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## Effects of nonsteroidal anti-inflammatory drugs on long-term pain in calves castrated by use of an external clamping technique following epidural anesthesia

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**Objective**—To compare efficacy of flunixin meglumine versus carprofen in controlling pain under field conditions following castration by use of an external clamping technique in calves that received epidural anesthesia.

**Animals**—40 male 5- to 6-month-old calves.

**Procedures**—Calves were allocated to 4 groups: castrated only (control calves; n = 8); castrated 5 minutes after epidural injection of 2% lidocaine (epidural-alone treated calves; 8), castrated after epidural anesthesia and SC flunixin meglumine administration (epidural-flunixin treated calves; 12), and castrated after epidural anesthesia and SC carprofen administration (epidural-carprofen-treated calves; 11 [1 calf not included]). Plasma cortisol concentration was measured before and 6, 24, and 48 hours after castration. Time of arrival at the feed trough at 24 and 48 hours was observed. Calves were observed at 24 and 48 hours for 4 pain-related behaviors.

**Results**—At 6 hours, control calves had significantly higher plasma cortisol concentrations, compared with baseline values and those of epidural-flunixin- and epidural-carprofen-treated calves. At 24 hours, epidural-carprofen-treated calves had significantly lower plasma cortisol concentrations, compared with control calves. At 48 hours, epidural-carprofen-treated calves had plasma cortisol concentrations that were similar to baseline values and significantly lower than epidural-flunixin- and epidural-alone-treated calves. At 24 and 48 hours, epidural-carprofen-treated calves were first to arrive at the feed trough and had fewer pain-related behaviors.

**Conclusions and Clinical Relevance**—SC carprofen administration in combination with epidural injection of lidocaine may improve the welfare of calves castrated by use of an external clamping technique for up to 48 hours. (*Am J Vet Res* 2008;69:xxx-xxx)

Castration of cattle is performed to reduce aggressiveness and sexual activity and to modify carcass characteristics. Castration methods frequently used are those that involve surgical removal of the testicles, application of a constricting elastic band (rubber ring) at the base of the scrotum, and bloodless castration by use of external clamping with an appropriate device (ie, via a castration clamp to crush the spermatic cord). All of these procedures are known to cause inflammation and pain.<sup>1-5</sup> Castration by use of an external clamping technique produced the least severe responses of the methods tested. Some authors consider use of a castration clamp to cause only short-term pain in young calves.<sup>7</sup>

Castration by use of an external clamping technique is sometimes preferred because it is quick and bloodless, with therefore less likelihood of an infection. Nevertheless, it has some important disadvantages, which include severe inflammation, signs of distress and pain, leukogram changes, reduced appetite, and loss of body condition,<sup>8-11</sup> and it is considered the method with the least certainty of success.<sup>12</sup> Dangerous complications, which are possible when erroneous application of the castration clamp occurs, are necrosis and gangrene of the scrotum.<sup>8</sup>

Recommendations of the European Commission<sup>13</sup> are that protocols to control pain (local anesthesia and analgesia) should be used when calves are castrated. Even where legislation is not specific, analgesia should be maintained for the period during which pain is confirmed or probable.

Some anesthesia and analgesia protocols have been studied for different castration methods.<sup>1-4,7,11</sup> It is well documented that use of a castration clamp causes behavioral (eg, changes in gait, posture during standing and lying, and feeding; foot stamping; restlessness; tail wagging; and scrotum licking) and physiologic (eg, changes in cortisol and acute-phase protein concentrations and immune function) pain-related changes for

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≥ 8 hours and that these reactions can be reduced by administering a local anesthesia and an analgesic drug (eg, ketoprofen).<sup>1,4,7,11</sup> However, studies on long-term pain after castration are scarce. In 1 study,<sup>2</sup> the effects of repeated ketoprofen injection after surgical castration were evaluated. However, under field conditions, it is unlikely that 2 injections of an analgesic drug would be given. Therefore, because inflammation and pain associated with the castration will probably last for > 24 hours,<sup>1,5,6,9,14,15</sup> it would be advantageous to study longer-lasting ways to reduce pain.<sup>8</sup>

