

Effects of Meloxicam on Oxidative Stress in Calves Undergoing Dehorning with Caustic Paste

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ABSTRACT

Background: Dehorning is a common practice in cattle operations aimed at enhancing animal safety, reducing the risk of injury to other animals and humans, and limiting aggressive behaviors. All dehorning methods, including thermal, surgical, and caustic paste application, cause pain, stress, and physiological changes in the animals. Non-steroidal anti-inflammatory drugs (NSAIDs), particularly meloxicam, are used to alleviate pain and inflammation associated with the procedure. This study aimed to investigate the effect of dehorning using the caustic paste method, with and without the administration of painkillers, on both clinical indicators and blood parameters in calves.

Materials, Methods & Results: The study involved 24 calves, arbitrarily divided into 2 groups, each comprising 12 calves. In group I, each calf received an intravenous (IV) dose of 2.2 mg/kg of meloxicam dispensed 30 min before dehorning, followed by caustic paste for horn blunting. In group II, the calves' horns were blunted using the same technique but without administering analgesia. Blood samples were collected at 0, 15, 30, and 60 min from calves in both groups, and clinical observations were documented. Cortisol levels (measured via ELISA), glucose levels (measured via ELISA), total oxidant capacity (TOC), total antioxidant capacity (TAC) (measured via ELISA), and glutathione (GSH) levels (measured via ELISA) were assessed in the collected blood samples. Cortisol levels in Group I showed a significant reduction at 15, 30, and 60 minutes compared to baseline ($P < 0.001$), whereas no significant changes were observed in Group II throughout the measurement times ($P > 0.05$). Glucose levels in Group I were lower than those in Group II at 30 and 60 min; however, the difference was not statistically significant ($P > 0.05$). When evaluated in terms of oxidative stress parameters, Group I showed significantly lower total oxidant capacity (TOC) values ($P < 0.001$) and total antioxidant capacity (TAC) values ($P < 0.05$) compared to Group II, whereas glutathione (GSH) values were higher ($P < 0.001$). These findings indicate that administering analgesics before dehorning reduces cortisol levels, alleviates oxidative stress, and supports the antioxidant system.

Discussion: Dehorning is an economically significant practice in cattle operations; however, it induces considerable stress, particularly in adult animals. Performing the procedure as early as possible, within the 1st 2 months after birth, minimizes discomfort in the animals. The method used also represents a key factor affecting stress levels; in calves aged 8-14 days, the caustic paste method emerges as the least painful and most practical approach. In this study, physiological parameters (heart rate, respiratory rate, and rectal temperature) generally remained within reference ranges, with a brief increase in heart rate indicating acute stress. In the group receiving meloxicam, lower cortisol and glucose levels demonstrated that analgesia effectively reduces pain-induced stress and inflammation. Regarding oxidative stress parameters, TOC decreased, while TAC and GSH levels increased in the analgesia group. Conversely, opposite trends were observed in the non-analgesia group, indicating higher stress and oxidative load in animals that did not receive analgesic treatment. In conclusion, the use of sedation and local anesthesia combined with analgesia effectively alleviates pain and stress during caustic paste dehorning, rendering this method a safe, effective, and practical procedure for calves.

Keywords: dehorning, meloxicam, oxidative stress, calves.

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INTRODUCTION

Dehorning is a common management practice in cattle operations to enhance safety, prevent injuries, reduce aggressive behavior, and improve handling efficiency [9,24,25]. It protects other animals, handlers, and infrastructure while optimizing space in feeding and housing areas [12]. Standard techniques for young calves include thermal disbudding, surgical removal, and caustic paste application [17,20,21]. Thermal methods destroy dermal and epidermal tissues using heat, while surgical methods remove horn buds and surrounding skin. Caustic pastes chemically inhibit horn growth using substances like calcium hydroxide [3,13]. Regardless of the technique, dehorning causes pain, behavioral changes, and physiological stress, including elevated heart rate and cortisol levels, which may impact growth and milk production [8,14,15]. Anesthesia and nonsteroidal anti-inflammatory drugs (NSAIDs), particularly meloxicam, reduce pain and inflammation by inhibiting cyclooxygenase and prostaglandin synthesis, with a particular focus on COX-2 [1,4,5,7]. Although studies have examined behavioral and physiological stress, oxidative stress, and antioxidant imbalance resulting from chemical dehorning, these aspects remain less understood.

