



Stress responses in lambs castrated with three different methods

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ABSTRACT

The present work was conducted to evaluate the animal response to stress in lambs caused by three different castration techniques. Forty-six male lambs aged 4-5 months were randomly allocated to one of four groups including Burdizzo (B), scrotal ablation (SA), orchiectomy (OR) and control handling (H). Local anaesthesia (lidocaine 2%) was administered in both spermatic cords and the scrotal neck of lambs before each treatment. Blood samples were collected at -30, -10, +1, +20, +40, +60, +120, and +180 minutes. Serum cortisol concentrations were determined using a competitive immunoassay and the area under the curve (AUC) was calculated for each lamb. The following biochemical parameters were assayed for each animal at each time point: alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and glucose (GLU). The time needed for total lesion resolution and weight gain of each animal was recorded. Orchiectomy elicits the greatest cortisol response, significantly greater than that seen in similarly handled controls ($P \leq 0.01$), Burdizzo and scrotal ablation groups ($P \leq 0.05$). The serum cortisol AUC was higher in the scrotal ablation group ($P \leq 0.05$) than controls, but lower than in the orchiectomy group ($P \leq 0.05$). The Burdizzo group didn't differ from controls. Serum glucose levels of the castrated lambs differed significantly from the control group, following a trend similar to cortisol. No change was seen in ALT, AST, LDH or CK. No difference in weight gain was seen among the groups. Our results suggest that use of the Burdizzo is the preferable castration technique for adult lambs, while scrotal ablation is a valid surgical alternative to orchiectomy and permits more rapid wound healing that is ideal for extensive management where flocks are not under close observation.

Key words: Stress, Cortisol, Castration, Lambs, Animal welfare.

RIASSUNTO

EFFETTI DI TRE DIFFERENTI TECNICHE DI CASTRAZIONE SUL BENESSERE DEGLI AGNELLI

Il presente lavoro si poneva quale obiettivo la valutazione dello stress provocato da tre differenti tecniche di castrazione in agnelli di 4-5 mesi di età. Quarantasei agnelli di sesso maschile sono stati suddivisi in quattro gruppi: un gruppo in cui gli animali venivano castrati con la tecnica Burdizzo (B), tramite asportazione chirurgica dello scroto (SA), tramite orchietomia (OR) ed un gruppo controllo (H). Prima di ciascun trattamento si eseguiva un'anestesia locale (lidocaina 2%) a livello dello scroto e dei cordoni spermatici. Sono stati prelevati campioni di sangue a -30, -10, +1, +20, +40, +60, +120, e +180 minuti dall'intervento, al fine di misurare il cortisolo sierico e i seguenti parametri biochimici: AST, ALT, LDH, CK e glucosio. Inoltre, si registrava il tempo necessario alla completa risoluzione delle lesioni provocate dall'intervento e gli incrementi ponderali degli agnelli dei vari gruppi. Nel gruppo OR si è riscontrato il maggior incremento della concentrazione di cortisolo sierico differendo significativamente rispetto al gruppo H ($P \leq 0,01$) e ai gruppi B e SA ($P \leq 0,05$); il gruppo SA ha manifestato una risposta maggiore rispetto a H ($P \leq 0,05$) e inferiore rispetto a B ($P \leq 0,05$), mentre il gruppo B non differiva da H. Gli esami biochimici hanno messo in evidenza come la glicemia degli animali castrati sia risultata superiore rispetto ai controlli, manifestando un andamento nel tempo simile a quanto osservato per il cortisolo. Non si sono evidenziate differenze negli incrementi ponderali degli agnelli appartenenti ai vari gruppi. In conclusione i nostri risultati suggeriscono che la tecnica Burdizzo è la più rispettosa del benessere degli agnelli di 4-5 mesi di età, mentre l'asportazione chirurgica dello scroto rappresenta una valida alternativa chirurgica all'orchietomia in quanto in grado di garantire un più rapido processo di guarigione, importante soprattutto in condizioni di allevamento estensivo.