Nonsteroidal anti-inflammatory drugs are effective in controlling pain in many clinical situations, such as after surgery, and in the treatment of arthritis, colic, mastitis, and traumatic lesions. Nonsteroidal anti-inflammatory drugs are cyclooxygenase-1 and cyclooxygenase-2 inhibitors that reduce inflammation by decreasing the production of prostaglandins, thromboxane A<sub>2</sub>, and other inflammation mediators.<sup>16</sup> Some NSAIDs also exert their analgesic action at the CNS level. Results of studies<sup>1-4,14,17</sup> on pain after castration in calves revealed that several NSAIDs (ketoprofen<sup>1-4,17</sup> and carprofen<sup>14</sup>) are beneficial. The effect of diclofenac has been studied<sup>18</sup> in lambs castrated by use of a castration clamp. Faulkner et al<sup>19</sup> showed a beneficial effect on performance and health of castrated bulls when butorphanol and xylazine are administered.

Flunixin meglumine is an NSAID known to inhibit mainly cyclooxygenase-1 and is considered to have excellent analgesic properties. Flunixin meglumine has a short half-life of 7 hours,<sup>20</sup> but its analgesic effect usually lasts longer as a result of accumulation and slow release from inflamed tissues.<sup>16</sup> Carprofen is an NSAID with a mode of action that is not entirely known, although it is considered a relatively poor cyclooxygenase inhibitor. However, results of a study<sup>16</sup> on dogs and horses reveal that it is an excellent analgesic drug for the treatment of arthritis and for use after surgery, similar to opioids. The half-life of carprofen depends on the species, but has been established to be > 34 hours in 17-week-old calves and 44 to 64 hours in adult cows.<sup>21,22</sup> A long-lasting anti-inflammatory effect of carprofen has been found for cattle.<sup>23</sup> The duration of the analgesic effect of carprofen in calves has not been established.

It is recognized that the combination of local or regional anesthesia with analgesic drugs controls pain after surgery more efficiently than the use of an NSAID alone.<sup>3,24-26</sup> Thüer et al<sup>27</sup> showed that local anesthesia (injected into the spermatic cord and SC at the neck of the scrotum) reduced acute pain during and immediately after calves were castrated by use of a castration clamp, but this is not a practical procedure in the field when many calves have to be castrated. Epidural anesthesia is more safely performed in cattle than scrotal injections; therefore, it provides a better way for the operator to guarantee anesthesia of the scrotum during and immediately following castration. Results of some studies<sup>1,26</sup> indicate that epidural anesthesia was more efficient in preventing increases in cortisol concentrations and pain behaviors than local anesthesia. No study has been conducted to look at the efficacy of epidural injection in reducing pain at the moment of external clamping of the spermatic cord.

Behavior changes are useful tools for the recognition and evaluation of pain in animals.<sup>28</sup> Cattle are a species in which concealment of vulnerability and weakness seems to be adaptive.<sup>29</sup> However, some signs and attitudes, even if subtle, may be used to detect pain.<sup>1,6,7,28</sup>

The purpose of the study reported here was to compare the efficacy of flunixin meglumine versus carprofen in controlling pain under field conditions following castration by use of an external clamping technique in calves that also received epidural anesthesia for castration. We investigated whether increases in plasma cortisol concentration, reluctance to approach the feed trough, and pain-related behaviors are elicited by daily movements of 5- to 6-month-old calves at 6, 24, and 48 hours after castration and compared the efficacy of 2 NSAIDs with different half-lives. We also looked at the efficacy of epidural anesthesia with lidocaine in reducing the pain reaction to external clamping of the spermatic cord.