This study aims to examine the anesthesia protocol used and assess the impact of meloxicam, a non-steroidal anti-inflammatory analgesic, on the oxidative stress associated with the horn-blunting procedure using calcium hydroxide, as well as the resultant antioxidant imbalance. The findings will provide insights into reducing stress during the dehorning process.

MATERIALS AND METHODS

Materials

The animal subjects in the study consisted of 24 Simmental calves of varying genders, with a mean age of 2 weeks (± 2 days) and a mean live weight of 50 kg (± 15 kg). These calves were procured from the Haliloğulları Dairy Farm in the Eleşkirt District of Ağrı Province. They were randomly separated into 2 groups: Group I (analgesia group), consisting of 12 animals each, received a painkiller 2% Meloxidyl[®] [2.2 mg/kg meloxicam – intravenously (IV)] in addition to caustic paste application. Group II (non-analgesia group) was further subdivided into 2 groups, where the dehorning process was conducted solely through the application of caustic paste.

Methods

In Group I, cases receiving the painkiller along with the caustic paste, only calves that underwent a thorough general examination and were deemed healthy were included in the study. Before any procedure was carried out on the animals in this group, their respiratory rate, heart rate, and rectal temperature were noted. A 1.0×32 mm diameter intravenous (IV) catheter was inserted into the left jugular vein for blood collection and medication-applied purposes. For biochemical analyses, 7 mL of blood was drawn from the jugular vein into glass vacuum tubes without an anticoagulant. This time point was documented as min 0 in the study. Simultaneously, the animals were sedated by administering Xylazine HCl² [2% Rompun[®] - 0.2 mg/kg, IV]. Five min after sedation, meloxicam was administered intravenously to the cases at a dosage of 2.2 mg/kg. During the same timeframe, 2% Lidocaine¹ [5 mL of Lidocaine HCl] was administered to each horn area to anesthetize the cornual nerve. Fifteen min after sedation, the horn blunting was conducted using the same method. For the dehorning procedure, the calf was securely held by an assistant to prevent movement, and the hair surrounding the horn button was trimmed with scissors to expose the horn button. Vaseline was then applied around the horn button to safeguard the skin and eyes from the chemicals to be utilized. The chemical substances employed were cylindrical calcium hydroxide sticks³ (Redfort Caustic Stick - 10 g), specially crafted for this intent. After moistening the tips of the caustic sticks slightly with water, they were pressed onto the horn button and applied in circular motions. The process was concluded when the horn button initially turned white and began to display signs of bleeding. Clinical parameters were assessed and recorded at the 15th, 30th, and 60th min following the initial application, and blood samples were obtained for biochemical analysis. In group II, only cases in the caustic paste group were included. The anesthesia protocol and blunting procedure were administered to the cases in this group using the same method as in group I. However, unlike Group I, no analgesia was administered in Group II.

Enrofloxacin⁴ [10% Baytril - single dose of 2.5 mg/kg, IV] was administered to all cases in the

study for prophylaxis. Furthermore, a skin ointment containing oxytetracycline⁴ [Terramycin ointment] was applied to the wound resulting from the blunting procedure.

Biochemical analysis

Blood samples collected at 0, 15, 30, and 60 min post-application were promptly transported to the laboratory, where they were centrifuged at 6000 rpm for 10 min at room temperature. The serum was then isolated and preserved at -80°C until analysis. Cortisol concentrations were measured using a commercial ELISA kit⁵ [Bovine (Cortisol) ELISA kit] designed for bovine cortisol detection. At the same time, glucose levels were assessed with a Glucose Colorimetric Assay Kit⁶ [Glucose Colorimetric Assay Kit] via the ELISA method. Total oxidant capacity (TOC)⁵ [Bovine (TOC) ELISA kit] and Total Antioxidant capacity (TAC) 5 [Bovine (TAC) ELISA kit] were determined to evaluate the oxidative stress index, and Glutathione (GSH)⁶ [Glutathione Assay kit] levels were measured to evaluate antioxidant potential. These assessments were conducted using commercial (ELISA kits) designed explicitly for bovine samples.

RESULTS

Clinical findings

In terms of heart rate, no statistically significant differences were observed either within groups or between groups at any time point in Group I and Group II ($P > 0.05$). Regarding respiratory rate, intra-group comparisons in Group I showed no statistically significant differences across all measurement times ($P > 0.05$). In Group II, no significant difference was observed between 0 and 15 min ($P > 0.05$); however, the decrease between 0 and 30 min was statistically significant ($P < 0.05$). No significant differences were found between 0 and 60 min ($P > 0.05$). A significant decrease was detected between 15 and 30 min ($P < 0.05$), whereas no significant differences were observed between 15 and 60 min or between 30 and 60 min ($P > 0.05$). Intergroup comparisons revealed no significant differences at 0 and 15 min ($P > 0.05$), while statistically significant differences were found at 30 min ($P < 0.05$) and 60 min ($P < 0.05$).

