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Pain management with flunixin meglumine at dehorning of calves

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ABSTRACT

Dehorning (DH) of calves is a common procedure on commercial dairy farms. Pain management of calves has been investigated in several studies. It is generally accepted that the use of local anesthesia before DH is essential for pain management. Postoperative inflammatory pain should be treated by using a nonsteroidal antiinflammatory drug. The objective of this controlled, randomized, and blinded clinical trial was to determine the effects of the nonsteroidal antiinflammatory drug flunixin meglumine before DH on cortisol concentrations in sera of 5- to 9-wk old calves. Furthermore, selected behavioral characteristics and heart and respiratory rate were examined to assess pain in the hours after dehorning. A total of 80 calves were allocated to 4 groups. In each of 20 replicates, 4 calves were randomly assigned to the following groups: in 3 treatment groups, calves received a local anesthetic (10 mL of procain hydrochloride) and a first treatment (i.v.) with flunixin meglumine or a placebo 20 min before hot-iron dehorning, and a second treatment with flunixin meglumine or a placebo (0.9% saline) 3 h after DH. Calves in the control (CON) group were not dehorned and did not receive any treatment. Groups received 2.2 mg of flunixin meglumine/kg followed by a placebo (FP), 2.2 mg of flunixin meglumine/kg for both treatments (FF), or a placebo for both treatments (PP). Blood samples were collected from all calves, including CON calves, 20 min before restraint in a headlock for DH, 2 min after DH, as well as 30 min and 1, 2, 4, 6, and 8 h after DH. Samples were analyzed for concentration of cortisol by enzyme immunoassay. It was found that concentration of cortisol, calculated as area under the curve, was greater in PP compared with FF and tended to be greater compared with FP. Significant differences between PP and FF were detected at 30 min and 2 h after DH. Throughout the observation period, cortisol concentrations were in both flunixin meglumine-treated groups at a similar level as in the CON group. The heart and respiratory rates showed neither difference between the CON group and the 3 dehorned groups nor between the treatment groups.
Key words: dehorning, cortisol, pain management, flunixin meglumine

INTRODUCTION

Dehorning (**DH**) of calves is a routine management procedure on most dairy farms, performed to prevent injury to stockpeople and other cattle. With regard to animal welfare, it is important to reduce unnecessary suffering and pain caused by this procedure. The most common methods for DH of calves are heat cauterization as well as trephine or gouging the horn bud by the Roberts dehorner or Barnes dehorner (Sutherland et al., 2002). Other procedures such as the use of caustic paste are forbidden in Austria. Beside the technique of DH, the management of pain around this procedure is an important animal welfare issue. The use of local anesthesia (Doherty et al., 2007) is routinely carried out for dehorning of calves and is obligatory in Austria by the National Animal Welfare Act (Bundesministerium für Gesundheit, 2004). The additional treatment with nonsteroidal antiinflammatory drugs (**NSAID**) has been recommended by several authors. Studies with the NSAID ketoprofen or meloxicam have demonstrated the efficacy of these treatments with respect to the release of cortisol and the behavioral expressions of pain (Faulkner and Weary, 2000; Sutherland et al., 2002; Heinrich et al., 2009; Heinrich et al., 2010). The cortisol concentration in serum or plasma was used in several studies to assess pain (Milligan et al., 2004; Stafford and Mellor, 2005; Duffield et al., 2010). Some authors have described that despite the use of an NSAID before or at DH, a later increase in the concentration of cortisol occurred 3 to 8 h after DH, depending on the type of the local anesthetic and the half-life of the NSAID (McMeekan et al., 1998; Sutherland et al., 2002; Milligan et al., 2004; Stafford and Mellor, 2005). Thus, it is conceivable that a second dose of an NSAID after dehorning could suppress a subsequent rise of cortisol and contribute to calf welfare. The effect of the NSAID flunixin meglumine, which is approved for the treat-

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Table 1. Description of groups

Abbreviation of group	Local anesthesia	Treatment before dehorning	Treatment 3 h after dehorning
FP	Yes	2.2 mg of flunixin meglumine/kg (i.v.)	Placebo (i.v.)
FF	Yes	2.2 mg of flunixin meglumine/kg (i.v.)	2.2 mg of flunixin meglumine/kg (i.v.)
PP	Yes	Placebo (i.v.)	Placebo (i.v.)
CON	No	No dehorning and no treatment	No treatment

ment of inflammation and pain suppression, has not been previously described in the literature on pain relief after DH of calves.