## Materials and Methods

**Animal housing and management**—The study was conducted in a Holstein-Friesian farm that is used for the fattening period. Calves are bought at dairy farms at 8 to 15 days of age, milk fed and weaned at 2 months of age at another farm, and then transported at 4 months of age to the fattening farm. Castration in this unit is performed on all calves between the ages of 5 and 6 months. Calves entering the farm are usually grouped by age and size in 2-hectare, outdoor, sandy-soiled paddocks. Pine trees provide shade. Food is distributed once a day (10 AM) and consists of a total mixed ration of wheat straw and concentrate (corn, soybean meal, and barley). As a rule, most calves are already near the feed trough when food is delivered.

**Experimental procedures**—Forty calves with a mean age of 173 ± 11 days and an estimated weight of 180 kg were taken 2 days before the study began from the large paddocks and placed in a smaller pen with an adjacent chute. The feed trough area was large enough for all calves to access. Water was permanently available. Weather conditions during the study were dry with mild temperatures (22° to 26°C). The day before castration, calves were moved once through the race and chute to reduce the effect of novelty on the study.

This study was approved by the Committee of the Interdisciplinary Centre of Research in Animal Health of the Lisbon Veterinary Faculty, which is responsible for approving studies that involve experiments with animals. The study was performed on a farm that does not use anesthesia or analgesia for castration by use of an external clamping technique in 5- to 6-month-old calves. Owner consent was obtained from the farmer before the start of the study. At the conclusion of the study, results obtained were useful in convincing the farmer to use anesthesia and analgesia for calves undergoing castration.

On the day of the castration, calves were moved, 4 at a time, through the race to the chute where treatments and castrations were performed beginning at 9 AM. The order of entrance in the race depended only

on the location of the calf in the pen, and calves were driven quietly by stockmen blinded to the treatments. Each of the 4 calves were allocated to 1 of the following 4 groups according to their order in the chute: control group, calves were castrated and treated SC with 5 mL of saline (0.9% NaCl) solution; epidural anesthesia (epidural-alone) group, calves were castrated 5 minutes after a caudal epidural administration of 2% lidocaine (4 mL) and SC administration of 5 mL of saline solution; epidural anesthesia plus flunixin meglumine (epidural-flunixin) group, calves were castrated 5 minutes after caudal epidural administration of 2% lidocaine (4 mL) and SC administration of 8 mL (approx 2.2 mg/kg) of flunixin meglumine<sup>a</sup>; and epidural anesthesia plus carprofen (epidural-carprofen) group, calves were castrated 5 minutes after caudal epidural administration of 2% lidocaine (4 mL) and SC administration of 5 mL (approx 1.4 mg/kg) of carprofen.<sup>b</sup>

The remaining 8 calves were allocated alternatively to the epidural-flunixin group or epidural-carprofen group. One calf that was originally assigned to the epidural-carprofen group was not included in the study because of severe lameness. Therefore, 8 calves were assigned to the control group, 8 to the epidural-alone group, 12 to the epidural-flunixin group, and 11 to the epidural-carprofen group.

All SC injections were given on the neck region, and epidural injection was given between the last sacral and the first coccygeal vertebrae. It was assumed that the NSAIDs would not have taken effect until after the castration had been performed, but the epidural anesthesia was confirmed before castration by absence of resistance to tail lifting.

Castration was performed by closing a castration clamp<sup>c</sup> on each side (first left then right) after assuring that the spermatic cord was pushed to the edge of the scrotum. The procedure was done with the calves standing. The efficacy of castration was not possible to assess, but no complication (eg, necrosis) was detected in any of the calves in the following weeks. After leaving the chute, all calves were left free in a study pen with water and feed available.

Blood samples were collected immediately after the calves entered the chute into 7-mL heparinized tubes<sup>d</sup> by venipuncture of the coccygeal vein at the following times: 5 minutes before castration (baseline) and 6, 24, and 48 hours after castration. Plasma was separated from blood samples by use of centrifugation and subsequently stored at  $-20^{\circ}\text{C}$  until assayed.

