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Research Article

The Effect of Hot-Iron Disbudding on Thiol-Disulphide Homeostasis in Calves

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Abstract

We aimed to examine the effect of hot-iron disbudding on serum thiol-disulphide homeostasis levels as a marker of oxidant stress in relationship with trauma in calves under sedation, local anaesthesia, and the non-steroidal anti-inflammatory drug ketoprofen. A total of 30 Holstein calves were enrolled in the study and allocated into three groups: disbudded following sedation with xylazine (n=10) (group I); disbudded following sedation (xylazine) and local anaesthesia with lidocaine (n=10) (group II); and disbudded after sedation (xylazine), local anaesthesia (lidocaine), and ketoprofen (n=10) (group III). Blood samples were withdrawn before (0. min) and 30, 60, 90, and 120 min after dehorning. Serum native thiols, total thiols, and disulphide levels were detected with a novel assay. Native thiol and total thiol levels were reduced in all groups without any significance during the study period. At the 90th min of the study, native thiol levels in group II were significantly lower than in groups I and III. There were no significant alterations in total thiol levels in both groups. Disulphide levels showed no significant changes in group, time, and group by time interactions, but at the 60th min, groups I and III had the lowest levels. Disulphite/native thiol, disulphite/total thiol, and native thiol/total thiol levels had significant group alterations in the 60th min. The reduction of native thiol and total thiol levels in all groups without significance might be related to the antioxidant activity of plasma; however, it is thought that the pain management procedures should be related to the sensitive oxidative balance by thiols.

Keywords: *Calf, Disbudding, Sedation, Thiol-disulphide*

Buzağılarda Sıcak Koter İle Boynuzsuzlaştırmanın Tiyol-Disülfit Homeostazı Üzerine Etkisi

Öz

Bu çalışmada sedasyon, lokal anestezi ve non-steroidal bir ilaç olan ketoprofen uygulanmış sıcak koterizasyon işlemi ile boynuzsuzlaştırılan buzağılarda oksidatif stresin değerlendirilmesinde tiyol-disülfüt homeostazına olan etkilerinin belirlenmesi amaçlandı. Çalışmaya toplam 30 adet Holstein buzağı dahil edilerek üç gruba ayrıldı: sedasyon (xylazine) işlemini takiben boynuzsuzlaştırılan (n=10) (grup I); sedasyon (xylazine) ve lidokain ile lokal anestezi işlemi sonrasında boynuzsuzlaştırılan (n=10) (grup II); ve sedasyon (xylazine), local anestezi (lidocaine) ve ketoprofen uygulaması sonrasında boynuzsuzlaştırılan (n=10) (group III). Kan örnekleri, boynuzsuzlaştırmadan önce (0. dak) ve 30, 60, 90 ve 120 dakika sonra olacak şekilde toplandı. Serum natif tiyol, total tiyol ve disülfür seviyeleri yeni bir test ile belirlendi. Çalışma süresince natif tiyol ve total tiyol seviyelerinde istatistiksel anlamlı olmayan azalmalar belirlendi. Araştırmanın 90. dakikasında grup II'de bulunan hayvanların natif tiyol seviyelerinin grup I ve III'e göre istatistiksel anlamlı düşüktü. Total tiyol seviyelerinde ise gruplar arasında istatistiksel anlamlı farklılıkların olmadığı belirlendi. Disülfit seviyelerinde grup, zaman ve grup zaman arasında farklılıklar belirlenmezken, araştırmanın 60. dakikasında grup I ve III'te en düşük seviyelere geldiği belirlendi. Disülfit/natif tiyol, disülfit/total tiyol ve natif tiyol/total tiyol seviyelerininde 60. dakikada gruplar arasında istatistiksel anlamlı değişimler gösterdiği belirlendi. Natif tiyol ve total tiyol seviyelerindeki bu azalmaların plazmadaki antioksidan kapasite ile ilişkili olabileceği, ancak uygulanan ağrı yönetimi prosedürlerinin tiyol ile oluşturulan hassas oksidatif dengeyi etkilediği düşünüldü.