The objectives of the present study were to test 2 hypotheses. First, the systemic administration of 2.2 mg of flunixin meglumine/kg before DH can reduce the pain response indicated by a decrease of cortisol in serum after DH. Second, a second dose of 2.2 mg of flunixin meglumine/kg 3 h after DH results in a further reduction of pain parameters.

MATERIALS AND METHODS

The study was approved by the institutional ethics committee and the national authority according to section 8ff of the Austrian law for animal experiments (reference number: bmwf GZ 68.205/0177-II/3b/2010) in compliance with the guidelines of Good Scientific Practice (GSP). The study was carried out as a controlled, randomized, and blinded study on the Teaching and Research Farm Kremesberg, University of Veterinary Medicine, Vienna (Vetmeduni Vienna), between October 2010 and February 2011.

Animals

Calves included in the study were between 5 and 9 wk of age and had to be clinically healthy. The calves were housed in groups on straw bedding and were fed with milk twice daily. They had free access to water, concentrates, and hay. The following data were documented: age (d), sex, breed (Simmental, Brown Swiss, or Holstein-Friesian), and weight (kg) using a weigh tape.

Randomization and Blinding

This study tested 3 different treatment regimens [flunixin meglumine/placebo (**FP**), flunixin meglumine/flunixin meglumine (**FF**), and placebo/placebo (**PP**); Table 1]. A fourth group of not dehorned calves served as an untreated control (**CON**). The study was conducted in 20 replicates. Within each replicate, 4 calves were randomly assigned to 1 of the 4 groups. A randomization list was created before the beginning of the trial (Excel 2010; Microsoft Corp., Redmond, WA) with blinded treatment groups A to C and an untreated and not dehorned CON. Flunixin meglumine (Finadyne; MSD Animal Health, Vienna, Austria) and placebo (0.9% saline) were bottled in identical 10-mL vials and labeled with serial numbers (1 to 60), blinded group (A to C), and order of treatment (1 = before DH; 2 = 3 h after DH). Personnel involved in DH of the calves were unaware of the randomization of the A to C treatment groups or the contents of the vials. Because flunixin meglumine and placebo were transparent and aqueous fluids, a blinding of the study was ensured. The randomization list with the assignment of each calf to treatment groups was stored in a sealed envelope at the Vetmeduni Vienna, and at MSD Animal Health. Unblinding was carried out after finishing all practical work, analyses in the laboratory, and entering all data in a database.

Treatments and Procedures

Dehorning was always performed 60 min after morning feeding (0730 h). The calves received a local infiltration anesthesia for DH (nerve block of ramus cornualis of the nervus maxillaris; McMeekan et al., 1998) with 10 mL of procaine hydrochloride (2% procaine hydrochloride; VMD NV, Arendonk, Belgium) on each side, midway along the lateral edge of the frontal bone crest. Calves in the CON group were only restrained and a blood sample was taken. At the time of administration of the local anesthestic, calves also received the first treatment of the assigned drug intravenously into the jugular vein. Dehorning was performed 20 min later by using a hot electric cautery iron (Kerbl Austria Handels GmbH, Klagenfurt, Austria) to remove the horn bud. Calves received a second treatment with flunixin meglumine or the placebo 3 h after DH.