**Plasma cortisol concentration**—Cortisol was assayed in duplicate and measured with a validated solid radioimmunoassay without extraction.<sup>e</sup> The intra-assay coefficient of variation for all samples was 6.98%, and the interassay coefficients of variation were 11.4% for 1  $\mu\text{g}/\text{dL}$  and 4.4% for 5  $\mu\text{g}/\text{dL}$ .

**Behavioral assessment**—Behavioral assessment was all done by 1 experienced observer, who was blinded to the study. Identification of the calf was made by ear-ring number with the help, when needed, of binoculars.<sup>f</sup> Behavior was recorded at the time of castration and at 24 and 48 hours after castration at the time of feeding and also at the time of blood sample collection.

At the time of castration, behaviors were recorded during and just after external clamping of the spermatic cord. The 3 behaviors recorded were vocalization, kicking with hind limbs, and lifting forelimbs off the ground.

At feeding time at 24 and 48 hours after castration, the time of arrival of calves at the feed trough was recorded during the 15 minutes following feed distribution at 10 AM. A score was given to each calf according to the following specifications: a score of 1 was assigned to calves with immediate arrival at the feed trough (already at or near the trough when feed was distributed), a score of 2 was assigned to calves with an early arrival to the feed trough (walked to feed trough within 5 minutes of starting food distribution), a score of 3 was assigned to calves with a late arrival to the feed trough (walked to feed trough after 5 minutes from the start of feed distribution), and a score of 4 was assigned to calves that did not approach the feed trough during the 15-minute observation period.

At 24 and 48 hours after castration, the behavior of calves was assessed after going through the race and chute for blood sample collection. Calves were observed for a 15-minute period for the following behaviors: abnormal walking with the hind limbs abducted, an arched back, raising the hind limbs, and looking at or licking the scrotum area.

**Statistical analysis**—Shapiro-Wilk and Levene tests were used to study the presuppositions of normal distribution and variance homogeneity of plasma cortisol concentration data. Because plasma cortisol concentration data fulfilled these presuppositions, a 1-way ANOVA model was used for each period and *t* tests for paired samples were used to compare periods within each group. When the ANOVA *F* test was significant, the Tukey test was used to compare plasma cortisol concentrations among groups and the Dunnett test was used to compare plasma cortisol concentrations of groups with those of the control group. Distributions of these variables of gait behavior and arrival time at the feed trough were determined on the basis of results of the Shapiro-Wilk and Levene tests to be non-normal, so nonparametric analyses were used. Significant differences between the 4 calf groups at each time were determined by use of the Mann-Whitney *U* test followed by a Kruskal-Wallis 1-way ANOVA. Values of  $P < 0.05$  were considered significant.

A correspondence analysis was also performed to find correlations between the treatment groups and different behaviors. This method aims at reducing multivariate data into a manageable number of variables to obtain a global view of the data that is useful for interpretation. A cluster analysis, considering Euclidian distances and the Ward method, was performed; graphics are not shown.

## Results

**Plasma cortisol concentrations**—Baseline plasma cortisol concentrations were similar among all groups ( $P = 0.164$ ; Table 1). Compared with control calves, both epidural-carprofen- ( $P = 0.009$ ) and epidural-flunixin- ( $P = 0.025$ ) treated calves had significantly

Table 1—The effect of no treatment and treatment with epidural anesthesia alone, epidural anesthesia plus flunixin-meglumine, and epidural anesthesia plus carprofen on mean  $\pm$  SD plasma cortisol concentrations of calves following castration by use of an external clamping technique.