Parole chiave: *Stress, Cortisolo, Castrazione, Agnelli, Benessere animale.*

Introduction

Castration of male lambs is generally performed during the first weeks after birth using bloodless methods (rubber rings, Burdizzo clamp) or surgical methods (Wolfe *et al.*, 1998; Stafford, 2007). However, to meet particular breeding needs, to shorten the time needed to reach the slaughter weight and to improve meat quality, it is sometimes necessary to employ these techniques in older lambs. Rubber ring castration is the most common technique used on lambs up to 7 days-old (Hosie *et al.*, 1996), but this is considered to be a practice that causes a high level of distress and pain, especially in older lambs (Molony *et al.*, 1993; Kent *et al.*, 1998). Castration causes stress as animals are handled, restrained and above all subjected to painful procedures. Assessment of distress occurring in lambs during castration can be measured by the use of indirect physiological indices. Changes in

the activity and functioning of the hypothalamic-pituitary-adrenal axis (HPA) are widely used to quantify animals' response to stress. Serum cortisol concentration has been evaluated in different animal species as an important marker of stress in large animals under common husbandry conditions (Mellor and Murray 1989a, 1989b; Lester *et al.*, 1991; Mellor *et al.*, 1991; Kent *et al.* 1993; Molony and Kent 1995; Lester *et al.*, 1996; Thornton and Waterman-Pearson, 1999; Molony *et al.*, 2002; Stafford *et al.* 2002; Thuer *et al.*, 2007).

As well as changes in serum cortisol levels, animal response to stress can be detected by modifications of other haematochemical parameters (Steyn, 1975; Adams and Rinnie, 1982; Kent and Ewbank, 1986). Measurements of most serum biochemical parameters are more appropriate for long-term welfare assessment (Broom *et al.*,

2000), but some of them can change rapidly enough to provide a useful indicator of short-term effects.

Castration carried out by both bloodless and surgical methods inevitably causes acute and chronic pain until a complete resolution of lesions occurs (Mellor *et al.*; 2000). To properly assess welfare of male lambs undergoing castration it is useful to consider the possibility of chronic pain and the time needed for complete resolution of lesions. Indeed, an optimal castration technique should be both effective and have a low incidence of complications, and this is particularly important in large flocks under extensive management conditions at pasture where animals are more likely to contract infections or parasite infestations and are generally less available for close observation.

The aim of this work was to evaluate stress responses of four-five month old lambs following three different castration methods by measuring serum cortisol concentration, biochemical parameters and weight gain. Furthermore, the current work examines lesion resolution in the different groups to evaluate which technique might be best for use in flocks under extensive management where close monitoring of individual animals is impractical.

Material and methods

Animals

Forty-six Sarda lambs aged 4-5 months were allocated randomly to four groups, with adjustments made to keep group mean bodyweights similar. Animals, all of which belonged to the same flock, were gathered together after the weaning and were allowed to graze in a 200 square meter paddock during the daytime and moved to a pen at night.

Experimental design

The four treatment groups were Burdizzo

castration (B, n=12), scrotal ablation (SA, n=11), orchietomy (OR, n=11) and control handling (H, n=12). Briefly, each lamb was held in a seated position and, after disinfection of the site with diluted povidone iodine solution, lidocaine hydrochloride 2% (4 mg per kg body weight) diluted 1:1 with saline to a final volume of 10 mL was injected, five minutes before castration, into each spermatic cord and subcutaneously around the scrotal neck. To prevent infectious complications 20,000 IU benzyl-penicillin and 20 mg streptomycin (Neo Vet-Cillin LA, Vetem) per kg body weight were administered intramuscularly to each animal. Antibiotic solution (oxytetracycline hydrochloride, Neo Spray CAF, Gellini) was sprayed on the surgery sites in the OR and SA groups. All lambs received insect repellent lotion.