In Group I, intra-group comparisons revealed that the increase in rectal temperature observed at

15 and 30 min compared to 0 min was statistically significant ($P < 0.05$). No significant differences were detected between 0 and 60 min, 15 and 30 min, 15 and 60 min, or 30 and 60 min ($P > 0.05$). In Group II, no significant difference was found between 0 and 15 min ($P > 0.05$), whereas the increase between 0 and 30 min was statistically significant ($P < 0.05$). Similarly, the difference between 15 and 30 min was substantial ($P < 0.05$). No significant differences were observed between 0 and 60 min, 15 and 60 min, or 30 and 60 min ($P > 0.05$). Between-group comparisons revealed no statistically significant differences at any time point ($P > 0.05$).

The assessment findings of clinical parameters are provided in Table 1.

Endocrine response

-Cortisol

In the intra-group comparison of Group I, significant differences were observed between baseline (0 min) and the measurements at 15, 30, and 60 min. Cortisol levels decreased significantly at 15, 30, and 60 min compared to pre-application values ($P < 0.001$). Moreover, cortisol levels at 15 min differed considerably from those at 30 and 60 min ($P < 0.001$), whereas no significant difference was detected between 30 and 60 min ($P > 0.05$). In Group II, no statistically significant differences were observed among all time points ($P > 0.05$). Between-group comparisons revealed that cortisol levels were significantly lower in Group II than in Group I at 0, 15, 30, and 60 min ($P < 0.001$). At all measurement times, cortisol levels in Group II remained lower than those in Group I. In Group II, cortisol levels remained low and relatively stable throughout the measurement period. This pattern can be attributed to the baseline (pre-application) cortisol levels, which were already lower compared to Group I. The cortisol level assessment outcomes of the groups are depicted in Figure 1.

-Glucose

In terms of glucose level findings, no statistically significant discrepancy was observed among the assessment results at any time in both intra-group and inter-group comparisons in both groups ($P > 0.05$). The glucose concentration assessment outcomes of the groups are depicted in Figure 2.

Biochemical findings

-Total Oxidant Capacity (TOC)

In intra-group comparisons, Group I showed no statistically significant changes in TOC between baseline (0 min) and 15, 30, or 60 min ($P > 0.05$). The increase observed between 15 and 30 min was not significant ($P > 0.05$), whereas the rise between 15 and 60 min reached statistical significance ($P < 0.05$). No significant differences were detected between 30 and 60 min ($P > 0.05$). In Group II, no statistically significant differences were observed across all time points in intra-group comparisons ($P > 0.05$). In the intergroup comparison, TOC values at baseline (0 min) did not differ significantly between the groups ($P > 0.05$). Significant differences were observed at 15 and 30 min ($P < 0.05$), whereas the 60-min measurements showed no significant difference ($P > 0.05$). Overall, Group II exhibited a greater increase in TOC compared to Group I.

-Total Antioxidant Capacity (TAC)

In intra-group comparisons of TAC assessments, no statistically significant difference was found between groups I and II at any assessment time ($P >$

0.05). However, in the comparison between groups, there was a statistically important discrepancy observed at all assessment times ($P < 0.05$), with TAC values being higher in group II.

-Glutathione (GSH)

In intra-group comparisons, Group I showed statistically significant increases in GSH levels between baseline (0 min) and 15 ($P < 0.05$), 30 ($P < 0.001$), and 60 min ($P < 0.001$). No significant difference was observed between 15 and 30 min ($P > 0.05$), while a significant increase was noted between 15 and 60 min ($P < 0.05$). No significant difference was detected between 30 and 60 min ($P > 0.05$). In Group II, intra-group comparisons revealed no statistically significant changes at any time point ($P > 0.05$). In intergroup comparisons, GSH levels at baseline (0 min) did not differ significantly between the groups ($P > 0.05$). However, significant differences were observed at 15, 30, and 60 min ($P < 0.001$). Overall, GSH levels in Group I showed a greater increase compared to those in Group II relative to the baseline.

The measurement results of the biochemical parameters are presented in Table 2.

Table 1. Changes in heart, respiratory rate, and body temperature are observed depending on the time of the groups.

