Effect of flunixin meglumine on pain associated with cautery disbudding on dairy goat kids

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Research Article

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Abstract

At 7 days of age, 30 goat kids were randomly allocated to three groups: Sham (Sh) control, (i.e., simulating disbudding); Disbudding (Di), using thermal cauterization; Disbudding + Flunixin (DiFl), thermal cauterization + flunixin meglumine (2.2 mg/kg body weight). Each kid was video-recorded for 30 min before and after treatment to evaluate the frequency of head shaking, head scratching, body shaking, grooming, head rubbing, jumping, running, and bleating. Blood samples were taken 30 minutes after disbudding to evaluate serum cortisol concentrations and white blood cell count. Heart rate (HR) and respiratory rate (RR) were measured using a stethoscope. Liveweight of goat kids was recorded at birth, the day before and 7 days after treatment, and at 25 days of age. The mean frequency of behaviors, such as head shaking (Sh: 3.42 ± 3.58; Di: 37.5 ± 47.8; DiFl: 4.02 ± 2.76) and head scratching (Sh: 2.2 ± 1.8; Di: 33.32 ± 31.2; DiFl: 4.42 ± 3.72) after treatment (corrected by pre-treatment values) were higher (p = 0.0001) in Di than in Sh and DiFl, and the frequency of jumping was higher (p = 0.022) for Di (3.74 ± 2.24) than that of DiFl (0.39 ± 0.92). The remaining behaviors did not show differences (p > 0.05). HR and RR were higher (HR: p < 0.0048; RR: p < 0.035) in group Di (HR: 156 ± 13.6; RR: 66 ± 14.8) than in Sh (HR: 138 ± 8.48; RR: 55.6 ± 5.4) and in DiFl (HR: 136 ± 6.38; RR: 52.8 ± 4.13). No differences were detected between liveweight, white blood cell count data (p > 0.05). Based on the results, it appears that flunixin meglumine reduced pain associated with disbudding and improves animal welfare.

Introduction

In natural settings, horns play a role in defence against predators and as a weapon in agonistic confrontations between males to gain mating access to females; the same can occur in domestic ruminants (Al-Sobayil 2007). Dehorning or disbudding are the most commonly used tools to reduce the risk injuries and improve the safety of the operators handling these animals (Harjinder et al. 1980).

In goats, disbudding is generally recommended over dehorning as it causes less pain and tissue damage, and with reduced healing times (Stafford and Mellor 2011; Neely et al. 2014; Adcock and Tucker 2018). Disbudding involves the use of a hot iron (electric or gas-powered) to remove of the horn buds at a very young age, on average 10.6 ± 5.7 (mean ± SD) days old to prevent horn growth (Hempstead et al. 2020). At this early age, disbudding is assumed to cause less pain and tissue damage as at approximately 14 days of age, the horn bud starts to attach to skull and become differentiated as a horn. Incorrect application of the hot iron can cause thermal damage to the skull and cerebrum, which may lead to a bacterial infection, meningoencephalitis, and death (Thompson et al. 2005).

Other disbudding methods used in goat kids that were originally designed for calves, such as cryosurgical disbudding or the application of a chemical caustic paste. Both techniques appear more painful than cautery disbudding in goat kids (Hempstead et al. 2018a, b).

Regardless of the method used, disbudding causes pain and stress for goat kids, which negatively impacts on animal welfare. However, the impact can be mitigated with treatments involving anesthetics
and/or analgesics (Álvarez et al. 2009; Ajuda et al. 2020; Hempstead et al. 2020b).

Stress in animals can be measured using blood parameters, such as cortisol, hematocrit and neutrophils to lymphocytes ratio, catecholamine, corticotropin-releasing factor, glucose, β-hydroxybutyrate, creatinine phosphokinase, and lactate, which are correlated with physiological variables, such as body temperature, heart rate, respiratory rate (Caballero and Sumano 1993; Tadich et al. 2000).