Burdizzo castration

The bloodless castration method (B) was performed using a standard 23 cm Burdizzo clamp used for crushing each spermatic cord and associated scrotal tissue for 30 seconds.

Orchietomy

A vertical incision was made through the skin and the scrotal sac to reveal the testis that were gently pulled away from the scrotum after ligament resection. The blood vessels and spermatic cords were tied proximally and clamped distally with a haemostat to prevent blood loss during excision of the testis.

Scrotal ablation

An horizontal incision was made around the scrotal neck, and after the isolation and ligation of the blood vessels and spermatic cords, the testis within the scrotum were excised and the skin sutured.

Control handling

Control animals were restrained in a

seated position for five minutes to mimic the conditions occurring in the different castration techniques.

Blood sampling and laboratory analysis

Blood samples were taken by jugular venipuncture 30 minutes and 10 min prior to and 1, 20, 40, 60, 120 and 180 minutes after each treatment (castration or control handling). The samples were centrifuged at 800g for 10 minutes and the serum was stored at -20°C pending the laboratory analysis. Serum cortisol concentrations were determined using a competitive immunoassay (Eclética cortisol, Adaltis) with an automatic laboratory analyser (Eclética, Adaltis). The integrated cortisol response, defined as the area underneath the cortisol curve during the period when concentrations were greater than pre-treatment values, was calculated for each lamb. The following biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and glucose (GLU) were determined by a clinical chemistry analyzer using a spectrophotometric method (Dimension RXL, Dade Behring).

Scrotal condition and animal weight

The local effects of the different castration techniques were assessed by visual inspection immediately after treatment and on weeks 1, 2, and 5 post-treatment (p.t.). Time needed for complete wound healing was recorded in castrated animals. Lambs from the four groups were weighed on the day of castration and after 5 weeks.

Statistical analysis

Data were analyzed using PROC MIXED implemented with SAS software. The model was:

$$Y_{ijkl} = \mu + G_i + T_j + G_i * T_j + C_k + L_l + R_{ijkl}$$

Where:

Y_{ijkl} is one of the following biochemical

parameters: ALT, AST, LDH, CK and GLU

μ is the overall mean;

G_i is the group treatment (B, SA, OR and H);

T_j is the blood sample time: 1, 20, 40, 60, 120 and 180 minutes after castration;

$G_i * T_j$ is the interaction effect between group treatment and blood sample time;

C_k is a covariate. It is the mean value for Y calculated 30 and 10 minutes prior castration

L_l is the lamb;

R_{ijkl} is the residual.

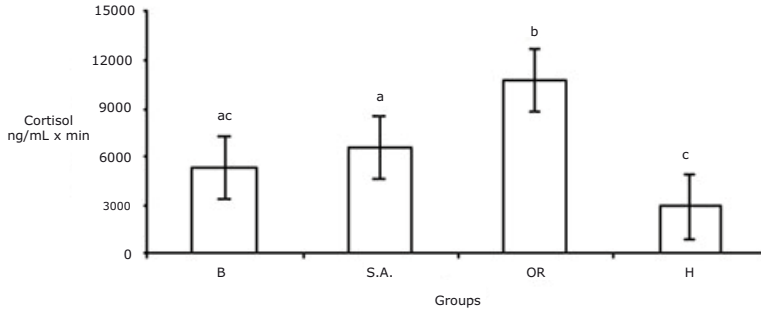
G and T were assumed as fixed factors, L was the random effect of lamb in group treatment. The C covariate was included in the model because pre-treatment values were different between groups. Differences between the cortisol AUC and weight gains in the four groups were assessed by analysis of variance (ANOVA) using Minitab version 12.1 (Minitab inc.).

