Effects of Dexketoprofen Trometamol on Stress and Oxidative Stress in Cattle Undergoing Claw Trimming

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ABSTRACT

Background: Animal welfare raised a great deal of attention and associated with ethical and moral aspects. In animal, evaluation of wellness is a great tackle and has a complex entity. Pain and stress are closely associated with poor animal welfare and animal suffering should be removed in order to achieve wellness. Today, good welfare in animal is defined as the absence of stress, thus most of the welfare studies focused on it. The aim of study was to investigate the effect of dexketoprofen trometamol (DEXT) on stress and oxidative stress in cattle undergoing claw trimming.

Materials, Methods & Results: This study was conducted on 14 female dairy cows aged 3 - 6 old years with mean body weight of 475 kg (7 Simmental, 5 Holstein Friesian and 2 Montafon). Each cow was randomly allocated into two groups. DEXT group (n = 8) was administered intravenously DEXT at a dose of 2 mg/kg body weight 30 min before claw trimming. Control group (n = 6) was administered physiological saline at the same dose 30 min before procedure was carried out. Trimming was performed using a mobile walk-in crush where the head of cow was restrained by a stanchion and the body supported thoracic and thigh belts in all procedures. The heart rate was measured by auscultation and respiratory rate by counting thoracic excursions before and after claw trimming. Blood samples were collected by jugular venepuncture into glass tube with gel, at 30 min before claw trimming and 15 and 30 min after trimming for measuring serum cortisol, nitric oxide (NO), malondialdehyde (MDA) and total antioxidant activity (AOA). Serum cortisol concentrations were determined by ELISA with used commercial bovine ELISA kit. In both groups no difference was observed in heart rate however the respiratory rate at 15 and 30 min was significantly different (P < 0.05). The heart rate was increased at 15 min after claw trimming but it reached normal values after 30 min in CO and DEXT groups. Respiratory rate was increased at 15 min when compared to onset of the values and it was significant in both groups (P < 0.05). There was no significant difference in serum cortisol concentrations between CO and DEXT groups at any of the time periods measured (P > 0.05). However in CO and DEXT groups the cortisol level in serum was significantly increased at 15 min after trimming (P < 0.05). There was slight drop in cortisol level at 30 min after trimming but this was never reach the level of onset of the value in both groups. No difference was observed in MDA and AOA concentrations between groups (P > 0.05) however the alteration in NO level between groups at 15 min after trimming was statistically significant (P < 0.05).

Discussion: The concentration of cortisol in blood is widely used as an indicator of stress. Pesenhofer et al., showed that cows trimmed with the tilt table had significantly lower concentrations of faecal cortisol metabolites than cows that remained standing during claw trimming in the walk-in crush. In our study, claw trimming was carried out at standing position (walk - in crush) and similar to other studies cortisol concentration increased in both groups. This elevation with respect to time was significant in control and DEXT groups. In conclusion, claw trimming itself in walk-in crush can be considered a stressful procedure, as evidenced by the increases in circulating cortisol in blood. However application of pre-emptive DEXT was showed slight reduction or preventive effect on stress and oxidative stress after claw trimming in cattle.

Keywords: claw trimming, cortisol, cattle, dexketoprofen, oxidative stress.
INTRODUCTION

Animal welfare raised a great deal of attention and associated with ethical and moral aspects [21]. In animal, evaluation of wellness is a great tackle and has a complex entity [25]. Pain and stress are closely associated with poor animal welfare and animal suffering should be removed in order to achieve wellness [4]. Today, good welfare in animal is defined as the absence of stress, thus most of the welfare studies focused on it [21]. According to Webster et al. [28] animal welfare is fit and feeling good, indicating that animal is capable of keeping health status during their life.

Lameness is a major concern in dairy cattle and claw disorders are closely related to the farm management [9,19,20]. Routine claw trimming should be carried out to sustain proper physiological and biomechanical function of the digits, prevent claw disorders before they become clinical concern.Trimming is now fundamental part of lameness management programme in any dairy farms because of its high preventive effects [11,27].

