 10 h (approximately 250 km) to the slaughterhouse, totalizing approximately 48 h. During transport lambs were deprived of feed and water. On arrival at the slaughterhouse, after unloading, the lambs were bled again (transport) and were kept in lairage for approximately 10 h following the usual commercial procedures; they were provided with water but not food, remained in pens with concrete floor, metallic fences, no roof. After lairage lambs were bled again (lairage), driven to the stunning box and stunned by means of a captive bolt pistol, impelled by cartridges, and a final blood sample was collected during exsanguination (bleeding). Considering the time since the lambs were rounded up until they were slaughtered, the total fasting period reached around 68 hours.

2.2 Analysis of the blood constituents
Packed cell volume (PCV) was obtained using the microhaematocrit technique. The blood samples were centrifuged and the plasma was removed and stored at -20 °C for subsequent analysis. Plasma cortisol concentrations were determined by radioimmunoassay (RIA), glucose plasma concentrations were determined using the GOD-PAP test without deproteinization (GL 2623, RANDOX®), plasma concentration of lactate was determined using the LOD enzymatic test, the plasma creatinphosphokinase activity (CK, EC 2.7.3.2) was measured by the UV-kinetic method optimized according to the Deutsche Gesellschaft für Klinische Chemie. Plasma concentration of haptoglobin was obtained by the peroxidase method, using a commercial kit (Tridelta®); for processing all these samples an autoanalyzer Cobas Mira Plus (Roche®) was used. The plasma concentration of β-hidroxiibutirate (β-HOB) was determined by the enzymatic technique that used the 3-hydroxybutyrate dehydrogenase enzyme. The change from NAD+ to NADH was measured by spectrophotometer (HITACHI 2040 at 340nm).

2.3 Statistical analysis

The variables were checked for normal distribution by visual inspection of histograms and using the Kolgomorov-Smirnov test. For variables with a normal distribution a repeated measures analysis of variance, was used. To compare non parametric variables a Kruskall Wallis test was used. All the analyses used the Statistix version 8.0 for Windows (Statistix 8, Copyright© 1985-2003, Analytical Software, USA).

3. Results

Figure 1 shows that plasma cortisol concentration increased significantly (P<0.05) after 48 hours of transport compared with on-farm concentration, whilst lairage resulted in a significant decrease (P<0.05); however the concentration of cortisol at exsanguination increased again (P>0.05). Glucose concentration decreased following transport and during lairage, becoming significantly lower than on farm after lairage (P<0.05, Figure 2). The plasma concentration of lactate followed a similar pattern to glucose, showing a significant (P<0.05) decrease following transport and lairage, compared with on farm concentration (Figure 3). PCV values were high and similar during the different periods of sampling (P> 0.05, Figure 4). Plasma activity of CK tended to decrease after transport (P>0.05) and decreased significantly after lairage (P<0.05) compared with on farm values. At exsanguination the plasmatic activity of CK increased (P<0.05) compared with means obtained at all other sampling points (Figure 5). The concentration of β-HOB increased after transport and lairage compared with on farm concentration (Figure 6). There was a significant increase (P<0.05)
of the plasma concentration of haptoglobin following transport and values remained high until exsanguination (Figure 7).

4. Discussion

The initial blood sample taken on farm showed that most blood constituents, except β-HOB and haptoglobin, were above values obtained in weaned, cannulated resting lambs of the same breed and weight (Barrientos et al 2006), although not always above the range of reference for sheep (Kaneko, 1997; Radostits et al 2000). The high initial values obtained in this study indicate that weaning and handling related to it (rounding up, penning) had in general a greater effect on blood constituents than the transport itself, except for cortisol, haptoglobin and β-HOB.

According to Shaw and Tume (1992) plasma cortisol concentrations increase in response to stress and this response is immediate; plasma concentrations increase rapidly and can reach several folds the initial values, being the response proportional to the magnitude of the stressor (Cunningham 1999). The high on farm cortisol concentration found in the present study (Figure 1) shows that handling procedures on the farm, such as rounding up, penning, handling, weaning and bleeding the lambs increased the release of cortisol. These results are similar to those of Sowinska et al (2006), who compared cortisol concentrations before and 15 h after weaning in lambs and reported an increase in the plasma cortisol concentrations. Accordingly, plasma concentrations of glucose and lactate were highest on farm (Figures 2 and 3).

The even higher concentration of cortisol found post transport does not agree with results of Broom et al (1996) and Hall et al (1999) who reported an increase in plasma cortisol concentration soon after loading and starting of travel, but a subsequent decline during transport. The increased cortisol concentration in the lambs after transport could be due to the unloading, handling and bleeding procedure, more than to transport itself, especially considering that since the start of the unloading to the time of bleeding there was a 30 minute interval. Broom and Johnson (1993) indicated that the handling of the animals to obtain blood samples can produce an increase in the concentration of glucocorticosteroids, masking the real effect of the stressor agent in study; therefore it is important that the blood sample should be taken as soon as possible, before unloading, because in most species the increase in corticoid concentration starts around two minutes after handling the animal. However Navarro et al (2007) took blood samples of lambs after being
submitted to the same transport journey, but before unloading, and also found an increase in cortisol concentration after the prolonged transport.

