

Effect of Pre-Emptive Dexketoprofen Trometamol on Acute Cortisol, Inflammatory Response and Oxidative Stress to Hot-Iron Disbudding in Calves ^[1]

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Abstract

The aim of this study was to evaluate the effect of dexketoprofen trometamol (DEX) on acute cortisol, inflammatory response and oxidative stress following hot-iron disbudding in calves. Twelve male Holstein Friesian dairy calves, weighing between 52 and 82 kg and 6-8 weeks of age were used for disbudding procedure. While calves (n = 6) in DEX group were treated with DEX (IV, 2 mg/kg) 30 min before disbudding, calves (n = 6) in control group were received only 0.9% saline (IV, 2 mg/kg). All calves were disbudded by an electrically heated hot-iron dehorner under local anaesthesia. The heart and respiratory rate were recorded before and after disbudding. Blood samples were collected at 30 min before and 15, 30 and 60 min after disbudding for analysis of serum cortisol, tumour necrosis factor alpha, interleukin-1 beta, interleukin-6, total antioxidant activity, malondialdehyde, glutathione and nitric oxide. In both groups, cortisol concentration was immediately increased after disbudding but dropped to lower level than baseline at 60 min after disbudding in DEX group (P<0.05). It was concluded that combination of a lidocaine and DEX may decrease or prevent acute cortisol, inflammatory response and oxidative stress during disbudding procedure.

Keywords: Acute phase response, Calves, Cortisol, Dexketoprofen, Disbudding

Boynuz Köreltme İşlemi Uygulanan Buzağılarda Pre-emptif Uygulanan Dexketoprofen Trometamol'un Akut Kortizol, İnflamatuar Yanıt ve Oksidatif Stres Üzerine Olan Etkisi

Özet

Bu çalışmanın amacı, koterle boynuz köreltme işlemi uygulanan buzağılarda, pre-emptif dexketoprofen trometamol'un (DEX) akut kortizol, inflammatuar yanıt ve oksidatif stres üzerine olan etkisinin değerlendirilmesidir. Boynuz köreltme işlemi için ağırlıkları 52-82 kg arasında, 6-8 haftalık yaşında 12 erkek Holştayn Frizyan ırkı buzağı kullanıldı. DEX grubunda bulunan buzağılara (n=6) boynuz köreltme işleminden 30 dk önce DEX (IV, 2 mg/kg) uygulanırken, kontrol grubuna (n=6) serum fizyolojik (IV, 2 mg/kg) uygulandı. Bütün buzağılarda boynuzları lokal anestezi altında elektrokoterle köreltildi. Kalp frekansı ve solunum sayısı boynuz köreltme işleminden önce ve sonra kaydedildi. Serum kortizol, tümör nekrozis faktör alfa, interlöykin-1 beta, interlöykin-6, total antioksidan aktivite, malondialdehit, glutatyon ve nitrik oksit düzeylerinin belirlenebilmesi için boynuz köreltme işleminden 30 dk önce ve 15, 30, 60 dk sonra kan örnekleri toplandı. Her iki grupta boynuz köreltme işleminden hemen sonra kortizol konsantrasyonlarının yükseldiği ancak DEX grubunda boynuz köreltme işlemi sonrası 60. dk'da başlangıç değerlerin altına indiği gözlemlendi (p<0.05). Sonuç olarak, boynuz köreltme işlemi sırasında lokal anestezi ve DEX kombinasyonunun akut kortizol, inflammatuar yanıt ve oksidatif stresi azalttığı veya önlediği belirlendi.

Anahtar sözcükler: Akut faz yanıt, Buzağı, Kortizol, Dexketoprofen, Boynuz köreltme

INTRODUCTION

Disbudding is a routine but also painful procedure performed in young calves in dairy farms ^[1,2]. Two methods are frequently used such as caustic paste and hot-iron ^[2,3].

The physiological, behavioural and production responses before, during and after the procedure with or without local anaesthesia or systemic analgesia are the main factors for the evaluation of the presence of pain-related distress caused by various techniques of dehorning and



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disbudding^[3,4]. Restraining or holding of horned animals may be associated with both human and animal safety issues. Therefore, horn buds of young calves normally removed to decrease the risk of injuries to farm workers or other animals^[5].