Treatment group	Time after castration			
	5 minutes before castration	6 hours	24 hours	48 hours
Control (n = 8)	15.45 $\pm$ 3.20 <sup>a,A</sup>	36.78 $\pm$ 5.24 <sup>a,B,C</sup>	46.99 $\pm$ 7.15 <sup>a,C</sup>	24.89 $\pm$ 4.97 <sup>a,b,A,B</sup>
Epidural (n = 8)	16.22 $\pm$ 3.45 <sup>a,A</sup>	21.56 $\pm$ 5.90 <sup>a,b,A,B</sup>	36.46 $\pm$ 7.15 <sup>a,B</sup>	36.28 $\pm$ 4.07 <sup>a,B</sup>
Epidural-flunixin (n = 12)	19.48 $\pm$ 2.62 <sup>a,A</sup>	17.69 $\pm$ 4.28 <sup>b,A</sup>	32.57 $\pm$ 5.82 <sup>a,b,B</sup>	32.45 $\pm$ 4.06 <sup>a,B</sup>
Epidural-carprofen (n = 11)	10.61 $\pm$ 2.73 <sup>a,A</sup>	15.12 $\pm$ 4.47 <sup>b,A,B</sup>	24.66 $\pm$ 6.07 <sup>b,B</sup>	15.81 $\pm$ 4.25 <sup>b,A,B</sup>

<sup>a,b</sup>Means within each column that do not have a common lowercase superscript letter differ significantly ( $P < 0.05$ ). <sup>A-C</sup>Means within each row that do not have a common uppercase superscript letter differ significantly ( $P < 0.05$ ).

Table 2—The effect of no treatment and treatment with epidural anesthesia alone, epidural anesthesia plus flunixin-meglumine, and epidural anesthesia plus carprofen on behaviors indicative of pain in calves following castration by use of an external clamping technique.

Behavior	Time	Treatment group			
		Control (n = 8)	Epidural alone (n = 8)	Epidural-flunixin (n = 12)	Epidural-carprofen (n = 11)
Arrival at feed trough*	24 h	2.88 $\pm$ 0.64 <sup>A</sup>	2.88 $\pm$ 0.64 <sup>A</sup>	2.42 $\pm$ 0.79 <sup>A,B</sup>	2.00 $\pm$ 0.77 <sup>B</sup>
	48 h	2.50 $\pm$ 0.53 <sup>A,B</sup>	2.63 $\pm$ 0.74 <sup>A</sup>	2.33 $\pm$ 0.65 <sup>A,B</sup>	1.64 $\pm$ 0.79 <sup>B</sup>
Gait alterations					
	Abnormal walking (No.)†				
	24 h	4	7	6	4
	48 h	1	1	5	1
Arched back (No.)†	24 h	4	3	2	1
	48 h	4	2	1	1
Lifting hind leg (No.)†	24 h	2	0	1	1
	48 h	0	2	0	0
Scrotum licking (No.)†	24 h	2	1	3	2
	48 h	2	1	2	1
Total gait alterations (mean $\pm$ SD)‡	24 h	1.50 $\pm$ 0.53 <sup>A</sup>	1.38 $\pm$ 0.98 <sup>A,B</sup>	1.00 $\pm$ 0.74 <sup>A,B</sup>	0.73 $\pm$ 0.65 <sup>B</sup>
	48 h	0.88 $\pm$ 0.35 <sup>A</sup>	0.75 $\pm$ 0.46 <sup>A</sup>	0.67 $\pm$ 0.65 <sup>A,B</sup>	0.18 $\pm$ 0.40 <sup>B</sup>

\*Mean  $\pm$  SD score in which a score of 1 = immediate arrival at the feed trough, 2 = an early arrival to the feed trough, 3 = a late arrival to the feed trough, and 4 = no approach to the feed trough during a 15-minute observation period. †Number of times each behavior was observed. ‡Number of times behaviors were observed divided by the number of calves in each group.

<sup>A,B</sup>Different uppercase letters in the same row indicate significant ( $P < 0.05$ ) differences.

lower plasma cortisol concentrations at 6 hours after castrations. Calves in all groups had a significant increase in plasma cortisol concentration at 24 hours after castration, compared with baseline values for each group; however, at 24 hours after castration, only epidural-carprofen-treated calves had a significantly ( $P = 0.016$ ) lower plasma cortisol concentration, compared with control calves. At 48 hours after castration, epidural-carprofen-treated calves had a significantly lower plasma cortisol concentration, compared with epidural-flunixin- ( $P = 0.002$ ) and epidural-alone- ( $P = 0.026$ ) treated calves, but not compared with control calves ( $P = 0.129$ ).