Pain can be objectively evaluated using physiological parameters, such as respiratory rate and/or heart rate, which increase after the procedure (Heinrich et al. 2010). Moreover, neuroendocrine biomarkers associated with the neuroendocrine axis are used to evaluate painful procedures related to inflammation, such as prostaglandins, catecholamines or cortisol. While blood cortisol concentrations are widely used as a stress indicator, it should be considered with caution because a cortisol increase may be due to other stressors (Stock et al. 2013).

Pain can also be evaluated through behavioral changes (Weary et al. 2006). The absence of normal behavior is the most remarkable sign of pain in animals. Although there are individual and species variations, some common signs that an animal is in pain include changes in behavioral patterns, appearance, posture, gait, appetite, and weight (Anil et al. 2002). The analysis of behavior using ethograms is a mechanism for assessing animal welfare (Weary et al. 2006; Stock et al. 2013).

One of the most widely used pharmacological therapies to reduce pain in farm animals are non-steroidal anti-inflammatory drugs (NSAIDs) (Mathews 2002; Smith et al. 2008). NSAIDs inhibit the synthesis of inflammatory mediators, such as prostaglandins, involved in inflammatory response and responsible for producing vasodilation and leukocyte accumulation (Clark-Price 2014). At the same time, the use of an NSAID relieves pain and inflammation without the immunosuppressive and metabolic side effects of corticosteroids (Hassan et al. 2016). Flunixin meglumine is versatile NSAIDs used in veterinary medicine to alleviate inflammation and fever, and as efficient analgesic to control post-surgical pain of soft tissues (Huber et al. 2013).

The objective of this research was to evaluate the physiological and behavioral parameters in Saanen goat kids treated with flunixin meglumine compared with no analgesic treatment after cautery disbudding using a device fueled by propane gas.

**Materials And Methods**

This preliminary study was carried out in Cerrillos city, in the Goat Dairy Unit of the Experimental Station of Salta (24° 53’36”S and 65° 28’14”W) during July 2020. The working protocol was approved by the Institutional Committee for the Care and Use of Experimental Animals of the Instituto Nacional de Tecnología Agropecuaria de Argentina of Salta – Jujuy, Acta N°16/21.

Thirty Saanen goat kids (15 females and 15 males), according birth weight and sex, were randomly assigned to three treatments: Sham (Sh): control – disbudding simulated for 10 s (following the method
described by Hempstead et al. 2018a), Disbudding (Di): disbudding using a cautery iron (The Coburn Company, Inc., reaches 600°C) fuelled by propane for 5 s per horn bud, as described by Hempstead et al., 2017, and Disbudding + Flunixin (DiFl): same procedure as for Di + treatment with intravenous flunixin meglumine (0.08 ml; BANAMINE® – 50 mg / ml, MSD Animal Health, Argentina) (following method and doses described by Ajuda et al. 2020).

Within the first 12 hours of life, the goat kids were separated from their dams, fed with colostrum ad libitum extracted manually from their dams. Kids were allocated to 3 pens (10 per pen) prior to the beginning of treatment. During the experiment, kids were provided with goat milk in feeders (DeLaval Plastic Five Feeder) twice a day (1.5 liter/animal/day); water and alfalfa hay were provided ad libitum.

At 7 days of age, kids were disbudded.

**Behavioral measurements**

The day before disbudding and immediately after the procedure, each animal was housed individually in a pen and recorded using a phone camera placed on one of the corners of the pen for 30 minutes. Phone camera was it on a stand 1,60 m from ground. Then, each video recording was downloaded as video file and analyzed by an experienced operator to evaluate the behavior according to the ethogram used by Hempstead et al. (2018a) for goat kids (Table 1). Data were stored in excel file until statistical analysis.
Table 1
Ethogram of goat kid behavior sourced from Hempstead et al. (2017).