Nonsteroidal anti-inflammatory drugs (NSAIDs) prevent inflammation by inhibiting cyclooxygenase (COX) enzyme which involved in the synthesis of prostaglandins. COX-1 and COX-2 are two primary forms of COX. Prostaglandins associated with COX-1 isoform mainly regulate maintenance of the gastrointestinal tract, renal function and other homeostatic processes, whereas COX-2 isoform are commonly related to pain and inflammation associated with tissue injuries^[6]. Ketoprofen is a NSAID, inhibits COX-1 and COX-2 enzymes reversibly and effectively reduces inflammation-related pain responses to dehorning^[7]. Dexketoprofen is the water soluble salt of the S-isomer of racemic NSAID ketoprofen. Dexketoprofen has some advantages as compared to ketoprofen, since it has faster onset of action, increasing potency and decreasing potential for gastrointestinal side effects^[8]. It is hypothesized that dexketoprofen trometamol (DEX) may have more adequate pain-relieving effect during painful procedures such as disbudding in calves. Therefore, this study was designed to evaluate the effect of DEX and local anaesthetic lidocaine on acute cortisol, inflammatory response and oxidative stress following hot-iron disbudding in calves.

MATERIAL and METHODS

Twelve male Holstein Friesian dairy calves, housed in the same farm, weighing between 52 and 82 kg and 6-8 weeks of age were used for disbudding procedure. The study was approved by the Animal Ethics Committee, Afyon Kocatepe University (registration number 295-13). The day before each trial, calves were kept in individual pens, bedded with straw. Calves were randomly allocated into two groups. "DEX" group (n = 6) was treated by intravenous injection of DEX (Arveles, UFSA, Turkey) at a dose of 2 mg/kg body weight 30 min before disbudding. Whereas 0.9% saline (2 mg/kg) was administered 30 min before procedure in "Control" group (n = 6).

Twenty minutes before disbudding, local anaesthesia was achieved by subcutaneous injection of 6 mL of 2% lidocaine hydrochloride with 0.01 mg/mL epinephrine (Adokain, Sanovel, Turkey) to each cornual nerve, just ventral to the lateral edge of the frontal bone midway between the base of the horn and the lateral cantus. Local anaesthesia was confirmed by pricking the horn-base with a needle. For disbudding procedure, an electrically heated hot-iron dehorner was used. Before procedure, iron was preheated for at least 10 min to a temperature of approximately 600°C. Disbudding was then carried out with the electro-cautery by applied over each horn bud for 30-60 sec. All cases were disbudded by the same

person and at the same time of the day.

The heart rate (HR) was measured by auscultation and the respiratory rate (RR) by counting thoracic excursions before and after disbudding procedure.

Blood samples (8 ml) were collected by jugular vein-puncture into glass tube containing gel at 30 min before disbudding and at 15, 30 and 60 min after disbudding for analysis of serum cortisol, tumour necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), total antioxidant activity (AOA), malondialdehyde (MDA), glutathione (GSH), and nitric oxide (NO). Blood samples were collected by a same person during the study and the procedure was completed in 15 sec. Samples were kept on ice then centrifuged, serum was removed and stored at -20°C until analysis.

An ELISA method was used to determine cortisol concentrations (Bovine Cortisol ELISA Kit, Hangzhou Eastbiopharm, China), TNF- α (Bovine TNF- α ELISA Kit, Hangzhou Eastbiopharm, China), IL-1 β (Bovine IL-1 β ELISA Kit, Hangzhou Eastbiopharm, China) and IL-6 (Bovine IL-6 ELISA Kit, Hangzhou Eastbiopharm, China) in serum samples.

NO level was quantified indirectly by measuring nitrites (NO₂) and nitrates (NO₃), using the Griess method^[9]. Plasma lipid peroxidation^[10], serum GSH^[11] and the total AOA^[12] levels were measured spectrophotometrically by the previously described methods.

Statistical Analysis

The comparison of parameters measured at same sampling times was evaluated by Mann-Whitney U test between groups. Data of measurement obtained at -30, 15, 30 and 60 min for each parameters were analysed using the analysis of variance (ANOVA) followed by Tukey test (SPSS 16.0) within groups. All data were presented as mean \pm standard error (SEM). Data were considered to be significantly different at P<0.05.

RESULTS

It was observed that HR was increased at 15 min after disbudding and dropped to baseline levels at 30 and 60 min after disbudding in control groups (Table 1). This alteration in HR was noted at 15 and 60 min after disbudding within control group was statistically significant (P<0.05). However, the time-dependent changes of HR did not show any significant within DEX group (P>0.05). The HR values detected at the same sampling time periods between control and DEX groups did not show any significant changes (P>0.05). The RR reached its peak value at 15 min after disbudding, but it was dropped to baseline value at 30 and 60 min after disbudding in control group. The RR detected at 15 and 30 min after disbudding was statistically significant when compared to baseline value in control

group ($P<0.05$). The RR in DEX group was significantly increased at 15 min after disbudding ($P<0.05$). There was significant difference in RR at 15, 30 and 60 min after disbudding between control and DEX groups ($P<0.05$).