Ketoprofen is a non-steroidal anti-inflammatory drug with analgesic and antipyretic effects [23]. In canine patients, it is one of the choice of analgesic widely used [7,14]. Ketoprofen is composed of racemic mixture of two enantiomers. Dexketoprofen [S (+) -ketoprofen] is the major enantiomer responsible for analgesic effect. It has less adverse effects than racemic ketoprofen. Dexketoprofen has faster onset of action, increased potency, and possibly decreased potential for gastrointestinal side effects with respect to ketoprofen [3,15].

The aim of study was to investigate the effect of DEXT on stress and oxidative stress in cows undergoing claw trimming.

MATERIALS AND METHODS

Animals and study design

This study was conducted on 14 female dairy cows aged 3 - 6 years with mean body weight of 475 kg (7 Simmental, 5 Holstein Friesian and 2 Montafon). The study was approved by the Animal Ethics Committee of Afyon Kocatepe University. Each cow was randomly allocated into two groups. DEKT group (n = 8) was administered intravenously DEXT1 at a dose of 2 mg/kg body weight 30 min before claw trimming. Control group (n = 6) was administered saline at the same dose 30 min before procedure was carried out.

Trimming was performed using a mobile walk - in crush where the head of cow was restrained by a stanchion and the body supported thoracic and thigh belts in all procedures. Animals were in the standing position during functional claw trimming which was achieved by grinders with 7 blades and a hoof knife. Claw trimming was performed by the same person and the procedure was accomplished within 15 min.

The heart rate was measured by auscultation and respiratory rate by counting thoracic excursions before and after claw trimming.

Blood Collection and Biochemical Analysis

Blood samples (8 mL) were collected by jugular venepuncture into glass tube with gel, at 30 min before claw trimming and 15 and 30 min after trimming for measuring serum cortisol, nitric oxide (NO), malondialdehyde (MDA) and total antioxidant activity (AOA).

Serum cortisol concentrations were determined by a commercial bovine ELISA kit2.

NO level was quantified indirectly by measuring nitrites (NO2) and nitrates (NO3), using the Griess method [16]. Briefly, 100 μL sample into 96 well plate and incubated for 30 min in presence of 50 μL of NEDD [N -(1 - Naphthyl) ethylenediamine dihydrochloride] (0.1%, w/v), 50 μL of sulfanilamide (2%, w/v) and 100 μL of vanadium (III) chloride (50 mM) at 37°C. After incubation, the absorbance of each sample was measured with plate microplate reader3, with an emission filter set at 545 nm. NO2 / NO3 concentration was calculated using NO2 standard curve (0.25 - 200 mM).

Plasma lipid peroxidation was determined using the procedure described by Yoshko et al. [29], in which MDA, an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a coloured complex with a maximum absorbance at 530 nm. The sensitivity of test was 0.093 μmol/mL.

The total AOA was determined using the method described by Koracevic et al. [13]. The assay measures the capacity of the serum to inhibit the production of TBA reactive substances (TBARS) from sodium benzoate, under the influence of the reactive oxygen free radicals derived from the Fenton’s reaction. The reaction was measured spectrophotometrically at 532 nm. Antioxidants from the added sample cause suppression of the production of TBARS, and the inhibition of the colour development is defined as AOA. A solution of 1 mmol/L uric acid was used as standard.
Statistical analysis

Differences in concentrations of cortisol, NO, MDA and AOP during sampling times (-30, 15, 30 min) within and between groups were compared using the analysis of variance (ANOVA) followed by Tukey test (SPSS 16.0). Data were considered to be significantly different at $P < 0.05$.

RESULTS

In both groups no difference was observed in heart rate however the respiratory rate at 15 and 30 min was significantly different ($P < 0.05$) (Table 1). The heart rate was increased at 15 min after claw trimming but it reached normal values after 30 min in CO and DEXT groups. The increase at 15 min after trimming was statistically significant in CO group ($P < 0.05$). Respiratory rate was increased at 15 min when compared to onset of the values and it was significant in both groups ($P < 0.05$).