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head shaking</td>
<td>A rapid and continuous tilting of the head from side to side, concluding with a return to a neutral position. Head shakes separated by &gt; 1 s are considered separate events.</td>
</tr>
<tr>
<td>Head scratching</td>
<td>The rear foot scratches any part of the head or neck. Scratches separated by &gt; 1 s are considered separate events.</td>
</tr>
<tr>
<td>Grooming</td>
<td>The mouth contacts any part of the body or legs (excluding the hoof) with a rhythmic back and forth motion. A separate grooming event was considered to occur after a pause of &gt; 1 s.</td>
</tr>
<tr>
<td>Head rubbing</td>
<td>Tilting of the head so that horn buds contact any surface while moving head back and forth (lasts more than 1 s). Head rubs separated by &gt; 3 seconds are considered separate events.</td>
</tr>
<tr>
<td>Jumping</td>
<td>Ears pulled back with head raised followed by raised ears so front legs are lifted off the ground and knees bend towards the body. Event concludes with return of front feet to the ground.</td>
</tr>
<tr>
<td>Body shaking</td>
<td>Body moves from side to side. Body shakes separated by &gt; 1 s are considered separate events.</td>
</tr>
<tr>
<td>Running</td>
<td>A burst of forwards or sideways motion faster than a walk that lasts &gt; 2 s.</td>
</tr>
<tr>
<td>Bleat</td>
<td>Vocalization produced from an open or closed mouth. A separate event is defined by &gt; 3 seconds are considered separate events.</td>
</tr>
</tbody>
</table>

Goat kids were weighed at birth, one day before disbudding, 7 days after disbudding, and at 25 days of age.

**Heart and respiratory rate**

After the 30-minute video recording period ended, the following was performed: heart rate was assessed via auscultation of left hemithorax using a stethoscope and using a stopwatch was counted for X s, and then the value multiplied by X (beats per minute). Respiratory rate was assessed by palpation of the thorax and counting thoracic movements (breaths per minute).

**Blood sampling**

Blood sampling was carried out with the help of another operator, the order for extract sample was defined conform behavior observation was concluded. Samples (10 ml) were collected by jugular venipuncture (21 needle gauge) for serum cortisol concentrations and white blood cell count. Cortisol was determined only in animals of treatments Di and DIFI and by chemiluminescence method.

The kids' blood samples were collected with anticoagulant (EDTA) for further relative and absolute leukocyte formula. The reading of the total white blood cells was carried out by means of 10x and 40x optical microscopy in a Neubauer chamber (blood sample preparation with a 1/20 ratio of white blood cells in Turk's solution). For the relative and absolute leukocyte formula, count and identification of the
different profiles of leukocytes, the May-Grunwald-Giemsa staining method under light microscopy (at 100x) was utilized. Leukocyte values obtained are expressed as number of cells per cubic millimeter.

**Statistical analysis**

InfoStat 2018v Statistical Software was used to analyse data. Due to the lack of normality of all the variables, the data were logarithmically transformed (x = log10 (x + 1). Differences in behaviour frequency, live weight, leukocytes data, cortisol concentration, and heart and respiration rate were compared among groups using an analysis of variance with sex as a fixed effect, and its interaction and pre-treatment weight as covariance for live weights analysis and pre-disbudding behaviour frequency as a covariant for post-disbudding behaviour frequency analysis. Differences among groups were assessed using Fisher's least significant differences test, with a significance level of 5%.