Time (min)	Group	Heart Rate (rate/min)	Respiratory Rate	Rectal Temperature (°C)
0	I	141.33 ± 18.20	60.33 ± 3.80	38.65 ± 0.21 ^a
	II	138.66 ± 10.46	54.33 ± 4.91 ^a	38.63 ± 0.21 ^a
15	I	147.00 ± 23.35	60.00 ± 6.77	39.23 ± 0.11 ^b
	II	158.00 ± 14.18	55.33 ± 6.05 ^a	38.81 ± 0.16 ^a
30	I	144.66 ± 19.19	62.00 ± 3.96*	39.26 ± 0.08 ^b
	II	166.00 ± 15.85	38.00 ± 5.72b*	39.31 ± 0.13 ^b
60	I	145.66 ± 23.30	61.33 ± 3.21*	39.00 ± 0.30 ^{ab}
	II	146.00 ± 11.25	44.66 ± 4.88ab*	39.05 ± 0.13 ^{ab}

I= Disbudding with painkiller + caustic paste. II= Disbudding with caustic paste application only. ^{ab}In the within-group comparison, statistically meaningful differences were found between the values represented by different letters in the same column ($P < 0.05$). *In the comparisons between groups, Assessments marked with asterisks (*) at the same period are considered statistically meaningful ($P < 0.05$).

Table 2. Changes in Total Oxidant Capacity (TOC), Total Antioxidant Capacity (TAC), and Glutathione (GSH) levels are observed over time within the groups.

Time (min)	Group	TOC	TAC	GSH
0	I	46.67 ± 5.57	3.89 ± 0.18*	16.61 ± 0.84 ^{abc}
	II	53.33 ± 7.14	4.68 ± 0.10*	15.93 ± 2.08
15	I	35.00 ± 4.28 ^{ab}	3.95 ± 0.21*	20.44 ± 0.68 ^{ad} **
	II	56.67 ± 4.94*	4.54 ± 0.10*	12.87 ± 0.79**
30	I	43.33 ± 4.21*	3.35 ± 0.27*	21.95 ± 0.55 ^b **
	II	68.33 ± 9.09*	4.48 ± 0.13*	13.65 ± 0.32**
60	I	51.67 ± 4.01 ^a	3.56 ± 0.35*	23.56 ± 0.70 ^{cd} **
	II	76.67 ± 12.29	4.54 ± 0.10*	13.24 ± 0.32**

I= Disbudding with painkiller + caustic paste. II= Disbudding with caustic paste application only. ^{abc}In the within-group comparison, a statistically meaningful difference was found between the values denoted by different letters in the same column ($P < 0.05$). *In the comparisons between groups, the Assessments marked with asterisks at the same period are considered statistically meaningful ($P < 0.05$). * $P < 0.05$; ** $P < 0.001$.

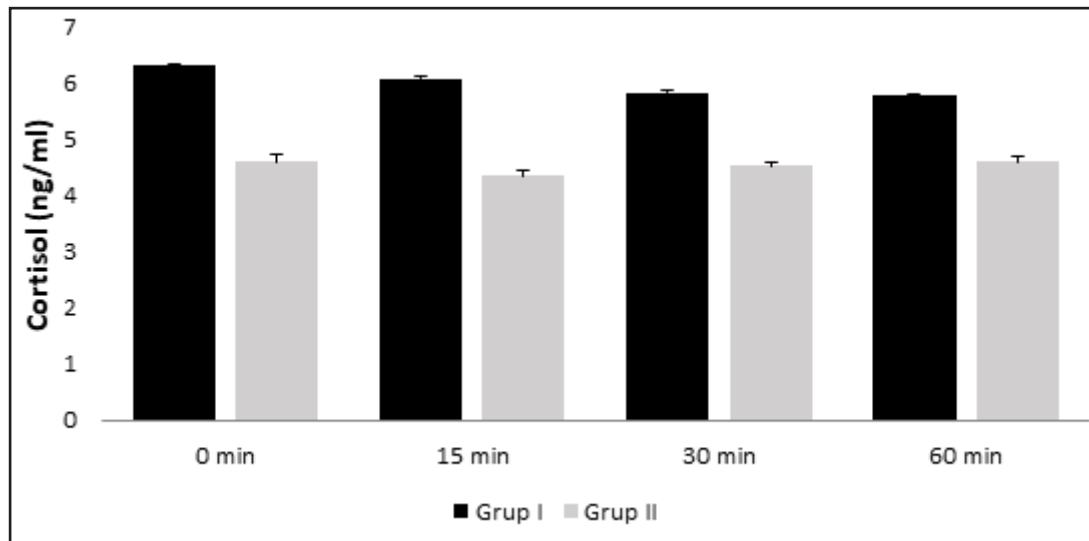


Figure 1. Changes in cortisol levels are observed depending on the time of the groups.