Results

Cortisol response

Following all four treatments, animals exhibited transient increases in the serum cortisol concentrations. As shown in Figure 1, the area under the curve of serum cortisol concentration for each group was calculated as mean \pm SD. Orchiectomy elicited the greatest cortisol response among the four treatments, significantly different from control handling ($P \leq 0.01$), Burdizzo, and scrotal ablation ($P \leq 0.05$). Integrated cortisol response caused by SA was higher ($P \leq 0.05$) than H and lower than OR ($P \leq 0.05$). AUC for Burdizzo castration didn't differ significantly from the control handling group. The effects of the four castration methods on serum cortisol concentrations at different sam-

Figure 1. Area under the curve of serum cortisol concentration in response to Burdizzo (B), Scrotum Ablation (SA), Orchiectomy (OR) castration and control handling (H).



min: minutes.

Different superscripts indicate significant differences ($P \leq 0.05$).

pling times in each group and between different groups are presented in Figure 2. As shown, cortisol concentration peaked after 20 minutes post treatment (p.t.) in groups B, SA and H, while the OR group reached its highest value after 40 minutes. Serum cortisol didn't return to pre-treatment levels after 180 minutes in any groups, except for the control handling group. Significant differences in cortisol levels between the two sampling times were seen in B ($P \leq 0.05$), SA ($P \leq 0.05$) and OR ($P \leq 0.01$). Differences between treated groups and the control group (H) at each of the sampling times were as follows: B differed from H after 40 ($P \leq 0.05$), 60 ($P \leq 0.01$) and 120 ($P \leq 0.05$) minutes p.t. SA differed from H after 20 ($P \leq 0.01$), 40 ($P \leq 0.01$), 60 ($P \leq 0.01$), and 120 ($P \leq 0.01$) minutes p.t. and OR differed from H after 20 ($P \leq 0.01$), 40 ($P \leq 0.01$), 60 ($P \leq 0.01$), 120 ($P \leq 0.01$) and 180 minutes ($P \leq 0.01$).

Biochemical parameters

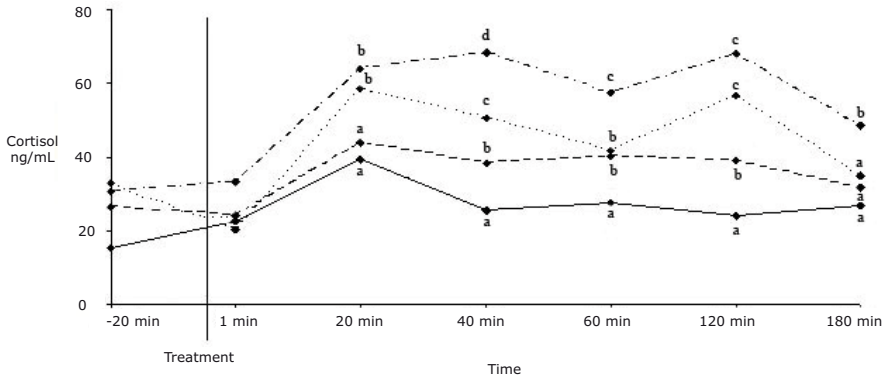
ALT, AST, LDH or CK did not show any castration related changes (Table 1). Blood glucose was the only analyte that differed significantly from the control group, similar to the pattern seen with cortisol levels. Glu-

cose concentration peaked at 20 minutes p.t. in all groups. As shown in Figure 3, B significantly differed from H at 20 ($P \leq 0.01$) and 40 ($P \leq 0.01$) minutes p.t. Glucose values in the B group had returned to levels similar to the control group at 60 minutes. SA significantly differed from H at 20, 40, 60, 120 ($P \leq 0.01$) and 180 ($P \leq 0.05$) minutes p.t.; while OR differed from H at 20 ($P \leq 0.01$), 40 ($P \leq 0.01$) and 60 ($P \leq 0.05$) minutes p.t. Glucose values in the OR group were similar to those seen in controls at 120 minutes p.t. Serum glucose returned to pre-treatment levels after 180 minutes only in SA and H groups.