The decrease in glucose (P>0.05) and lactate (P<0.05) concentrations after transport compared with on farm values also indicated that the procedures on farm were more stressful than the transport itself. Knowles et al (1993, 1995) transporting lambs and ewes for 14 and 24 h, respectively, found that the loading and beginning of transport had a large effect on glucose values; these authors attributed this increase to a cathecolamine-stimulated glycogenolysis. However, Knowles et al (1995) found a decrease of glucose and cortisol after 9 hours suggesting that the sheep became adapted to transport. Short periods of exercise and stressors as those provoked by rounding up, loading and unloading increase the circulating adrenalin, producing a degradation of the muscular glycogen and an increase of the plasma concentration of lactate (Mitchell et al 1988). The decrease of glucose after transport in this study could be attributed to its utilization as a source of energy before transport, which is coincident with the low muscle (Longissimus thoracis) and liver glycogen concentrations (5.1 ± 4.4 and 5.2 ± 9.0 µmol/g, respectively) found in the carcasses and livers of the same animals (Carter and Gallo 2008).

The significant decrease (P< 0.05) in the plasma concentrations of cortisol, lactate and glucose after lairage (10 h approximately) agrees with the findings of Knowles et al (1993), who after transporting lambs for 14 h, found that the values of cortisol decreased after 6 and 12 h of resting. Knowles et al (1995) after transporting ewes for 24 h found that the cortisol and glucose concentrations decreased near to basal pretransport values after 24 h of resting.

The increase (P>0.05) of cortisol and glucose at exsanguination could be a result of the handling in the race when driving the lambs to the stunning box and to the process of stunning and sticking itself. Shaw and Tume (1992) indicated that samples collected at exsanguination could be expected to provide information on the stress of preslaughter treatments. Mitchell et al (1988) and Shaw and Tume (1992) pointed out that glucose concentration increases at slaughter due to the peak of cathecolamines produced at the time of stunning.

The decrease of the lactate concentrations until the stunning does not agree with Knowles et al (1993) nor with previous studies of the same authors in cattle (Tadich et al 2002), where they observed an increase in lactate concentration at bleeding. Coincidently with the low concentrations of glycogen found post-mortem in the Longissimus thoracis muscle of the same lambs (Carter and
Gallo 2008), it is possible that exercise before loading as well as the prolonged transport without food exhausted the glycogen reserves and there was not enough glycogen left to produce lactate.

The PCV values during the four sampling periods (Figure 4) were above ranges described by Barrientos et al (2006) in cannulated resting lambs of similar characteristics, although within the reference range for sheep given by Radostits et al (2000). The high PCV values could indicate a release of erythrocytes from the spleen to the blood stream as a result of a sympathetec-adrenal stimulation but in this case it is attributable mainly to dehydration (Knowles et al 1995). It has to be considered that these lambs were still lactating and from the start of the rounding up until their arrival to the slaughterhouse (around 58 hours) they had no access to any water sources, which could produce an important degree of dehydration, notwithstanding the stress due to transport and handling. The PCV values did not recover after lairage. Knowles et al (1993) indicated that lambs will not drink readily from an unfamiliar source after being transported, even when they have been deprived of water for up to 24 hours. In this case the lambs were deprived of water for much longer; however proceeding from very extensive conditions in the Patagonia, where they had access to milk from their mothers and natural water sources, but had never drank water from troughs, they probably did not drink during lairage.

Mean values of plasmatic activity of CK during the four different sampling points (Figure 5) were all above reference values (Smith 2002, Barrientos et al 2006). According to Warriss et al (1995), there is a direct relationship between the duration of transport and the plasma activity of CK in cattle. However, in this study the plasma activity of CK decreased after transport and lairage. This could be attributed to the high initial values found on farm, due to the rounding up (6 hours walk) and the handling during weaning, considering that Tadich et al (1999) found that the maximum activity of this enzyme in the case of transported cattle was reached between 2 and 12 after the initial muscular damage. Kannan et al (2000) working with goats also indicated that vigorous physical activity such as penning, loading and unloading are more important in determining the plasma activity of CK than the transport or fasting. Furthermore Navarro et al (2007) found that mean CK before loading was 100% increased when lambs were brought immediately before from distant fields compared to lambs penned and rested 2 hours prior to loading. Although the CK activity decreased in the lambs after transport and lairage, the values were still above the reference values for the species (Radostits et al 2000). The decrease of the CK activity observed after transport could be due to the fact that transport was less physically demanding than handling on farm. Resting during lairage at the abattoir helped to decrease the plasma activity of the enzyme (P<0.05) as observed before in cattle (Tadich et al 2005). The final raise in the plasma activity of
CK (P<0.05) during bleeding could be due to the pre stunning handling (driving) and to severing
the blood vessels in the neck, rather than to stunning itself. This result is not coincident with
previous findings of the same authors in cattle, where no significant differences between the activity
of the enzyme preslaughter and at exsanguination were found, suggesting that this procedure does
not induce a raise in the enzyme activity in cattle (Tadich et al 2002).