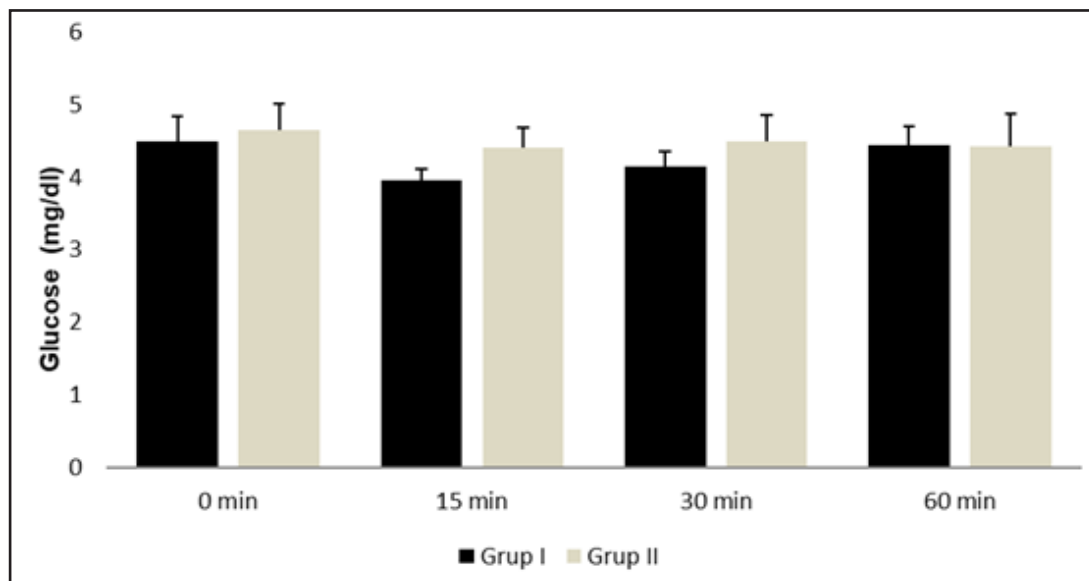


Figure 2. Changes in glucose levels are observed depending on the time of the groups.

DISCUSSION

In painful procedures, especially dehorning, physiological parameters such as respiratory rate, heart rate, and rectal temperature are frequently monitored to assess the pain-related stress response in animals [1,5,18]. Studies have shown that respiratory rate and heart rate findings vary depending on the combination of the dehorning method applied and the anesthesia and analgesia protocol used [5,24,25]. Alvarez *et al.* [1] found that the heart and respiratory rates of goats subjected to horn blunting via the cauterization method were comparable between the groups that received local anesthesia and those that did not. Heinrich *et al.* [7] observed that heart and respiratory rates increase as a result of pain in horn blunting methods. Their study noted that the heart and respiratory rate findings in calves subjected to dehorning via the cauterization method were near physiological limits in the groups receiving local anesthesia along with meloxicam, compared to those given only local anesthesia. Huber *et al.* [9] found no discrepancy in heart and respiratory rate findings among calves undergoing dehorning with electrocautery across various groups, including the control group, local anesthesia group, flunixin meglumine group, and local anesthesia combined with flunixin meglumine group. In the study by Kupczynski *et al.* [12], it was noted that heart rate meaningfully increased, particularly during the horn blunting process performed without anesthesia. Yanmaz *et al.* [24] reported that in calves subjected to horn blunting via surgical and cauterization methods, the respiratory rate remained unchanged regardless of whether analgesia was administered. Additionally, in the group that underwent the procedure without analgesia, a decrease in heart rate was observed up to the 6th h as a response to the stress stimulus. In our study, the calves subjected to horn blunting with caustic paste were separated into two groups: one receiving analgesia, sedation, and local anesthesia, and the other receiving only sedation and local anesthesia. Respiratory rate changes remained within physiological limits in both groups. Regarding heart rate, an increase was observed at the 15th min of blunting compared to the initial value in both groups. We believe that this increase indicates acute stress. It was observed that heart rate findings approached the baseline value after the 15th min in both groups. In our study, which also assessed rectal temperature, we observed an increase in analgesia in the analgesia

group compared to the initial value. But, while this rise was statistically meaningful, it remained within physiological limits, necessitating no medical intervention. Conversely, in Group II, no statistically meaningful findings in rectal temperature were observed for 60 min. We attribute the physiological parameter changes observed within reference ranges during the experiment to the anesthesia protocol and methodology used.