Anahtar sözcükler: *Buzağı, Boynuzsuzlaştırma, Sedasyon, Tiyol-disülfit*

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INTRODUCTION

Dehorning is one of the frequently applied practices in livestock and is depended on to keep animals safe from injuries. Dehorning is a stressful and painful process that results in many homeostatic changes in animals [1]. The disbudding procedure is another term for dehorning in calves up to 3 months of age. The disbudding procedure can be applied to calves in different ways, such as hot-iron disbudding, chemical disbudding with caustic pastes, and surgical disbudding using scoop dehorners. In calves up to 8 weeks of age, hot-iron disbudding can be used [2]. However, this provokes third-degree burns in the area where it is performed [3]. Along with hot-iron disbudding, inflammatory changes, severe pain, behavioural/physiological alterations, and acute stress responses exist in calves [2,4-6]. Numerous reports have described behavioural and physiological reactions to disbudding in calves [1,7-9]. Many inflammatoric conditions cause an increase in oxidative stress mediators that are provoked by pro-inflammatory cytokines in castrated and dehorned calves [10,11]. Previous studies and the American Veterinary Medical Association indicate the essentiality of pain management by pharmacological agents. These studies point out several methodologies, such as local anaesthesia [4,12,13], non-steroidal anti-inflammatory drugs (NSAIDs) [13-15], and sedatives [16,17].

Oxidative balance is described as the equilibrium among free radical eradication and production. Cell damage initiated by free radicals is limited to oxidative balance, and the imbalanced free radical production derives oxidative stress. Thiol is a novel and substantial antioxidant used to eliminate reactive oxygen via non-enzymatic and enzymatic pathways [18,19]. The plasma thiol pool includes both low molecular weight thiols (e.g. glutathione, cysteine, and homocysteine) and protein thiols. Thiols have an antioxidant role in oxidation reactions by composing disulphide bonds. Dynamic thiol/disulphide homeostasis is crucial for detoxification, apoptosis, and the processes of controlling enzymatic reactions and signalling pathways. Many inflammatoric conditions are initiated by anomalous thiol/disulphide levels [20-25]. Korkmaz et al.[9] described the alterations of oxidative stress parameters in calves and mature cows undergoing hot iron dehorning, and defined the amputation process [9,26]. To our knowledge, dynamic thiol/disulphide homeostasis has not been studied previously in calves. Therefore, in the present study, we aimed to evaluate the alterations of thiol/disulphide homeostasis in calves undergoing disbudding with different analgesia and anaesthesia procedures.

MATERIAL and METHODS

Experimental Design, Calves, and Treatments

The study included 30 Holstein calves from both sexes (17 male and 13 female) at 10 weeks of age (BW= 85.6±8.9 kg). All calves were assigned to individual pens 7 days prior to the study and weaned at 7 weeks of age. Calves were fed with ad libitum access to water and a calf starter during the entire period. Study procedures was approved by Local Animal Ethic Committee of Adnan Menderes University with a number of 2017-058.

Calves were randomly divided into three groups. Study groups were designed as the treatment procedure: group I (n=10) xylazine group (with disbudding under sedation with an intramuscular injection of xylazine [Xylazinbio®, Interhas, Czech Republic (dose of 0.25 mg/kg)]); group II (n=10) to those of calves administered xylazine and local anaesthesia [with disbudding under xylazine sedation and subcutaneous infiltration of 20 mg of lidocain (Adokain®, Sanovel, Turkey) for horn buds prior to disbudding]; and group III (n=10) received xylazine, local anaesthesia, and subcutaneous injection of meloxicam (Maxicam®, Sanovel, Turkey) with a dose of 0.5 mg/kg before dehorning. All treatment and disbudding procedures were performed by the same researcher. Furthermore, the local anaesthesia procedure was carried out with a ring block and corneal nerve block between the horn bud and lateral canthus of the eye. Calves were deprived from calf starter 12 h prior to the study (in an attempt to decrease the risk of bloat whereas personnel controlled the calves during reanimation.

Sample Collection and Analysis

Peripheral blood specimens were withdrawn from *Vena jugularis* starting prior to the dehorning and after drug application at 0, 30, 60, 90, and 120 min in lithium heparinised tubes. Immediately after the blood samples were taken, plasma samples were removed using a portable centrifuge in the eppendorf tubes. Plasma samples were stored at -80°C, then moved to the laboratory and analysed.