The concentrations of cortisol in control group did not show any significant difference in time-dependent manner ($P>0.05$) (Table 2). In DEX group, the cortisol concentration was immediately increased at 15 min after disbudding but decreased to lower level than baseline at 60 min after disbudding. However, the decreased cortisol concentrations detected at 60 min after disbudding were significant, when compared to all measured time intervals ($P<0.05$). There was no significant difference in serum cortisol concentrations between control and DEX groups at any sampling time periods ($P>0.05$).

Serum TNF- α , IL-1 β and IL-6 levels in control and DEX groups were presented in Table 2. TNF- α concentration was significantly increased at 15 min after disbudding with respect to baseline value (30 min before disbudding) in control group ($P<0.05$). The concentrations of TNF- α were not significant within DEX group ($P>0.05$). The concentrations of IL-1 β were not significant within control

group, whereas IL-1 β concentrations in DEX group significantly decreased at 15, 30 and 60 min after disbudding, as compared to concentration detected at baseline value ($P<0.05$). The concentrations of IL-6 at 15, 30 and 60 min after disbudding were not significant, when compared to baseline value within both control and DEX groups ($P>0.05$). In addition, there was no significant difference in TNF- α , IL-1 β and IL-6 concentrations between control and DEX groups at any sampling time periods ($P>0.05$).

Serum oxidant and antioxidant status were presented in Table 3. The concentrations of NO and MDA within control and DEX groups did not reveal any significant difference ($P>0.05$). Similarly, the alteration of concentrations of GSH observed in time-dependent manner was not significant statistically in control group ($P>0.05$). However, GSH concentrations significantly increased at 15 min after disbudding in DEX group ($P<0.05$). The concentrations of AOA detected at 15 min after disbudding within control group significantly increased ($P<0.05$), whereas the alteration of AOA concentrations did not show any significant difference within DEX group ($P>0.05$). Furthermore, the concentrations of NO, GSH, MDA and AOP did not present any significant difference between control and DEX groups at all measured time intervals ($P>0.05$).

Table 1. Heart rate and respiratory rate in control (CO) and dexketoprofen trometamol (DEX) groups (Mean \pm SEM)

Tablo 1. Dexketoprofen trometamol ve kontrol gruplarında kalp frekansı ve solunum sayıları (Ort \pm SEM)

Time (Min)	Group (n=6)	Heart Rate (Pulse/min)	Respiratory Rate (Beat/min)
-30	CO	97.3 \pm 6.7 ^b	30.3 \pm 1.4 ^b
	DEX	119.3 \pm 4.1	25.3 \pm 1.0 ^b
15	CO	137.3 \pm 3.5 ^a	62.0 \pm 2.3 ^a
	DEX	127.0 \pm 8.5	32.6 \pm 2.7 ^a
30	CO	113.3 \pm 3.7 ^b	57.0 \pm 4.1 ^a
	DEX	110.3 \pm 5.4	26.3 \pm 2.1 ^b
60	CO	108.6 \pm 5.2 ^c	36.0 \pm 2.2 ^b
	DEX	94.6 \pm 5.7	21.3 \pm 1.0 ^b

Small (abc) letters in the same column indicate significant differences within the group ($P<0.05$); * Indicate significant differences between the groups ($P<0.05$)

DISCUSSION

Evaluation of changes in cortisol concentrations widely used as an indicator of acute distress responses to a wide range of painful husbandry practices such as castration, disbudding or dehorning procedures [4,7,13]. When calves are disbudded by cautery after a cornual blockade with lidocaine, there is a small transient increase in cortisol concentrations which returns to baseline concentrations at 60 min after disbudding [14]. Similarly, the cortisol concentrations of calves disbudded by hot-iron after a cornual nerve blockade with lidocaine instantly increase without any significant difference but return to the

Table 2. Serum TNF - α , IL - 1 β , IL - 6 and cortisol concentrations of calves disbudded with hot - iron in control (CO) and dexketoprofen trometamol (DEX) groups (Mean \pm SEM)

Tablo 2. Dexketoprofen trometamol ve kontrol gruplarında boynuz köreltme uygulanan buzağuların serum TNF - α , IL - 1 β , IL - 6 and kortizol konsantrasyonları (Ort \pm SEM)