To define initial values, the calves were observed and blood sampled 75 min before DH (i.e., approximately 15 min before morning feeding). Calves were restrained 20 min before DH in a head lock, the next blood sample was taken, and the calves received the first treatment and the local anesthetic. This second blood sample was used to assess the effect of restraint on cortisol concentration in serum. Further blood samples were taken

Table 2. Ethogram used for behavioral analyses (modified by Heinrich et al., 2010)

Behavior	Description
Head shaking	Calf rapidly moves head from one side to the other
Head rubbing	Calf lifts hind leg to scratch top of head with foot
Foot stamping	Calf raises one foot and brings it down again immediately
Ear flicking	Calf rapidly moves one or both ears; no movement of the head
Groaning/moaning	Calf vocalizing
Head protrusion	Calf puts head in streched position; under a line of the withers

immediately after DH, +30 min, and +1, +2, +4, +6, and +8 h. The clinical parameters heart and respiratory rate were assessed 4 times (before blood sampling at −75 min, +30 min, +4 h, and +8 h). Heart rate was measured using an electrode belt T 31 transmitter and a heart rate monitor S810 (both by Polar Elekro Oy, Kempele, Finland). Respiratory rate was measured by counting the breaths for 30 s, multiplied by 2 to to get the respiratory rate per minute.

The frequency of behavioral events was assessed by direct observation of the calves for 5 min before the next blood sampling by the same person. Table 2 shows the ethogram used for behavioral analysis.

Blood Samples

Blood samples (8 mL) were collected from the jugular vein using a Vacuette system (Greiner Bio-One GmbH, Kremsmünster, Austria). Times of sampling are shown in Table 3. Samples were stored for 3 to 4 h in a warm room (15–20°C) to ensure complete clotting. Afterward, blood samples were centrifuged at 2,000 × *g* for 10 min (Eppendorf centrifuge; Eppendorf AG, Hamburg, Germany). Subsequently, 2 aliquots of serum were pipetted in 2-mL vials (BioScience Inc., Salt Lake City, UT) and frozen at −20°C until biochemical analysis of cortisol. Cortisol analysis was performed at the Institute for Biochemistry, Vetmeduni Vienna, by an enzyme immunoassay as published by Palme and Möstl (1997). In brief, serum was thawed for analysis, 300 μL was extracted with 3 mL of diethyl ether, and the organic phase was transferred to new tubes. The solvent was evaporated under nitrogen and the extract was redissolved in 300 μL of assay buffer. Out of this, 3 μL were used in the assay.

Statistical Methods

Data were analyzed using the SPSS statistical software (version 17; IBM Corp., New York, NY). In a first step, the course of the measured concentrations of cortisol, heart rate, respiratory rate, and the observed

Table 3. Treatment and blood sampling scheme

Time relative to dehorning	Measure[1]	Rational for blood sampling
−80 min	5-min behavioral observation	
−75 min	Clinical examination HR, RR First blood sample (BS 1)	Baseline cortisol
−20 min	Restraint in head lock BS 2 and first treatment Local anesthesia	Cortisol at restraint
0	Dehorning with hot iron BS 3 Release from restraint	Acute reaction
+30 min	5-min behavioral observation Clinical examination HR, RR BS 4	
+1 h	5-min behavioral observation before BS 5	Expected maximum of cortisol
+2 h	5-min behavioral observation before BS 6	Expected decrease in cortisol
+3 h	Second treatment	
+4 h	5-min behavioral observation Clinical examination HR, RR BS 7	Expected second increase in cortisol
+6 h	5-min behavioral observation before BS8	Expected second maximum of cortisol
+8 h	5-min behavioral observation Clinical examination HR, RR BS 9	End of study

[1]HR = heart rate; RR = respiratory rate; BS = blood sample.

behavioral characteristics were compared between treatment groups using a general linear model with repeated measures. The Bonferroni post-hoc test was applied. As groups FF and FP were treated exactly the same until 3 h after DH, a biphasic analysis with the general linear model was performed. In the biphasic analysis, the periods from the second to the fifth blood sample (phase 1) and from the sixth to the ninth blood sample were analyzed seperatly. Phase 1 included 3 groups: both flunixin meglumine-treated groups (FF + FP), PP, and CON. In phase 2, all 4 groups (FF, FP, PP, and CON) were analyzed seperately.

Heart rate, respiratory rate, and cortisol concentrations of all 4 groups at each sampling time were compared using ANOVA by the Brown-Forsythe test for not-normally distributed data, followed by the Bonferroni post-hoc test. The cortisol concentration was additionally analyzed as area under the curve (**AUC**), and group AUC were compared by the Mann-Whitney U test. The number of calves in which the respective behavioral characteristics were recorded was compared between groups using the chi-squared test. The level of significance was set at $\alpha = 0.05$.