Total thiol (-S-S- + -SH) includes native and reduced thiol. A novel automatic and spectrophotometric technique established by Erel and Neselioglu [27] was used to determine the thiol/disulphide concentrations. The principle of this method is based on the degradation of dynamic disulphide bonds (–S–S–) to functional thiol groups (–SH) with a sodium borohydride ($NaBH_4$) solution. The remaining $NaBH_4$ residue was totally removed by formaldehyde. Thus, this inhibited extra reduction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) along with any disulphide bonds resulting from the reaction with DTNB. The following reaction with the DTNB-modified Ellman reagent was used to detect the amount of total thiol. The disulphide levels were counted automatically as half of the quantity of total thiol and native thiol. Disulphide/total thiol percent ratios, disulphide/native thiol percent ratios, and disulphide/total thiol percent ratios were calculated from the measured disulphide, total thiol, and native thiol parameters.

Statistical Analyses

All repeated measurements were tabulated as means and standard errors according to descriptive statics. Normality tests were confirmed using the Shapiro-Wilk test. Obtained data were evaluated using both parametric repeated measures of ANOVA and non-parametric and Kruskall Wallis tests for group, time, and group-time interactions. The SPSS 22.0 packet program was used for all tests and P<0.05 was considered significant.

RESULTS

There was no statistical difference in native thiol levels in any of the calves in the study groups. Native thiol levels were determined to be statistically lower in group I and III compared to group II at the 90th min following disbudding. The native thiol levels decreased in a statistically insignificant manner regarding all the treatment groups depending on time. Total thiol levels were found to be lowest at the 60th min of the study, while no significant differences were observed in group, time or group-time interaction in any treatment group. The total thiol levels increased from the 60th min of administration to the 90th and 120th min, but were lower than values measured at min 0. In the disulphide levels, the differences in terms of group, time, and group-time interaction in the application groups were not statistically significant. The disulphide concentrations decreased to the lowest level in groups I and III at the 60th min of application but increased in group II compared to min 0. There was no statistical difference in terms of time and group-time interactions in any of the disulphite/native thiol, disulphite/total thiol, and native thiol/total thiol levels. There were differences at the 60th min of application between all groups *(Table 1)*.

DISCUSSION

In livestock, the pain management of dehorning or disbudding procedures is an important animal welfare issue [28]. In addition to local anaesthesia, NSAID analgesia appears to be generally beneficial, but the lack of specific recommendations for analgesic protocols may reflect the diversity studied in the literature. It is the current recommendation of clinicians and veterinarians that local anaesthetic and NSAIDs in North America can be obtained in full compliance [29,30]. Different methodologies might be used for dehorning (e.g. surgical amputation, chemical methods, or cautery), but disbudding with a cautery is still the most preferred method by livestock producers in the United States, Canada, and North America [29-31]. For managing pain and cortisol spikes in calves after the cautery disbudding process, local anaesthetic agents, non-steroidal anti-inflammatory drugs, and sedatives are used together or solely [30]. Stock et al.[1] reported that the suppression of increases in cortisol levels reduced the pain-related inflammatory response. In our study, the calves undergoing the disbudding process were divided into groups based on commonly used pain management methods.

In a study that evaluated serum oxidant and antioxidant status, the concentrations of nitric oxide (NO) and malondialdehyde (MDA) levels did not reveal any difference

Table 1. *Time-dependent disulfide concentrations pursuant to treatment groups*

Parameter	Group	0. min	30. min	60. min	90. min	120. min	Interactions	P value
Native thiol (µmol/L)	Group I	273.4±13.2	271.5±11.7	249.1±19.6	254.1±16.8	247.6±9.3	Group	0.028
	Group II	240.0±11.6	230.0±11.9	221.4±16.9	224.7±9.2	232.7±14.4	Time	0.211
	Group III	269.6±8.1	254.4±5.4	260.2±2.7	263.3±2.9	251.7±13.2	Group & time	0.779
Total thiol (µmol/L)	Group I	317.2±12.7	318.3±14.8	284.1±24.0	299.4±19.2	290.3±12.2	Group	0.134
	Group II	285.4±14.8	274.6±17.2	269.7±19.1	268.8±8.1	281.6±11.7	Time	0.168
	Group III	313.5±7.2	289.2±9.6	287.8±4.2	292.5±2.8	294.1±16.5	Group & time	0.823
Disulphide (µmol/L)	Group I	21.9±3.7	23.4±3.4	17.5±4.2	22.5±3.3	21.3±2.8	Group	0.106
	Group II	22.7±2.3	22.3±3.0	24.2±2.8	22.0±2.8	24.4±2.1	Time	0.271
	Group III	22.0±1.6	17.4±2.7	13.8±2.1	14.6±1.5	21.2±2.4	Group & time	0.451
Disulphide/native thiol (%)	Group I	7.4±1.4	8.6±1.3	6.9±1.6	9.1±1.3	8.6±1.1	Group	0.014
	Group II	9.4±0.8	9.5±1.0	11.2±1.4	10.1±1.6	11.0±1.5	Time	0.435
	Group III	8.2±0.8	6.8±1.0	5.3±0.8	5.6±0.6	8.4±0.7	Group & time	0.284
Disulphide/total thiol (%)	Group I	6.7±1.1	7.3±0.9	5.8±1.2	7.5±0.9	7.3±0.8	Group	0.017
	Group II	7.9±0.6	7.9±0.7	9.0±1.0	8.2±1.9	8.9±1.0	Time	0.342
	Group III	7.0±0.6	5.9±0.8	4.8±0.7	5.0±0.5	7.1±0.5	Group & time	0.312
Native thiol/total thiol (%)	Group I	86.3±2.3	85.5±1.9	88.4±2.5	84.8±1.8	85.5±1.6	Group	0.019
	Group II	84.2±1.1	84.2±1.5	82.0±1.9	83.6±2.2	82.3±2.0	Time	0.342
	Group III	85.9±1.1	88.2±1.6	90.5±1.3	90.1±1.0	85.7±1.0	Group & time	0.285