Time (Min)	Group (n=6)	TNF- α (ng/L)	IL - 1 β (pg/mL)	IL - 6 (ng/L)	Cortisol (nmol/L)
-30	CO	187 \pm 6.4 ^b	116.3 \pm 28	148.4 \pm 12.9	38.2 \pm 11.7
	DEX	150 \pm 6.7	103.1 \pm 11.1 ^a	136.1 \pm 12.5	51.2 \pm 3.1 ^a
15	CO	225.8 \pm 4.1 ^a	116.2 \pm 19.8	166.3 \pm 30.8	47.1 \pm 11.4
	DEX	169 \pm 9.9	69.6 \pm 9.5 ^b	118 \pm 14.5	63.4 \pm 6.6 ^a
30	CO	172.8 \pm 9.9 ^b	146.5 \pm 37.1	166.6 \pm 41.3	59.3 \pm 5.6
	DEX	154.1 \pm 18.1	63.6 \pm 12.1 ^b	174.3 \pm 12.1	50.4 \pm 3.8 ^a
60	CO	191.3 \pm 10.1 ^b	148.4 \pm 31.8	168.9 \pm 13.1	39.1 \pm 6.1
	DEX	198.5 \pm 18.9	62.6 \pm 8.5 ^b	161.8 \pm 22.1	34.1 \pm 6.3 ^b

CO: calves disbudded after regional lidocaine application, DEX: calves disbudded after treatment with dexketoprofen trometamol and regional lidocaine
^{ab}Superscript letters in the same column indicate significant differences within the groups ($P<0.05$)

Table 3. Serum oxidant and antioxidant levels of calves disbudded with hot - iron in control (CO) and dexketoprofen trometamol (DEX) groups (Mean±SEM)**Tablo 3.** Dexketoprofen trometamol ve kontrol gruplarında boynuz köreltme uygulanan buzağuların serum oksidan ve antioksidan düzeyleri (Ort±SEM)

Time (Min)	Group (n=6)	NO (µmol/L)	GSH (nmol/mL)	MDA (nmol/mL)	AOA (mmol/L)
-30	CO	14.5±1.6	15.9±0.7	7.9±0.5	6.2±0.6 ^b
	DEX	15.5±2.5	14.4±0.3 ^b	7.6±0.9	7.4±0.4
15	CO	15.3±2.1	15.1±0.4	7.7±0.3	7.9±0.3 ^a
	DEX	14.2±1.5	17.7±1.9 ^a	6.7±0.5	6.9±0.7
30	CO	13.6±2.3	15.1±0.5	8.5±1.4	7.1±0.3 ^{ab}
	DEX	13.4±1.9	16.2±0.1 ^{ab}	7.5±1.1	6.9±0.2
60	CO	14.4±2.4	14.7±0.8	6.3±0.4	7.2±0.1 ^{ab}
	DEX	14.6±1.1	15.9±0.2 ^{ab}	8.3±1.5	6.3±0.6

CO: calves disbudded after regional lidocaine application, DEX: calves disbudded after treatment with dexketoprofen trometamol and regional lidocaine; ^{ab}Superscript letters in the same column indicate significant differences within the groups (P<0.05)

initial levels approximately one hour after disbudding ^[1,2]. The observation of the temporary increment of cortisol concentration immediately after disbudding and the decreasing cortisol concentration at 30 min after disbudding and similar concentrations as initial cortisol levels detected at one hour after disbudding in control group as in results of the present study supports the observations of other reports ^[1,2].

There are several studies indicating the use of local anaesthetics and NSAIDs to prevent or decrease of distress after disbudding ^[2,7,13,15,16]. However, there is no report indicating the effect of DEX on distress. The use of ketoprofen has been shown to significantly reduce cortisol response to the scoop dehorning of calves, when it is combined with a local anaesthetic lidocaine ^[7]. Moreover, it has been stated that the cortisol response of disbudded calves given the NSAID ketoprofen and local anaesthesia is less than the response of calves given local anaesthetic alone in hot-iron disbudded calves ^[2,13]. Stilwell *et al.* ^[2] reported that the cortisol concentration detected at one hour after disbudding the calves following the treatment with regional lidocaine and carprofen had less concentration than initial cortisol level. In addition, the authors assessed no significant difference in decreasing cortisol concentration at first hour between the calves disbudded only regional lidocaine and the calves disbudded by regional lidocaine and carprofen. In the present study, a decreasing cortisol concentration at 30 min after disbudding following a transient increasing cortisol level was observed. It was also found that the cortisol level which was detected at one hour after disbudding was lower than initial cortisol concentration in DEX group. This non-significant decrement in DEX group might be due to pain-relieving effect by the inhibition of prostaglandin synthesis via COX inhibition in addition to that of local anaesthesia alone following DEX injection before hot-iron disbudding.