Scrotal condition

Scrotal swelling occurred in lambs belonging to the B group on the day of castration and lasted for one week. Hyperaemic areas could be seen at the clamp application sites on treatment day. These progressed to scabby lesions over the next few days, and were still present in only few lambs after 1 week. Animals castrated by orchiectomy showed severe swelling and serous and/or sanguineous discharge on the day of surgery. Swelling and purulent discharge were

Figure 2. Effect of Handling (—), Burdizzo (---), Scrotum ablation (····) and Orchiectomy (-●-) on serum cortisol concentration of lambs.



min: minutes.

Different superscripts indicate significant differences ($P \leq 0.05$) between groups at each sampling time.

present after 1 week in only one lamb, possibly due to the bacterial contamination of a scrotal haematoma. After 2 weeks, this lamb had an abscess in one hemi-scrotum. The SA group had no evidence of gross pathology at any time after surgery.

Live weight

There were no significant differences in weight gains of lambs at five weeks post treatment (Table 2).

Time needed for castration

The time needed for castration of all lambs in the SA group (14.7 ± 5.12) was significantly longer ($P < 0.01$) than that required for the B (9.1 ± 0.51) or OR (9.4 ± 3.82) groups.

Discussion

The present work was done to evaluate the stress created in adult lambs castrated using three different methods. Stress was measured by changes in physiological indices. Serum cortisol level is widely recog-

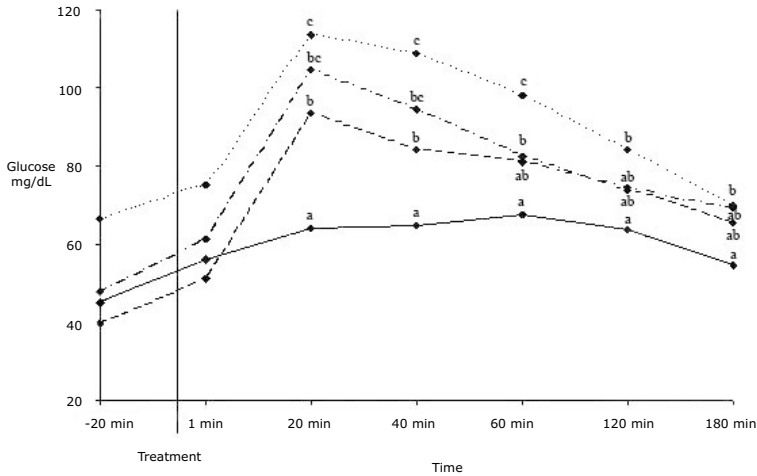
nized as a general marker of stress, and it has been largely used to assess the welfare of farm animals during different husbandry procedures (Fulkerson *et al.*, 1982; Shutt *et al.*, 1988; Hargreaves *et al.*, 1990; Graham *et al.*, 1997; Sutherland *et al.*, 2000). Our results showed that the area under the cortisol curve of lambs castrated by OR demonstrated the highest stress response while cortisol levels of lambs treated with the Burdizzo method were not significantly different from the control group. The AUC magnitude of the SA group showed an intermediate stress response. Cortisol levels at 180 minutes differed from control values only in the OR group. This suggests that lambs treated with orchiectomy suffered a higher level of stress, consistent with more extensive tissue damage in the site of castration. Complete surgical ablation of the scrotal region, with less attendant tissue trauma, resulted in lower cortisol levels. In the Burdizzo technique, the crushing of the afferent nervous fibres appeared to have reduced or prevented noxious sensory transmission to the central nervous system.

Table 1. Influence of different castration techniques upon various serum parameters.