**Behavioral findings**—Although 6 of 8 control calves had a detectable reaction of kicking ( $n = 3$ ) or lifting forelimbs (3) to external clamping of the spermatic cord, no significant differences were found among groups because some calves that received epidural anesthesia also had behaviors indicative of pain. These reactions were observed immediately and only

after external clamping of the first spermatic cord. In addition to observations in the control calves, 1 epidural-alone-treated calf, 2 epidural-flunixin-treated calves, and 2 epidural-carprofen-treated calves kicked when the clamp was applied. Vocalization was rare; only 1 control calf (that also kicked) and 2 epidural-alone-treated calves vocalized.

At 24 hours after castration, epidural-carprofen-treated calves arrived significantly sooner at the feed trough than control calves ( $P = 0.033$ ) and epidural-alone-treated calves ( $P = 0.033$ ; Table 2). At 48 hours after castration, epidural-carprofen-treated calves arrived significantly ( $P = 0.041$ ) sooner at the feed trough than epidural-alone-treated calves. At either time after castration, no significant difference was found in the time to arrival at the feed trough between epidural-flunixin-treated calves and epidural-carprofen-treated calves.

At 24 hours after castration, epidural-carprofen-treated calves had significantly ( $P = 0.033$ ) less gait and posture abnormalities than control calves. At 48 hours

after castration, epidural-carprofen-treated calves had significantly less gait and posture abnormalities than control calves ( $P = 0.009$ ) and epidural-alone-treated calves ( $P = 0.041$ ).

Results of correspondence analysis revealed a close correlation between different behaviors and the treatment groups at 24 hours after castration. Arched back and nonarrival at the feed trough (score 4) were closely correlated with control calves. Late arrival at the feed trough (score 3) and abnormal walking were correlated with epidural-alone treated calves. Immediate arrival (score 1) at feed trough was not related to any of the groups at 24 hours.

At 48 hours after castration, results of correspondence analysis revealed correlations between different behaviors and treatment groups. Nonarrival at the feed trough (score 4) and raising hind limbs were closely related with epidural-alone-treated calves. Delay of arrival at the feed trough (scores 2 and 3), arched back, and licking the scrotum were closely related to control calves. Epidural-flunixin-treated calves had more instances of abnormal walking than other group calves. Immediate arrival at the feed trough (score 1) was closely related to epidural-carprofen-treated calves. No pain-related behavior had a close relationship to epidural-carprofen-treated calves at 48 hours.

## **Discussion**

Acute pain is a known activator of the hypothalamic-pituitary-adrenal axis; therefore, measuring plasma cortisol concentrations has been extensively used to help evaluate the presence and severity of painful conditions.<sup>30,31</sup> Because other factors, such as fear and stress, may cause a similar increase,<sup>28</sup> it is important to reduce the effect of handling.

For calves in our study, no significant differences were found in baseline plasma cortisol concentrations among groups and our measurements were similar to those of calves of the same age group in other studies.<sup>1,5,11,14</sup> This indicates that initial handling had a negligible effect on plasma cortisol concentrations when calves of our study went into the race. Calves of our study were accustomed to humans and handling. Also, care was taken to obtain blood samples immediately after the calves had entered the chute because results of another study<sup>25</sup> indicate that the effect of handling on plasma cortisol concentrations is reduced if blood sample collection is performed within 2 minutes after a stressful event.