There was no significant difference in serum cortisol concentrations between CO and DEXT groups at any of the time periods measured ($P > 0.05$) (Table 2). However in CO and DEXT groups the cortisol level in serum was significantly increased at 15 min after trimming ($P < 0.05$). There was slight drop in cortisol level at 30 min after trimming but this was never reach the level of onset of the value in both groups. The cortisol level in CO group was generally higher than those in DEXT group.

Before and after claw trimming, variations in NO, MDA and AOA levels in CO and DEXT groups were given in Table 2. No difference was observed in MDA and AOA concentrations between groups ($P > 0.05$) however the alteration in NO level between groups at 15 min after trimming was statistically significant ($P < 0.05$).

Table 1. Heart rate and respiratory rate in dexketoprofen trometamol (DEXT) (n = 8) and control (CO) (n = 6) groups (mean ± SEM).

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Group</th>
<th>Heart Rate (Pulse/min)</th>
<th>Respiratory Rate (Beat/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>CO</td>
<td>60 ± 3.5$^b$</td>
<td>24.3 ± 0.5$^b$</td>
</tr>
<tr>
<td></td>
<td>DEXT</td>
<td>73.5 ± 3.3</td>
<td>17.6 ± 1$^b$</td>
</tr>
<tr>
<td>15</td>
<td>CO</td>
<td>79.3 ± 6.1$^a$</td>
<td>35.5 ± 3$^a$</td>
</tr>
<tr>
<td></td>
<td>DEXT</td>
<td>80.7 ± 4.1</td>
<td>26.3 ± 1.5$^a$</td>
</tr>
<tr>
<td>30</td>
<td>CO</td>
<td>64.1 ± 4.2$^{ab}$</td>
<td>29.1 ± 1.8$^{ab}$</td>
</tr>
<tr>
<td></td>
<td>DEXT</td>
<td>78.1 ± 4.3</td>
<td>21.6 ± 1.2$^{ab}$</td>
</tr>
</tbody>
</table>

$^a$Superscript letters indicate significant differences within the groups ($P < 0.05$); $^b$There is a significantly difference between groups ($P < 0.05$).

Table 2. Serum cortisol, oxidant and antioxidant concentrations of cows in dexketoprofen trometamol (DEXT) (n = 8) and control (CO) (n = 6) groups (mean ± SEM).

<table>
<thead>
<tr>
<th>Time (Min)</th>
<th>Group</th>
<th>Cortisol (µg/L)</th>
<th>NO (µmol/L)</th>
<th>MDA (nmol/mL)</th>
<th>AOA (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-30</td>
<td>CO</td>
<td>3.8 ± 0.2$^b$</td>
<td>10.6 ± 2.4</td>
<td>6.5 ± 0.6</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>DEXT</td>
<td>2.7 ± 0.4$^b$</td>
<td>16.3 ± 1.8</td>
<td>6.2 ± 0.3</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>15</td>
<td>CO</td>
<td>7.1 ± 0.7$^a$</td>
<td>11.3 ± 2.9$^*$</td>
<td>6.4 ± 0.3</td>
<td>6.5 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>DEXT</td>
<td>5.5 ± 0.7$^a$</td>
<td>23 ± 2.8</td>
<td>6.1 ± 0.4</td>
<td>6.2 ± 0.1</td>
</tr>
<tr>
<td>30</td>
<td>CO</td>
<td>4.9 ± 1.1$^{ab}$</td>
<td>15.6 ± 6.2</td>
<td>6.6 ± 0.2</td>
<td>6.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>DEXT</td>
<td>3.1 ± 0.4$^b$</td>
<td>22 ± 3.2</td>
<td>6.5 ± 0.3</td>
<td>6.5 ± 0.2</td>
</tr>
</tbody>
</table>