**Results**

Table 2 shows goat kid behavior data before and after disbudding. There were no differences between the most of pre-treatment behaviors, only body shaking differed pre-disbudding between groups (p = 0.0093). Post-treatment frequency differences were observed in head shaking (p = 0.0001), head scratching (p = 0.0001), jumping (p = 0.022) and running (p = 0.025). Regarding differences between treatments, Di showed a higher frequency (number of behavior/ 30 minutes) percentage of head shaking (83.4%) than Sh (7.6%) and DiFl (8.9%) and Di showed a higher frequency of head scratching (87.8%) than Sh (5.1%) and DiFl (11.1%). Di (52.8%) only differed from DiFl (5.5%) (p < 0.05) in the percentage of kids that running was registered. Figure 1 illustrates the difference in the number of behaviors observed during 30 min in each of the groups.
Table 2
Mean frequency (± standard deviation) of goat kid behaviors before and after treatment.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Pre-disbudding</th>
<th>Post-disbudding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Disbudding</td>
</tr>
<tr>
<td>Head Shaking</td>
<td>3.31 ± 1.98</td>
<td>6.31 ± 9.01</td>
</tr>
<tr>
<td>Body Shaking</td>
<td>0.19 ± 0.28 a</td>
<td>1.64 ± 2.14 b</td>
</tr>
<tr>
<td>Head Scratching</td>
<td>2.82 ± 2.8</td>
<td>2.81 ± 1.92</td>
</tr>
<tr>
<td>Self-Grooming</td>
<td>4.42 ± 6.1</td>
<td>6.42 ± 8.18</td>
</tr>
<tr>
<td>Head Rubbing</td>
<td>1.98 ± 5.31</td>
<td>0.28 ± 0.38</td>
</tr>
<tr>
<td>Jumping</td>
<td>5.91 ± 4.76</td>
<td>4.11 ± 3.74 ab</td>
</tr>
<tr>
<td>Running</td>
<td>6.83 ± 6.56</td>
<td>7.33 ± 13.82 b</td>
</tr>
<tr>
<td>Bleating</td>
<td>1.44 ± 1.39</td>
<td>1.36 ± 2.23</td>
</tr>
</tbody>
</table>

Means with a different superscript are significantly different (p < 0.05).

Results of physiological variables are presented in Table 3. No differences were recorded among treatments in birth weight (p = 0.38), and live weight on the day before (p = 0.52) and after (p = 0.159) treatment. Live weight at 25 days of age did also not show statistically significant differences among treatments (p = 0.43). Mean cortisol concentration 30 minutes after disbudding was similar in Di and DiFl treatments (p = 0.25).
Table 3
Mean values (± standard deviation) of birth weight, weight before and after treatment, and cortisol, heart rate and respiratory rate of goat kids after treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham</th>
<th>Disbudding</th>
<th>Disbudding + flunixin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3.31 ± 0.23</td>
<td>3.53 ± 0.5</td>
<td>3.58 ± 0.55</td>
</tr>
<tr>
<td>Pre-treatment weight</td>
<td>4.48 ± 0.53</td>
<td>4.73 ± 0.61</td>
<td>4.74 ± 0.58</td>
</tr>
<tr>
<td>Post-treatment weight</td>
<td>5.06 ± 0.51</td>
<td>5.43 ± 0.6</td>
<td>5.46 ± 0.66</td>
</tr>
<tr>
<td>25 day old weight</td>
<td>6.11 ± 0.77</td>
<td>6.26 ± 0.63</td>
<td>6.44 ± 0.58</td>
</tr>
<tr>
<td>Cortisol</td>
<td>sd</td>
<td>2.62 ± 0.55</td>
<td>2.87 ± 0.31</td>
</tr>
<tr>
<td>Heart rate</td>
<td>138 ± 8.49 a</td>
<td>156.4 ± 13.66 b</td>
<td>136.4 ± 6.38 a</td>
</tr>
<tr>
<td>Breathing frequency</td>
<td>55.6 ± 5.48 ab</td>
<td>66 ± 14.88 b</td>
<td>52.8 ± 4.13 a</td>
</tr>
</tbody>
</table>

Means with a different superscript are significantly different (p = 0.05).

Heart rate was higher in Di kids than in those in Sh and DiFl (p = 0.0048), whereas respiratory rate was higher in Di kids than in those of DiFl (p = 0.035).