between control and Dex (dexketoprofen trometamol) groups. In addition, glutathione (GSH) significantly increased at 15 min after disbudding in the Dex group, and total antioxidant activity did not show any difference between groups. There were no significant alterations in the examined parameters in both groups during the study period [9]. Among the adverse consequences of stress, oxidative stress, which is characterised by the accumulation of radical oxygen species (ROS), can affect life [32].

The thiol-disulphide homeostasis situation has important responsibilities in antioxidant protection, apoptosis, signal transduction, detoxification, regulation of enzymatic activity, and cellular signalling mechanisms [20,21]. Furthermore, thiol-disulphide homeostasis is increasingly being evaluated in various medical conditions in humans, such as diabetes [22], rheumatoid arthritis [23], cancer [25], multiple sclerosis [33], hepatic disorders [24], and surgery [34]. The plasma thiol pool is constituted by cysteine, GSH, homocysteine, and albumin. In expanded oxidative stress conditions, thiol concentrations are decreased to compensate for the reactive oxygen radicals, wherein the sulfhydryl groups of the thiols play an important role [35]. Under oxidative stress conditions, thiol molecules engage disulphide bonds, which are reduced back to thiols to tolerate thiol/disulphide homeostasis [36]. In our study, thiol-disulphide homeostasis was evaluated in calves undergoing the dehorning process with different pain management regimens. Native thiol and total thiol concentrations were decreased in all groups of calves during the study period. Native thiol concentrations were found to be significantly different at the 90th min of the study period between group II and both groups I and III. In contrast, total thiol levels showed no significant alterations in group, time, or group-time interactions, but the lowest levels were examined at the 60th min of the study. This might be explained by thiols' negative reduction properties as electron acceptors. Thiol groups interact with oxidants and are neutralised to a less toxic product called disulphide. Total thiol and native thiol concentrations have been shown to be reduced by oxidation [27]. In our study, another interesting finding was detected in disulphide levels. There were no significant alterations in disulphide concentrations in both groups. In the 60th min of the study, the disulphide concentrations reached minimum levels in groups I and III. However, in group II, the disulphide concentrations were greater than at the beginning of the study. Reductions of thiol concentrations without a rise in disulphide levels might be the outcome of inadequate intake or increased devastation because of its use in other syntheses instead of conversion to disulphide [37]. The calculated parameters of disulphite/native thiol, disulphite/total thiol, and native thiol/total thiol levels were significant in group interactions at the 60th min of the study. In this study, decreases in native thiol and total thiol concentrations without increases in disulphide levels might be related to nutritional factors instead of oxidative stress. Furthermore, oxidative stress might be suppressed by pain management strategies. This study is thought to be limited by the fact that pain management cannot be measured by cortisol levels.

In conclusion, to the best of our knowledge, this is the first study to examine thiol disulphide homeostasis in calves undergoing dehorning. Examining thiol/disulphide homeostasis during dehorning with different pain management procedures might be beneficial as an early evaluation test to recognise the best strategies in calves. Further studies are warranted to understand the association between oxidative stress and dehorning.

Declaration of Conflicting Interest

All authors have declared to be any financial and personal contest effected this study by other people or organizations.

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