In our study, control calves had an increase in plasma cortisol concentrations at 6 and 24 hours after castration, findings that are similar to those of Ting et al<sup>1,32</sup> and Pang et al.<sup>14</sup> In other studies, it has been reported that plasma cortisol concentrations of calves castrated at 1 week,<sup>6,7</sup> 1 month,<sup>27</sup> 2 to 4 months,<sup>4</sup> or 5.5 months of age<sup>11</sup> return to baseline concentrations after just 2 to 3 hours. It is important to consider that these calves were either young or were kept in individual pens with little movement permitted and a reduced possibility of additional trauma. In contrast, Ting et al<sup>1</sup> and Pang et al<sup>14</sup> found an increased plasma cortisol concentration at 24 and 72 hours after castration, respectively, compared with noncastrated calves. Results of other studies

in calves indicate that surgical castration or castration by use of latex rings results in increased plasma cortisol concentrations at 2 days<sup>5</sup> or at 7 and 14 days<sup>15</sup> after the procedures.

In our study, epidural-alone-treated calves did not have significantly different plasma cortisol concentrations at 6 or 24 hours after castration, compared with control calves. This finding suggested that epidural block with lidocaine, if effective, did not control nociception for more than a few hours or that it did not control pain originating from deep structures. This was to be expected because lidocaine nerve blocks do not last > 90 to 120 minutes.<sup>8</sup> In our study, epidural-alone-treated calves continued to have high plasma cortisol concentrations at 48 hours after castration, suggesting that calves castrated by use of a castration clamp can cause prolonged hyperalgesia and pain.

Low plasma cortisol concentrations of epidural-flunixin- and epidural-carprofen-treated calves at 6 hours after castration in our study suggest that these drugs are equally effective in controlling pain for the first 6 hours, as was found for ketoprofen in other studies.<sup>1,4</sup> In contrast, Pang et al<sup>14</sup> failed to find similar effects at 6 and 12 hours after castration in comparison to calves castrated by use of a castration clamp that did and did not receive carprofen IV.

In our study, the increase in plasma cortisol concentration from baseline at 24 hours after castration in calves of all groups indicates that the treatments studied here are not capable of totally controlling inflammation and pain. This was also suggested by Pang et al<sup>14</sup> for calves and by Price and Nolan<sup>33</sup> for lambs. Nevertheless, epidural-carprofen-treated calves in our study, but not the epidural-flunixin treated calves, did have significantly lower plasma cortisol concentrations at 24 hours after castration, compared with control calves and epidural-alone-treated calves, suggesting some analgesic effect of carprofen. This could be the result of the longer half-life of carprofen, compared with flunixin, or the accumulation of carprofen in inflamed tissues.

At 48 hours after castration, the plasma cortisol concentrations in epidural-carprofen-treated calves was similar to baseline values and significantly lower than those of epidural-alone- and epidural-flunixin-treated calves, indicating that carprofen treatment reduced overall cortisol response for the duration of our study. These results are similar to those of Pang et al.<sup>14</sup> Although Pang et al<sup>14</sup> did not find an effect of carprofen on plasma cortisol concentrations in calves from 0 to 6 hours after castration, they found that plasma cortisol and acute-phase protein concentrations remained high in castrated calves for 3 and 14 days, respectively, and that carprofen reduced overall cortisol response and inflammation.

In contrast, epidural-flunixin-treated calves of our study had high plasma cortisol concentrations at 48 hours after castration, indicating that the analgesic effect of flunixin does not last for 48 hours. The low plasma cortisol concentration of control calves at 48 hours after castration may be the result of reduced inflammation because of the powerful anti-inflammatory effect of high concentrations of glucocorticoids<sup>34</sup> produced by these calves during the previous 24 or more hours.

Pain-related behaviors that indicated that all groups of calves in our study were affected were observed at 24 and 48 hours after castration. No calf group had a close correlation with immediate arrival at the trough (score 1) at 24 hours, suggesting that reluctance to move was increased in all calves. However, control calves and epidural-alone-treated calves had higher numbers of gait alterations and a significant delay (scores 3 and 4) in getting to the trough, compared with other calf groups. Epidural-carprofen-treated calves were the first to arrive at the feed trough and had fewer pain-related behaviors, compared with control calves, at 48 hours after castration. This finding supports the proposal, following the data on plasma cortisol concentrations, that calves that are reluctant to move are those that have more pain when forced to do it. We suggest that the reduced appetite of castrated cattle, as described in a review by Bretschneider,<sup>9</sup> could be the consequence, among other factors, of this reluctance to move. No differences were found in the time of arrival at the feed trough and in the mean number of gait alterations between epidural-flunixin- and epidural-carprofen-treated calves. Although not complete, it appeared that some analgesia was provided by both these drugs.