RESULTS

Animals

A total of 44 male and 36 female calves were included in the study. One calf had to be excluded after dehorning because of signs of an anaphylactic reaction after treatment. After unblinding, it turned out that this calf was from group FF. The manufacturer's package leaflet indicates an anaphylactic reaction as a possible side effect of a treatment with flunixin meglumine. The calves were, on average, 46.1 ± 9.3 d old and weighed 75.9 ± 10.7 kg.

Cortisol Concentrations

The cortisol concentration in serum was used as the main criterion to evaluate the efficacy of the treatments. Because cortisol concentrations at the time points were not normally distributed, Figure 1 shows the median concentration of cortisol in the 4 groups throughout the observation period. All 4 groups showed from the first sampling (−75 min; presumed baseline) to the restraint before DH (−20 min) a decrease in cortisol concentration. Immediately after DH (or restraint without dehorning in CON), the cortisol concentrations in FP and CON reached their highest levels. In PP, cortisol concentrations increased up to +30 min, whereas in FF, concentrations remained below the initial value of the first blood sample throughout the entire observation period. In all groups, a more or less pronounced increase in cortisol concentration after the seventh blood sample (+4 h) was found. In summary, cortisol values in FP, FF, and CON groups showed a similar course until +6 h, whereas the curve of PP showed higher levels from +30 min to +4 h.

For the statistical analysis of cortisol concentration, the general linear model with repeated measures was applied. Statistically significant effects were found for time but not for treatment group ($P > 0.05$). The bi-

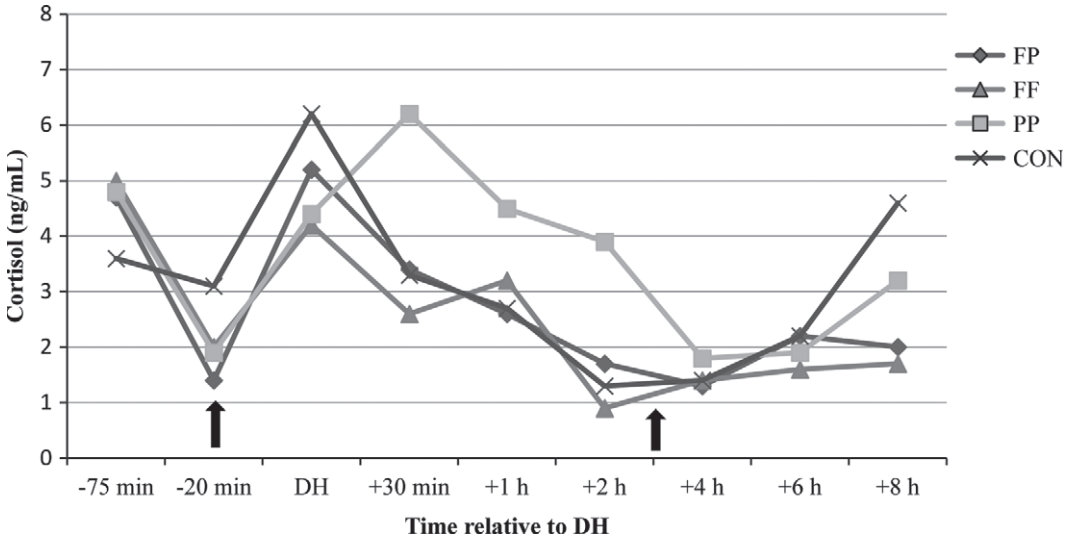

Figure 1. Median cortisol concentrations in the 4 groups before and after dehorning (DH). ↑ = time of treatment. FP = 2.2 mg of flunixin meglumine/kg at DH, placebo 3 h after dehorning; FF = 2.2 mg of flunixin meglumine/kg at DH, 2.2 mg of flunixin meglumine/kg at 3 h after DH; PP = placebo at DH, placebo 3 h after DH; CON = untreated, nondehorned control. Area under the curve is different for PP versus FF ($P < 0.05$).