The prolonged fasting period to which the lambs were submitted to during this study increased by
250 per cent the plasma β-HOB concentration (Figure 6). An increase in the plasma
concentrations of β-HOB is an indicator of prolonged fasting Horton et al, 1996, Knowles et al,
h, found that after each journey there was an increase in the plasma concentration of β-HOB.
Warriss et al (1989) deprived lambs of food, but not water, for more than 72 hours, and found that
plasma concentrations of β-HOB increased with increasing the period of fasting, pointing out that
this would be due to the mobilization of body fat reserves as a response to a prolonged food
restriction. The results of Warriss et al (1989) are coincident with the findings of the present study,
since the period where the lambs had a food restriction considering the start of the rounding up in
the fields up to the moment of the stunning was around 68 h in average. This increase in β-HOB
also agrees with the decrease found in the plasma concentration of glucose. According to Herdt
(1988), ketogenesis and gluconeogenesis are directly related. It is important to point out that the
lambs used in this study were in a transition period from lactating to full ruminants, therefore the
fasting period they were submitted to could have produced a faster mobilization of body fat reserves
than those reported in other studies for adult sheep or cattle. The live and carcass weight loss found
in the same lambs and the low glycogen concentrations in their muscle tissue (Carter and Gallo
2008) also agree with a mobilization of body reserves. According to Knowles et al (1993, 1995,
1996, 1998) the concentration of β-HOB could be recovered if the lambs had access to food after a
period of time between 24 to 96 hours. During these prolonged transport journeys, as well as
during lairage in the slaughterhouses, lambs do not have access to food; however the possibility of
feeding them either during transport or lairage, or resting the lambs on pasture either before or after
transport should be taken into account as possibilities to reduce stress, reduce weight and carcass
quality losses and improve animal welfare during these journeys.

Arthington et al (2003) evaluated the effect of weaning and weaning plus transport in calves and
found a raise in the concentration of haptoglobin in calves weaned but not in those weaned and
transported, concluding that it is not necessary to have an inflammatory process to increase the
concentration of this protein. Arthington et al (2005) found that calves weaned at 300 days and
transported had an increase in haptoglobin values compared with calves weaned at 89 days and transported. The results obtained in this study (Figure 7) do not allow to conclude whether the significant increase in haptoglobin concentration observed after arrival to the abattoir was due to the duration of the transport itself or an additive effect of on-farm management plus transport. However, Tapia et al (2007) found a lower increase in the concentrations of haptoglobin (0.16 g/L) in lambs of the same characteristics from the same farm but transported for 12 h only to a local abattoir, which would indicate that the duration of transport plays an important role in the raise of this protein. It would be necessary to further investigate about the role of weaning alone on the increase of plasma haptoglobin in lambs.

5.- Conclusions

According to the results of this study it can be concluded that the commercial procedures of weaning and prolonged transport immediately after in lambs destined for slaughter are stressful and exhaust body reserves; improvements in animal welfare and meat quality could be met by alternatives such as weaning the lambs sometime before transport (two or three weeks) and leaving them in pastures close to the loading ramps or feeding and watering during or after transport. It would be also recommendable to study whether reducing lairage time or feeding the animals during this period could also reduce the concentrations of blood constituents related to stress without continuing the depletion of body reserves after such a prolonged transport journey without food and water. All these alternatives will increase costs and should be confronted against benefits such as reducing losses in meat quantity and quality or just being able to reach higher price markets through complying with international animal welfare standards.

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Figure 1. Mean plasma concentrations (± E.E.) of cortisol (µg/dL) at the farm before transport, at the abattoir after 48 h of transport, after 10 hours of lairage, and at slaughter time.

Figure 2. Mean plasma concentrations (± E.E.) of glucose (mmol/L) at the farm before transport, at the abattoir after 48 h of transport, after 10 hours of lairage, and at slaughter time.

Figure 3. Mean plasma concentrations (± E.E.) of lactate (mmol/L) at the farm before transport, at the abattoir after 48 h of transport, after 10 hours of lairage, and at slaughter time.
Figure 4. Mean values (± E.E.) of PCV (%) at the farm before transport, at the abattoir after 48 h of transport, after 10 hours of lairage, and at slaughter time.

Figure 5. Mean values (± E.E.) of plasma activity of CK (U/L) at the farm before transport, at the abattoir after 48 h of transport, after 10 hours of lairage, and at slaughter time.

Figure 6. Mean plasma concentrations (± E.E.) of β-hidroxibutirate (mmol/L) at the farm before transport, at the abattoir after 48 h of transport, after 10 hours of lairage, and at slaughter time.
Figure 7. Mean plasma concentrations (± E.E.) of haptoglobin (g/L) at the farm before transport, at the abattoir after 48 h of transport, after 10 hours of lairage, and at slaughter time.

Photograph 1. Geographical location of the farm of origin and routes covered during transportation of the lambs: red line = stone and gravel road from farm to dock (400 km); light blue line = ferry crossing (494 km); green line = paved highway (250 km).

Photograph 2. Rounding up of ewes and lambs before weaning.
Photograph 3. Three deck truck used for the transportation of the lambs.