Table 4 shows the relative values of white blood cell count of goat kids after treatment. No statistically significant differences between treatments were recorded for different types of leukocytes and for the neutrophil to lymphocyte ratio (p = 0.57). However, Di had a tendency to show higher values of leukocytes, segmented neutrophils, band neutrophils and total neutrophils and of lymphocytes than Sh and DiFl.
Table 4
Mean values (± standard deviation) associated with the white blood cell count of goat kids relative to treatments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham</th>
<th>Disbudding</th>
<th>Disbudding + flunixin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes/mm³</td>
<td>12960.9 ± 4062.9</td>
<td>15015 ± 2860.7</td>
<td>13735.2 ± 2623.8</td>
</tr>
<tr>
<td>Segmented Neutrophils/mm³</td>
<td>4987.6 ± 1934.8</td>
<td>5603.1 ± 2212.9</td>
<td>4922.8 ± 1026.5</td>
</tr>
<tr>
<td>Band Neutrophils/mm³</td>
<td>142.2 ± 155.7</td>
<td>221.8 ± 264.2</td>
<td>300.8 ± 188.2</td>
</tr>
<tr>
<td>Total Neutrophils/mm³</td>
<td>4987.6 ± 2052.2</td>
<td>5603.1 ± 2212.9</td>
<td>4976.3 ± 1159.9</td>
</tr>
<tr>
<td>Lymphocytes/mm³</td>
<td>7604.4 ± 3310.1</td>
<td>9683.6 ± 2722.3</td>
<td>6294.3 ± 800.2</td>
</tr>
<tr>
<td>Monocytes/mm³</td>
<td>441.4 ± 441.3</td>
<td>324.8 ± 263.9</td>
<td>335.9 ± 228.6</td>
</tr>
<tr>
<td>Eosinophils/mm³</td>
<td>214 ± 221</td>
<td>136.8 ± 177.1</td>
<td>175.6 ± 238.2</td>
</tr>
<tr>
<td>Basophils/mm³</td>
<td>21.6 ± 64.7</td>
<td>11 ± 34.8</td>
<td>16.7 ± 44.2</td>
</tr>
<tr>
<td>Neutrophils/Lymphocytes</td>
<td>0.8 ± 0.6</td>
<td>0.7 ± 0.4</td>
<td>0.8 ± 0.3</td>
</tr>
</tbody>
</table>

Discussion

Cautery disbudding is applied to goat kids to avoid horn growth, but that causes pain and distress (Álvarez et al. 2009; Ingvast-Larsson et al. 2011; Hempstead et al. 2020a). During disbudding, kids usually exhibit have higher frequency of leg shaking, as well as intense and frequent vocalizations. These responses suggest acute pain (Álvarez and Gutiérrez 2010; Álvarez et al. 2015). In turn, immediately after disbudding, kids also have frequent shakes of body and head, and more prolonged head scratching events compared with non-disbudded kids, which also indicates pain (Hempstead et al. 2017, 2018a). Disbudding also induces an immediate elevation of cortisol secretion, which returns to basal levels approximately 30 minutes after disbudding (Álvarez et al. 2015; Hempstead et al. 2018c).

In the present study, the observed differences in behavior were evident due to the positive effects of anti-inflammatory and analgesic treatment. Frequent head shakes after disbudding may be the mode of manifesting pain in goat kids (Greenwood and Shutt 1990), whereas the successful administration of analgesics, like NSAIDs, may reduce the incidence of head shakes (Ingvast-Larsson et al. 2011). This positive effect was confirmed by the higher number of head shaking and head scratching of goat kids of group Di, as well as by a higher number of jumps. These data are in agreement with findings of Hempstead et al. (2017), who indicated that behaviors involving the head seem to be the best indicators of pain, since goat kids subjected to thermal cautery disbudding performed head shaking, scratching and rubbing more frequently than those subjected to simulated disbudding. The highest frequency of head-related behaviors may be due to the fact that this region of the body is directly affected by horn bud cauterization and the resulting tissue damage.
Liveweight was similar among goat kids of the three groups, both before and after treatment until 25 days of age. Our results are similar to those reported by Hempstead et al. (2018c), who did not find significant differences between weight of goat kids subjected to several disbudding techniques for 7 days after treatment. Physiological variables, like heart rate and respiratory rate, have been used as indicators of acute stress in animals (Mohr et al. 2002). However, Álvarez et al. (2009) did not find differences in heart or respiratory rate between disbudded goat kids, with or without local anesthesia.