Parameter	Sampling time						
	-20 min	1 min	20 min	40 min	60 min	120 min	180 min
ALT							
RI (15-55 U/L)							
B	50.7 ± 11.7	54.1 ± 14.7	54.2 ± 15.8	51.2 ± 13.8	51.3 ± 15.1	52.7 ± 14.5	50.3 ± 12
SA	48.2 ± 13.4	47.4 ± 11.7	48.3 ± 14.4	47.4 ± 12.1	49 ± 9.9	50.4 ± 12.9	50.1 ± 11.3
OR	52.2 ± 13.2	48.1 ± 12	49.8 ± 15.4	49 ± 17.6	51.1 ± 17.9	49.4 ± 18.8	52.8 ± 14.6
H	49.2 ± 11.8	54.4 ± 21.5	52.7 ± 15.4	51.9 ± 15.2	50.6 ± 13.8	51.4 ± 13.8	52.5 ± 13.9
AST							
RI (70-200 U/L)							
B	159 ± 48.1	158.8 ± 62.3	167.3 ± 47.7	169.2 ± 52	177.7 ± 66.8	182.2 ± 70.1	174.2 ± 82.3
SA	126.5 ± 27.5	119.1 ± 29.1	125.2 ± 25.7	124.4 ± 22	126.4 ± 22.7	131.9 ± 24.7	152.4 ± 24.3
OR	147.1 ± 49.1	137.6 ± 49.4	132 ± 29.4	155.7 ± 73.8	138 ± 54.5	158.2 ± 79.7	150.9 ± 50.8
H	126.1 ± 40.5	154.7 ± 91.5	147.7 ± 44.6	163 ± 57.9	146.5 ± 41.3	162.6 ± 57.3	164 ± 59.5
CK							
RI (80-260 U/L)							
B	95.7 ± 122.6	107.9 ± 54.1	101.5 ± 50	128.1 ± 34.3	138.3 ± 28.9	100.3 ± 55	127.2 ± 41
SA	115.8 ± 28.8	129 ± 29.2	129.5 ± 29.1	135.0 ± 30.4	132.4 ± 30.9	158.6 ± 36.6	141.4 ± 48.1
OR	124.3 ± 36.5	138.1 ± 51	132.4 ± 36.6	137.4 ± 43.4	134.1 ± 50.5	148.1 ± 61.9	138.7 ± 49.4
H	138.5 ± 37.2	140.7 ± 44	151.5 ± 48.3	146.2 ± 52.2	164.1 ± 61.1	140 ± 72.6	135.8 ± 62.70
LDH							
RI (200-600U/L)							
B	377.4 ± 73	412.1 ± 64.5	390.8 ± 65.1	369.3 ± 54.1	353.4 ± 55.1	380.7 ± 93.5	389.1 ± 59.1
SA	385.8 ± 57.7	371.3 ± 39.3	373.7 ± 53.9	381.3 ± 48.3	384.8 ± 50.5	378 ± 45.6	419.8 ± 28.4
OR	400.9 ± 66	401.1 ± 24.2	410.2 ± 57.6	404.3 ± 68	411.5 ± 70.9	393.1 ± 40.5	373.1 ± 46.8
H	410.3 ± 53.8	428.4 ± 98.8	403.7 ± 55.4	409.4 ± 94.4	381.5 ± 70.1	388.9 ± 78.3	397.7 ± 67.2

RI: reference intervals; min: minutes; B: Burdizzo; SA: Scrotum Ablation; OR: Orchiectomy; H: control handling.
No significant differences were found at different sampling time in each group and between groups.

Figure 3. Effect of Handling (—), Burdizzo (---), Scrotum ablation (····) and Orchiectomy (-•-) on glucose concentration of lambs.



min: minutes.

Different superscripts indicate significant differences ($P \leq 0.05$) between groups at each sampling time.

Table 2. Influence of different castration techniques on lambs average (\pm SD) weight gain (kg) after five weeks post treatment.

Groups	Day 0	Day 35	Weight gain
B	31.2 \pm 7.6	39.30 \pm 8.2	8.0 \pm 1.5
SA	31.1 \pm 8.1	38.10 \pm 9.5	7.0 \pm 2.05
OR	29.2 \pm 8.4	36.85 \pm 9.6	7.6 \pm 2.03
H	29.4 \pm 3.9	37.30 \pm 4.2	7.9 \pm 1.4

B: Burdizzo; SA: Scrotum Ablation; OR: Orchiectomy; H: control handling.