Table 4. Biphasic analysis of the cortisol concentration in serum

Phase[1]	Treatment A[2]	Treatment B	Mean difference A − B	95% CI	*P*-value
Phase 1 (BS 2–5)	FF + FP	PP	−0.20	−0.48; 0.08	0.26
		CON	−0.13	−0.41; 0.15	0.81
	PP	FF + FP	0.20	−0.08; 0.48	0.26
		CON	0.07	−0.25; 0.40	1.00
	CON	FF + FP	0.13	−0.15; 0.41	0.81
		PP	−0.07	−0.40; 0.25	1.00
Phase 2 (BS 6–9)	FP	PP	−0.20	−0.42; 0.01	0.74
		FF	0.13	−0.09; 0.35	0.69
		CON	−0.06	−0.28; 0.15	1.00
	PP	FP	0.20	−0.01; 0.42	0.07
		FF	0.33	0.11; 0.55	<0.001
		CON	0.14	−0.07; 0.35	0.48
	FF	FP	−0.13	−0.35; 0.09	0.69
		PP	−0.33	−0.55; −0.11	<0.001
		CON	−0.19	−0.41; 0.03	0.12
	CON	FP	0.06	−0.15; 0.28	1.00
		PP	−0.14	−0.36; 0.07	0.48
		FF	0.19	−0.03; 0.41	0.12

[1]BS = blood sample.

[2]FF = 2.2 mg of flunixin meglumine/kg at dehorning (DH), 2.2 mg of flunixin meglumine/kg 3 h after DH; FP = 2.2 mg of flunixin meglumine/kg at DH, placebo 3 h after DH; PP = placebo at DH, placebo 3 h after DH; CON = untreated, nondehorned control.

phasic analysis did not show any differences between FF + FP, PP, and CON groups (Table 4) in the first phase. A significant difference has been detected in the second phase between the groups FF and PP ($P = 0.001$).

The cortisol concentrations determined as AUC are shown in Table 5. Significant differences were found between PP and FF. The cortisol concentrations of each sampling time were analyzed using the Brown-Forsythe test because homogeneity of variances (Levene statistic) was not given, followed by the Bonferroni post-hoc test. It was found that the cortisol concentration in PP at +30 min and +2 h were significantly greater than in FF (7.6 vs. 2.6 ng/mL and 5.0 vs. 1.5 ng/mL, respectively). At +2 h, the concentrations in the PP group were also greater than in the CON group (5.0 vs. 1.5 ng/mL, respectively). Significant differences were also found between CON and FF groups at −20 min (4.7 vs. 1.6 ng/mL, respectively) and +8 h (5.4 vs. 2.8 ng/mL, respectively), and between CON and FP at +8 h (5.4 vs. 2.8 ng/mL, respectively).

Behavioral Observations

The assessment of behavioral characteristics regarded as response to pain is summarized in Table 6. Overall, head shakes and head rubs were recorded most frequently. Both features were observed most frequently in PP. Head shakes and head rubs were significantly more often identified in calves of the 3 dehorned groups (FP, PP, and FF) than in the nondehorned calves in the CON group. Foot stamping, groaning/moaning, and head protrusion were rarely observed and occurred only in a few animals (up to 2 per group).

Heart and Respiratory Rates

The mean heart and respiratory rates of all groups are presented in Table 7. Using a general linear statistical model with repeated measures, significant effects were only found within the respective treatment groups ($P < 0.05$) but not between the groups ($P > 0.05$).

Table 5. Area under the curve (AUC) for concentration of cortisol in 4 treatment groups

	Treatment group[1]			
Item	FP (ng/mL)	FF (ng/mL)	PP (ng/mL)	CON (ng/mL)
Median	1,445.8	1,113.9[b]	1,970.2[a]	1,721.0
First quartile	877.9	718.4	1,464.4	1,174.7
Third quartile	2,377.5	1,570.0	2,626.1	2,821.3

[a,b]Values within a row with different superscripts differ ($P < 0.05$).

[1]FP = 2.2 mg of flunixin meglumine/kg at dehorning (DH), placebo 3 h after DH (n = 20); FF = 2.2 mg of flunixin meglumine/kg at DH, 2.2 mg of flunixin meglumine/kg at 3 h after DH (n = 19); PP = placebo at DH, placebo 3 h after DH (n = 20); CON = untreated, nondehorned control (n = 20).