The present results show higher heart rates in goat kids of group Di than in those of Sh or DiFl, although values were within standard parameters expected in goat kids, between 145–240 beats/minute (Miller and Robertson 1945; Raggi and Boza 1986) and between 140–180 beats/minute reported by Álvarez et al. (2009) in goat kids subjected to disbudding (with and without anesthesia). On the other hand, while respiratory rate was also higher in goat kids of Di treatment than in those of DiFl, in the three groups this parameter was higher than the 12–20 breaths/minute suggested as standard for the species and age (Miller and Robertson 1945; Raggi and Boza 1986); however, in all cases of the present work, respiratory rate was within the range of 40–70 breaths/minute reported by Álvarez et al. (2009). The obtained results suggest that the high respiratory rate detected in all the goat kids maybe due to the stress associated with handling before (and during) treatment.

No statistically significant differences in cortisol concentrations were detected between goat kids of the Di and DiFl groups. However, the obtained values in the present study (2.75 ± 0.45 µg/dl) are similar to those reported by Hempstead et al. (2018c), which were measured 15 minutes after disbudding treatment using an electric iron. The same authors reported that the maximum level of cortisol was measured 15 min after disbudding, and had returned to baseline by 30 min (Hempstead et al. 2018c). The time, 30 minutes, after disbudding the cortisol was evaluated in this experiment, may explain lack of differences. A further limitation of the present study, is that due to human mistake, baseline cortisol concentrations sample taken got lost. Concentrations of some blood components used as stress indicators vary with increasing discomfort, fright and defense of animals. The stress leukogram presents mild mature neutrophilia, lymphopenia, monocytosis, and eosinopenia. Lymphopenia is the only specific parameter and indicates a chronic stress state (Schaefer et al. 1997; Pargas-Alvarado et al. 2014). The increase in neutrophils indicates a transient stress state; in this work, no differences among groups were observed, although the neutrophil count in Di would indicate a tendency to be higher than in the other group.

The differences observed in the DiFl group, i.e., reduced head shaking, head scratching and jumping, and lower heart and respiratory rates, suggest the efficacy of flunixin meglumine in relieving pain and improving welfare of goat kids that experience cautery disbudding. However, this conclusion is drawn from a preliminary trial; therefore, further studies should be conducted to confirm this finding.

Declarations

Funding
This research was funded by the project Animal Welfare of the National Institute of Agricultural Technology of Argentina.

Data availability

All data generated or analyzed during this study are included in this article.

Consent to participate

Authors have permission to participate.

Consent for publication

Authors have permission for publication.

Competing interests

The authors declare that they have no conflicts of interest.

Authors' Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by GMM, VHS, EA, JA and LCC. The first draft of the manuscript was written by GMM, VHS and MH. All authors commented on previous versions of the manuscript. All authors approved the final manuscript.

Ethics approval

This study has been approved by a research ethics committee of the National Institute of Agricultural Technology of Argentina (Regional Centre Salta - Jujuy). Acta No 16/21 of 1 July 2021.

References


17. Hempstead MN, Waas JR, Stewart M, Sutherland MA (2020a) Goat kids are not small calves: Species comparisons in relation to disbudding. Anim Welf 29(3):293–312


Figures

Figure 1

Back-transformed mean number with 95% confidence intervals of goat kid behaviours group frequencies (N°/30 min) post-treatment. Sham: control simulating disbudding; disbudding: disbudding using thermal
cauterization; disbudding+flunixin: disbudding using thermal cauterization plus flunixin meglumine. * Indicates the behaviours that differ between treatments at the 5% significance level.