The increase in cortisol levels immediately following the dehorning process is indicative of pain-induced stress, while the administration of local anesthesia effectively mitigates this cortisol response [18,23]. Studies have observed that administering cornual nerve block anesthesia using lidocaine 15-20 min before horn blunting through amputation effectively suppresses the cortisol response, with cortisol levels returning to baseline within 2 h post-procedure. Additionally, intravenously administered NSAIDs, given 15-20 min before horn blunting via cauterization, not only alleviate pain but also mitigate inflammation in the resulting wound, aiding in the healing process. Although xylazine does not impact the baseline cortisol response indicative of pain during horn blunting, its sedative properties effectively eliminate behavioral responses during the procedure [22]. Numerous studies have reported a sudden spike in cortisol levels following dehorning, which persists at elevated levels for 4-5 h [1,3,11,12,23,24]. In their research, Stafford and Mellor [22] reported that administering local anesthesia to the horn area to anesthetize the cornual nerve during horn blunting via cauterization, along with the application of the nonsteroidal anti-inflammatory drug ketoprofen for analgesia, prevented the increase in cortisol levels. According to the studies conducted by Huber *et al.* [9], cortisol levels were comparable between the research and control groups where calves underwent dehorning by cauterization and received flunixin meglumine. However, in contrast to findings from other researchers [1,3,12,23-25], our study found that cortisol levels were lower than baseline in the group administered meloxicam. The serum cortisol levels in calves receiving analgesic treatment were notably lower compared to those without analgesics, suggesting that meloxicam mitigates pain-induced stress. Elevated levels of stress and inflammation lead to increased glucose production, with hyperglycemia levels rising as the severity of disease or injury escalates [2]. In their study, Yanmaz *et al.* [24] noted that the

serum glucose levels in calves undergoing the operation and in the cauterization group with applied analgesia were lower compared to those without analgesia. In our study, similar to the findings of Yanmaz *et al.* [24], serum glucose levels were lower in the analgesia group, although this difference was not statistically significant.

In studies assessing pain-induced stress during dehorning procedures, various factors are examined, including behavioral reactions, physiological parameters (such as heart rate, respiratory rate, corneal and rectal temperatures), and endocrine responses (e.g., cortisol and glucose levels). Moreover, there is a growing importance in investigating oxidative stress resulting from horn blunting methods and understanding the impact of anesthesia protocols and non-steroidal anti-inflammatory analgesics on the antioxidant defense system [25].

Numerous molecules and techniques are available for assessing oxidative stress. Several studies suggest that TOC and TAC parameters can serve as valuable noninvasive markers for oxidative stress-related diseases. Consequently, there has been a recent recommendation to measure TOC and TAC to evaluate oxidant and antioxidant status, thereby assessing the balance [10,25]. In our study, we showed a decrease in TOC assessments starting from the 15th min in the analgesia group. In contrast, there was an increase in TOC assessments beginning from the 15th min in the non-analgesia group. Regarding TAC assessments, they were higher in the non-analgesia group than in the analgesia group. We speculate that the elevated TAC concentration in the non-analgesia group may have risen in response to increased TOC levels, aiming to counteract the free radicals generated due to oxidative stress. As a crucial component of the antioxidant defense system in living organisms, GSH functions as a non-enzymatic antioxidant. Highly sensitive to damage caused by free radicals, peroxides, heavy metals,

and reactive oxygen species, GSH serves as an endogenous antioxidant, offering vital defense mechanisms and playing a meaningful role in cellular function and defense [6,16]. In our study, the decreased levels of GSH in the group without analgesia suggest that stress occurs during the horn blunting process, irrespective of the method employed, emphasizing the necessity of combining anesthesia and analgesia.

CONCLUSION

Consequently, it has been shown that employing sedation and local anesthesia during the horn blunting process with caustic paste effectively eliminates pain-induced stress in calves. However, it underscores the importance of augmenting the anesthesia protocol with a combination of analgesia to mitigate the endocrine response. Our study's findings reveal that the horn blunting process, involving the application of caustic paste in calves, is a safe and practical method that minimizes stress on the animals.

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Declaration of of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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