Table 6. Behavioral expressions accumulated over all observation periods and relevant animals in the 4 treatment groups

	Behavioral expression[2]					
Group[1]	HS	HR	EF	FS	GM	HP
FP						
Behavioral expression (no.)	28	39	19	3	0	1
Relevant animals (no./total no.)	13/20[a]	16/20[a]	10/20	2/20	0/20	1/20
FF						
Behavioral expression (no.)	21	35	19	10	3	0
Relevant animals (no./total no.)	15/19[a]	12/19[a]	9/19	2/19	1/19	0/19
PP						
Behavioral expression (no.)	59	45	13	10	0	1
Relevant animals (no./total no.)	17/20[a]	14/20[a]	8/20	2/20	0/20	1/20
CON						
Behavioral expression (no.)	6	4	8	3	0	0
Relevant animals (no./total no.)	5/20[b]	3/20[b]	5/20	2/20	0/20	0/20

[a,b]Values within a column with different superscripts differ ($P < 0.05$).

[1]FP = 2.2 mg of flunixin meglumine/kg at dehorning (DH), placebo 3 h after DH; FF = 2.2 mg of flunixin meglumine/kg at DH, 2.2 mg of flunixin meglumine/kg at 3 h after DH; PP = placebo at DH, placebo 3 h after DH; CON = untreated, nondehorned control.

[2]HS = head shakes; HR = head rubs; EF = ear flicks; FS = foot stamps; GM = groaning/moaning; HP = head protrusion.

DISCUSSION

The management of pain during DH of calves has been investigated in numerous studies (reviewed by Stafford and Mellor, 2005). The use of local anesthesia is essential to ensure the welfare of calves (Grondahl-Nielsen et al., 1999). Pain management can be improved by an additional administration of an NSAID (e.g., ketoprofen or meloxicam; McMeekan et al., 1998; Faulkner and Weary, 2000; Sutherland et al., 2002; Milligan et al., 2004; Heinrich et al., 2010; Stilwell et al., 2012) or sedation of the calves (Stafford et al., 2003; Stafford and Mellor, 2005). In the present work and in most of the cited studies, an NSAID was tested against a negative control. Thus, these studies do not provide information about the most efficacious treatment. This should be evaluated in further studies. The present study for the first time evaluated the use of the NSAID flunixin meglumine to manage pain during DH of calves. Furthermore, the hypothesis was tested that a second administration 3 h after DH results in further reduction of pain parameters. Flunixin meglumine has a half-life of 3 to 8 h (Ungemach, 2006). The intention was to administer the second dose after the effects of flunixin began to wear off but before a measurable increase of pain (i.e., cortisol concentration) occurred. Other references, however, described a longer half-life for flunixin meglumine of up to 26 h (Odensvik and Johansson, 1995). These different assumptions for the half-life of flunixin meglumine and the best time for a second treatment might have resulted in no or only slight differences between the groups FF and FP.

This study included a total of 20 calves per group, which is comparable with similar studies in the upper range (McMeekan et al., 1998; Grondahl-Nielsen et al., 1999; Faulkner and Weary, 2000; Stafford et al., 2003;

Table 7. Heart rate and respiratory rate of the calves in the 4 treatment groups (mean ± standard deviation)

	Time of measurement relative to dehorning (DH)			
Group[1]	−75 min	+30 min	+4 h	+8 h
Heart rate				
FP	106 ± 24	136 ± 30	127 ± 41	124 ± 38
FF	106 ± 23	142 ± 27	126 ± 26	131 ± 22
PP	107 ± 23	143 ± 42	137 ± 30	138 ± 27
CON	103 ± 25	133 ± 35	130 ± 34	132 ± 25
Respiratory rate				
FP	23 ± 4.6	29 ± 6.7	28 ± 8.0	28 ± 3.9
FF	23 ± 5.5	26 ± 6.5	27 ± 5.2	27 ± 6.2
PP	23 ± 3.9	30 ± 4.9	29 ± 8.5	27 ± 4.8
CON	22 ± 3.7	27 ± 7.4	29 ± 7.3	28 ± 6.9

[1]FP = 2.2 mg of flunixin meglumine/kg at DH, placebo 3 h after DH; FF = 2.2 mg of flunixin meglumine/kg at DH, 2.2 mg/kg of flunixin meglumine 3 h after DH; PP = placebo at DH, placebo 3 h after DH; CON = untreated, nondehorned control.