Thüer et al<sup>27</sup> observed definitive signs of pain in nonanesthetized 1-month-old calves during castration by use of an external clamping technique. In another study<sup>11</sup> in which locally injected lidocaine was evaluated in the castration of older cattle, some differences were found in plasma cortisol concentrations for the first 2 hours after castration between lidocaine-treated and nontreated cattle, but no evaluation of behavior was attempted. We could not find a significant effect of epidural injection on the behavior of calves at the time of castration. Two factors could explain this: control calves could not express more signs of pain because of the limiting effect of having 4 calves in the chute at the same time, or lidocaine epidural anesthesia is not efficient in blocking deeper pain caused by the clamping of inner structures. The fact that only the first application of the castration clamp on the left side elicited pain-related behaviors could be explained by the fact that intense pain probably inhibits further reactions. It might also be that the first application of the castration clamp causes some kind of endogenous opioid-mediated analgesia, and as a result, the second application of the castration clamp on the right side elicits less pain.

External clamping of the spermatic cord by use of a castration clamp causes extensive tissue damage and severe inflammation.<sup>1,14</sup> Although age of cattle and the method of castration are important issues,<sup>8,9,11,35</sup> results of several studies<sup>1,5,6,11,14,27</sup> indicate that chronic pain occurs for several days following the use of a castration clamp on the basis of increases in acute-phase protein concentrations and pain-related behaviors.

It is well recognized that tissue and nerve damage, in association with the exposure of the nociceptors to an inflammatory environment, result in an increased sensitivity of the high-threshold nociceptors so that they will respond to low-intensity stimuli.<sup>36-40</sup> This pathologic pain process, possibly causing allodynia, may also follow castration. The consequence of this is that walking and other daily activities of calves in the paddock

could be responsible for regular activation of sensitized nociceptors and the release of cortisol in response to pain.<sup>41</sup> Molony et al<sup>6</sup> suggests that it may be that only during the most intense peaks of the experience of chronic pain does behavior change enough to permit its recognition. Accordingly, we suggest that routine movement and interaction between hyperalgesic calves at the feedlot could be responsible for perpetuating the signs of pain and the high plasma cortisol concentrations that we found in calves of our study.

In conclusion, calves castrated by use of a castration clamp under field conditions in a feedlot have increases in plasma cortisol concentrations and pain-related behaviors at 6, 24, and 48 hours after the procedure. We suggest that external clamping of the spermatic cord causes prolonged inflammation and a state of hyperalgesia that is responsible for acute pain and, consequently, for an increase in plasma cortisol concentrations when calves have to move. In our study, SC carprofen administration in combination with epidural injection of lidocaine at 5 minutes before castration was efficient in improving the welfare of 5-month-old calves for at least 48 hours by reducing signs of pain. Further studies are needed to determine whether carprofen treatment alone is as efficacious as SC carprofen administration in combination with epidural injection of lidocaine in reducing signs of pain following castration in calves.

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- a. Finadyne (50 mg/mL), Schering-Plough, Levallois-Perret, France.
  - b. Rimadyl (50 mg/mL), Pfizer Animal Health, Dundee, England.
  - c. Burdizzo clamp (40 cm), INSVET, Esplugas-Huesca, Spain.
  - d. Sarstedt tubes, Sarstedt AG & Co, Numbrecht, Germany.
  - e. Coat-A-Count, Diagnostic Product Corporation, Los Angeles, Calif.
  - f. Nikon 8×30 binoculars, Nikon UK Ltd, Kingston upon Thames, Surrey, England.
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