Weight gains did not differ significantly ($P \leq 0.05$) between groups.

Our findings are in accordance with other authors who compared different castration techniques, and demonstrated how surgery creates a greater hypothalamic-pituitary-adrenal axis (HPA) stimulation than bloodless castration methods (Stafford *et al.*, 2002; Melches *et al.*, 2006). As shown in our results, no animal returned to pre-treatment cortisol value after 180 minutes, but the values in lambs castrated with the

B and SA techniques approached pre-treatment levels. Further sampling at longer intervals after castration would have helped to determine when serum cortisol actually reached pre-treatment levels in all groups.

Local anaesthesia regime used in our experimental condition did not significantly reduce the cortisol response in all treated animals. Indeed, lidocaine injection in the spermatic cords and scrotal neck did not

eliminate acute pain suffered by lambs castrated with surgical methods. This could be due to the inability of lidocaine to reach sites where nociceptor impulses were generated (Stafford *et al.*, 2002). On the contrary, Thornton *et al.* (1999) reported that surgical castration following local anaesthesia did not produce changes in cortisol concentrations different from those seen in control lambs. The poor anaesthetic effect during Burdizzo castration had been reported by other authors (Kent *et al.*, 1998; Mellema *et al.*, 2005). In our study, it appeared that lidocaine failed to prevent pain produced during clamping, as confirmed by lambs vocalizing and kicking when the Burdizzo castrator was applied. If more time had been allowed between lidocaine injection and bloodless castration, the pain reduction method would probably have been more effective. Except for glucose, serum biochemical parameters didn't display any treatment-related changes. Serum ALT, AST, LDH, and CK values were within the normal reference intervals reported for ovine (Dimauro *et al.*, 2007). Other authors have found changes in these analytes in various species experiencing physical stress where muscle damage had occurred (Moss *et al.*, 1979; Stein *et al.*, 1985). Lambs used in this work were treated in their pen and animal-to-animal interactions were reduced. Hyperglycemia is recognized as a sign of stress in various animal species (Broom *et al.*, 2000; Perez *et al.*, 2002). Stress modifies metabolic balance, favouring anabolic processes and mobilization of energy stores, preparing the animal to cope with emergency conditions (Moberg *et al.*, 2005). Our results showed that levels of serum glucose in each group peaked at 20 minutes p.t., then gradually fell without reaching pre-treatment values, but approached those of the control group. The glucose curve was similar to the cortisol curve in each castration group while levels

in the H group remained at the baseline. These data confirm that lamb castration caused a glycaemia increase, that was higher following the use of surgical techniques than in the bloodless method.

The current study also examined the dynamics of lesion resolution in the different groups in order to determine the best castration technique for extensively managed adult lambs. Lambs castrated with the Burdizzo showed quicker wounds healing with respect to those who had undergone surgery, with the latter requiring a longer time for complete resolution of skin lesions. Orchiectomy, owing to the inevitable presence of blood or serum collecting in the scrotal sack in the early days post treatment, easily predisposed lambs to bacterial infection and parasitic infestation. Even though scrotal ablation is a more time-consuming procedure, no post-surgical complications occurred in this treatment group. Technical improvements may make this technique more easily applicable under field conditions.

The different castration techniques used in this work did not affect weight gain of lambs. This is in agreement with the results previously reported by Melches *et al.* (2006) that found no significant differences between weight gains of lambs castrated by bloodless and surgical methods at 5 weeks post treatment.

Conclusions

On the basis of our results, we suggest that the Burdizzo method is the preferable castration technique in 4-5 months old lambs, as it proved to be less stressful to the animals than surgical techniques. The cortisol response elicited by this bloodless method was lower with respect to the other methods tested. Moreover, the Burdizzo technique also required less time and

lesions were limited to the skin, avoiding the risk of complications that might occur in extensive management conditions. Nev-

ertheless, if a surgical technique is required, scrotal ablation is the preferred alternative to orchiectomy in extensively reared lambs.

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