Milligan et al., 2004; Stewart et al., 2009; Duffield et al., 2010; Stilwell et al., 2012). Only the work by Heinrich et al. (2010) with 30 calves per group enrolled more animals.

The concentration of cortisol in serum has been described as an indicator for pain and stress in calves (Mellor et al., 2002; Milligan et al., 2004; Duffield et al., 2010). The course of the cortisol concentration in the present study indicates that the pain response after DH, measured by cortisol in serum, can be reduced by the administration of flunixin meglumine before DH. The cortisol levels were similar to the nondehorned CON group. An increase in the concentration of cortisol in the CON group demonstrates that restraint itself seems to have an effect on cortisol concentrations, indicating stress. Behavioral characteristics, as discussed later, should also be taken into consideration for the evaluation of pain.

The concentration of cortisol analyzed as AUC showed significant differences between the placebo group PP and group FF, whereas the graphical and numerical differences between FP and PP were statistically not significant. This suggests a positive effect of a treatment with flunixin meglumine 3 h after DH. It can be speculated that with a larger number of calves differences between FP and PP could also be demonstrated as significant. With regard to individual sampling times, significant differences were found between PP and FF at +30 min and +2 h. At these time points, groups FP and FF had received the same treatment; thus, equal effects can be expected. Numerical differences between PP and FP at +30 min and +2 h, and between FF and FP, however, were statistically not significant.

Other studies examined the NSAID ketoprofen (McMeekan et al., 1998; Sutherland et al., 2002; Milligan et al., 2004; Duffield et al., 2010), meloxicam (Heinrich et al., 2010), or carprofen (Stilwell et al., 2012) and their effects on concentration of cortisol after DH. Cortisol has been described as a useful parameter to measure stress associated with painful husbandry procedures (e.g., DH or castration; Heinrich et al., 2010). In a recent study by Duffield et al. (2010), the administration of ketoprofen, however, showed no effect on cortisol concentration. This is in contrast to other works in which the effect of ketoprofen or meloxicam on the concentration of cortisol has been shown for a period of approximately 3 to 5 h after DH, before cortisol increased again (Sutherland et al., 2002; Milligan et al., 2004; Heinrich et al., 2009). The delayed or second increase in cortisol concentration has been observed after the local anesthetic wears off (McMeekan et al., 1998; Sutherland et al., 2002). In the study by Heinrich et al. (2009), this increase was less distinct in the meloxicam-treated group compared with the placebo-treated control group. In the present study, however, we did not find a second or delayed increase in cortisol concentration in any of the groups, clearly related to DH. It has to be discussed whether an increase in cortisol in groups FF, FP, and PP was not detected because of a 2-h interval between samplings. In a study that has described such a second or delayed increase in cortisol after DH, the sampling interval was every 30 min (Sutherland at al., 2002). At +8 h, an increase in cortisol concentration was found in the PP group but even more pronounced in the CON group. It can be speculated whether this increase was related to the forthcoming feeding in the groups or to diurnal changes in cortisol concentrations in blood (Clow et al., 2010). In groups FP and FF, this normal increase in cortisol concentration could have been suppressed by the lasting effects of flunixin meglumine.

A baseline for cortisol concentrations could not be defined. The first value (−75 min), which was supposed to be the baseline, was in all groups above the values measured after restraining the calves in the head lock. This indicates that the stress by manual fixation was higher than during restraint in the head lock. It was not possible to demonstrate clear minimum levels of cortisol, either at the beginning or at the final sampling +8 h. Sutherland et al. (2002) reported that the cortisol values reached a basal level approximately 12 h after DH. Heinrich et al. (2009) found differences in cortisol concentrations in treated and untreated calves at 6 h after DH, but not at the next sampling at 24 h. Thus, we cannot exclude the possibility that a longer observation period would have shown differences in cortisol concentrations in the groups.