The stressful events such as castration and disbudding raise the HR in cattle ^[15,17] and therefore can possibly be used as a measure of relative stress ^[17]. Schartzkopf-

Genswein *et al.* ^[17] reported that HR increased at 15 min after dehorning and started to decrease at 30 min after dehorning in calves as compared to initial HR. However, the HR increased during five min following cautery disbudding and returned the baseline values at 15 min after cautery disbudding with and without local anaesthetic ^[16]. Besides, Heinrich *et al.* ^[15] reported lower HR and RR following administration of meloxicam with local anaesthesia in comparison to calves those given only local anaesthesia in disbudding procedure. When compared to control group, DEX group had lower heart and respiratory rates after disbudding, as well as lower cortisol concentrations during 60 min after disbudding. Hence, our data may support the hypothesis that DEX reduces distress caused by the hot-iron disbudding.

The acute phase response is a complex systemic early defence system that is activated by trauma, infection, stress, neoplasia and inflammation ^[18]. There are three pro-inflammatory cytokines; TNF-α, IL-1β, and IL-6, are regarded to be the chief stimulators of the systemic reaction to inflammation and these cytokines are major mediators of acute phase response. Inflammation, infection or tissue injury triggers cytokine release by defence-oriented cells, thereby inducing acute phase protein synthesis. It is widely accepted that physical and psychological stress increase plasma IL-6 and acute phase proteins levels in humans and experimental animals. There is also evidence in cattle that physical stress can induce acute phase proteins ^[19]. Ballou *et al.* ^[20] reported that giving local anaesthetic and a systemic analgesic prevented the decrease in supernatant concentrations of TNF-α among surgical castration or dehorning procedure in calves. Leukocytosis and neutrophilia were previously reported following hot-iron dehorning in calves ^[21]. In our study, it was determined that TNF-α concentrations increased at 15 min after disbudding in both groups however, this increment was higher in control group than those in DEX group (P<0.05). Furthermore, it was seen that IL-1β and IL - 6 concentrations in control group increased after disbudding but decreased in DEX group as compared to initial levels. In addition, IL-6 concentrations in DEX group

decreased after disbudding but subsequently increased at 30 and 60 min after disbudding. It is suggested that no increasing TNF- α concentration and decreasing IL-1 β and IL-6 concentrations after disbudding in DEX group may be related to either inhibition of pro-inflammatory cytokine synthesis of glucocorticoids^[22] or anti-inflammatory effect of DEX itself.

The trauma activates the hypothalamic-pituitary-adrenal (HPA) axis which is the main response for stress^[23]. The cytokines, NO and prostaglandins are the molecules secreting from adrenal cortex induced by pituitary gland for adaption to stress^[24]. Furthermore, surgical trauma may lead to oxidative stress by increasing oxidation and lipid peroxidation or activation of inflammatory response in tissue^[25]. Lipid peroxidation and the plasma MDA concentrations have been stated to directly correlate with the damage severity^[26]. In this study, it was observed that the concentrations of MDA which was known as a marker of lipid peroxidation, decreased at 15 min after disbudding in both groups and raised at 30 min after disbudding without any significant difference. Moreover, it was determined that GSH concentration in DEX group increased at 15 min after disbudding ($P < 0.05$), while control group did not show any significant difference at 15, 30 and 60 min after disbudding ($P > 0.05$). NO concentration increased at 15 min after disbudding in control group ($P > 0.05$), while NO level decreased at 15, 30 and 60 min after disbudding in DEX group without any significant difference. It has been indicated that there is a positive relationship between COX enzymes and NO action. NO is elevated by COX enzymes which are considered highly significant receptor targets for the activation of NO^[27,28]. Hence, it is suggested that COX inhibition by DEX may cause in decreasing NO concentrations in DEX group.

In conclusion, the present study re-stated that hot-iron disbudding was a painful procedure in calves. Combination of DEX and local anaesthesia showed lower cortisol response than local anaesthetic alone in hot-iron disbudded calves. The combination of a local anaesthetic agent with DEX may decrease or prevent the acute inflammatory response and oxidative stress during disbudding procedure.

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