CO: cows claw trimmed after treatment with saline DEXT: cows claw trimmed after treatment with dexketoprofen trometamol; $^a$Superscript letters indicate significant differences within the groups ($P < 0.05$); $^b$There is a significantly difference between groups ($P < 0.05$).
DISCUSSION

The claw trimming is strongly suggested for the prevention of lameness however it can generate stress reaction in dairy cattle such as an interruption of the daily routine, the handling of the cattle in the immediate pre-trimming phase, the restraint procedure and claw trimming itself with optical, acoustical and mechanical disturbances [22]. The concentration of cortisol in blood is widely used as an indicator of stress [18]. Exposure to stress conditions would stimulate hypothalamic-pituitary adrenal axis leading to dramatically increasing in the ACTH and cortisol secretions [21,30]. In clinically lame cattle, xylazine sedation prior to trimming in lateral recumbency had lower cortisol concentration than those in control group [24]. They also reported cortisol concentration reached the normal level after 1 h of trimming. Cows trimmed with the tilt table had significantly lower concentrations of faecal cortisol metabolites than cows that remained standing during claw trimming in the walk-in crush [22]. In our study, claw trimming was carried out at standing position (walk-in crush) and similar to other studies cortisol concentration increased in both groups. This elevation with respect to time was significant in control and DEXT groups. However the increase in cortisol level was lower in DEXT group than in control group. This may be due to DEXT having some pain-relieving effect by the inhibition of prostaglandin synthesis.

Stressful events such as claw trimming, rectal palpation and dehorning can raise the heart rate and respiratory rate in cattle [4,10,24] and therefore these parameters widely accepted criteria used as a measure of relative stress [26]. After xylazine or saline administration, claw trimming in lateral recumbency heart and respiratory rates were slightly increased at 15 min in saline group whereas they decreased in xylazine group [24]. In our study, after claw trimming heart and respiratory rates were increased in control and DEXT groups but the increment in heart rate was significant in control group after 15 min of claw trimming. Moreover the increment in respiratory rate in both groups (control and DEXT) was also significant at 15 min.

Once hypothalamic-pituitary adrenal axis is stimulated by stress the changes in pituitary secretion secondarily lead to hormone secretion from the adrenal cortex, that adapts to stress by producing various molecules, such as cytokines, NO and prostaglandins that play a role in corticosterone release [5,12]. Also NOS activity is raised associated with stress and infection [17]. No studies into the evaluation of oxidant parameters on claw trimming have been observed by the authors (to the best of our knowledge, currently). It was shown elsewhere that rectal palpation in cattle increased the concentration of NO [4]. In our study, the claw trimming elevated the NO level in both groups.

The trauma caused by surgery may be associated with the oxidative stress due to increasing oxidation and lipid peroxidation by releasing free iron and copper from tissues and activation of inflammatory response [2]. To determine the involvement of free radical damage lipid peroxidation is the main parameters should be analysed and damage severity is closely related with MDA concentration [1]. Fidan et al. [6], observed elevated MDA concentration after dehorning procedure. In our study MDA levels, a marker for lipid peroxidation [8] were not affected with respect to baseline values in both groups after 15 and 30 min of trimming.

CONCLUSION

In conclusion, claw trimming itself in walk-in crush can be considered a stressful procedure, as evidenced by the increases in circulating cortisol in blood. However application of pre-emptive DEXT was showed slight reduction or preventive effect on stress and oxidative stress after claw trimming in cattle. This may be associated with a dose-dependent effect and relevant dose regime of DEXT in cattle should be evaluated.

SOURCES AND MANUFACTURERS
1 Arveles, UFSA, Istanbul, Turkey.
2 Bovine Cortisol ELISA Kit CK - E90577, Hangzhou Eastbiopharm, China.
3 MWGt Lambda Scan 200, Bio-Tek Instruments, USA.

Ethical approval. The study was approved by the animal local Ethics Committee (AKU, HADYEK Date: 21.11.2013. Number: 296 - 13), Afyon Kocatepe University.

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