Immediately after DH or restraint (CON), the cortisol concentration reached the maximum in FF and FP groups and in the untreated, nondehorned CON group as well. A clear effect of the handling of the calves on cortisol concentrations has been published previously (Boandl et al., 1989). Figure 1 shows that cortisol concentrations in FF, FP, and CON groups were similar, suggesting that the administration of flunixin can reduce the pain (measured as cortisol concentrations) to the level of the handling of the calves.

In accordance with Heinrich et al. (2009), we found that heart rate was higher at the end of the study period compared with the beginning in all groups. It remains speculative if this is a result of the treatments during the day or a reaction to different events (e.g., presence of observers or forthcoming feeding). As we did not find any differences between the 4 groups, this might indicate that DH or pain caused by DH were not associated with changes in heart rate and respiratory rate in our study.

Behavioral manifestations of stress caused by DH that can be considered as evidence for acute pain have been

described in several studies (Grondahl-Nielsen et al., 1999; Faulkner and Weary, 2000; Milligan et al., 2004; Sylvester et al., 2004; Stilwell et al., 2012). In the present study, head shakes, head rubs, and ear flicks were most frequently observed. This is in accordance with the results of other studies (Faulkner and Weary, 2000; Milligan et al., 2004; Heinrich et al., 2010). More calves in the 3 dehorned groups (FP, PP, and FF) showed head shaking and head rubs than in the not-dehorned CON group. This shows that the animals responded to DH independently of treatment with flunixin meglumine. This can be interpreted as acute pain or as indicative of irritation, itching, or healing of wounds (Mellor et al., 2002; Sutherland et al., 2002; Duffield et al., 2010). Also in the work by Heinrich et al. (2010), dehorned animals showed more frequent head shaking and ear flicking than sham dehorned calves. After administration of meloxicam before DH, these behavioral features were recorded less frequently than in a group that had received only a local anesthetic (Heinrich et al., 2010), which is similar to the numeric results in our study. Although Faulkner and Weary (2000) and Duffield et al. (2010) described similar effects by the administration of ketoprofen, this could not be shown by other authors (McMeekan et al., 1998; Milligan et al., 2004). Reasons for these different results could be due to different study designs (i.e., age of the animals, duration of observation periods, and type of DH procedure). Behavioral characteristics in our study have been recorded by direct observation for 5 min before blood samples were taken. Other studies have analyzed this aspect more detailed by video recording and over longer observation periods (Grondahl-Nielsen et al., 1999; Milligan et al., 2004) and found contrary results with regard to the effects the NSAID ketoprofen on behavioral characteristics after DH (Faulkner and Weary, 2000; Heinrich et al., 2010).

CONCLUSIONS

The DH of calves is associated with destruction of tissue and causes significant pain and stress. This stress can be measured by concentration of cortisol in serum. In this study, the effects of a treatment with flunixin before (and 3 h after) dehorning were tested with regard to changes in cortisol concentrations in serum and selected behavioral traits and heart and respiratory rates. All calves received a local anesthetic 20 min before DH. Calves that received 2.2 mg of flunixin meglumine/kg 20 min before DH showed lower concentrations of cortisol in serum than placebo-treated calves. This difference was significant for calves that received a second treatment with flunixin meglumin 3 h after DH (FF) and showed a strong tendency for calves that received flunixin meglumine only before DH (FP). The course of the cortisol concentration in the FF and FP groups was similar to the nondehorned CON group. The heart and respiratory rates showed no differences between the CON and the 3 dehorned groups. More calves in the PP, FP, and FF groups showed head shakes and head rubs than calves in the nondehorned CON group, with the greatest frequency in PP. The administration of flunixin meglumine before DH can be recommended for veterinary practice. Further administration 3 h after DH showed no clear additional positive effects. This study demonstrated the effects of a treatment with flunixin meglumine compared with a group that did not receive an NSAID. Further studies should compare commonly used NSAID (e.g., ketoprofen, meloxicam, and flunixin meglumine) to find the most efficacious treatment to reduce pain after DH.

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