Livestock Science 216 (2018) 61–66

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effects of castration and a lidocaine-plus-flunixin treatment on growth and indicators of pain, inflammation, and liver function in Korean cattle bull calves

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ARTICLE INFO

Keywords:
Animal welfare
Cortisol
Haptoglobin
Stress
Substance P

ABSTRACT

The aim of this study was to determine the effects of castration and a lidocaine-plus-flunixin (LF) treatment on growth and indicators of pain, inflammation, and liver function in Korean cattle bull calves. Forty Korean cattle bull calves (body weight 197.0 ± 2.94 kg and age 6.3 ± 0.09 months) were each assigned to one of four treatments (n = 10 heads/group): no castration with no LF injection (NC-NLF); no castration with LF injection (NC-LF); castration with no LF injection (C-NLF); and castration with LF injection (C-LF). LF treatment included a local anesthetic lidocaine hydrochloride injection (12 mL of 2% in the scrotum) and intravenous injection of a non-steroidal anti-inflammatory drug, flunixin meglumine (2 mg/kg body weight of 50 mg/mL solution), immediately prior to castration. For the NLF groups, a 0.9% NaCl placebo solution was used. Castration was performed surgically using a Newberry knife and a Henderson castrating tool. Blood was collected immediately before castration and at h 0.5, h 6, d 1, d 3, d 7, and d 14 after castration. Feed intake was recorded daily, and body weight was measured on the day prior to the experiment and at d 14 after castration. Castration tended (P = 0.07) to decrease average daily weight gain, but LF treatment did not affect weight gain. Castration increased both circulating cortisol concentrations (P < 0.001) at h 0.5 after castration and substance P (SP) concentrations (P = 0.001) at h 6 after castration. However, the LF treatment did not significantly reduce cortisol and SP concentrations in castrated animals. Castration increased (P < 0.001) circulating haptoglobin (Hp) concentrations on d 1 and d 3 after castration, and LF treatment tended (P = 0.09) to decrease Hp concentrations on d 1 and decreased (P = 0.02) Hp concentrations on d 3. Castration did not affect glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) concentrations. LF treatment increased GOT concentrations at h 6 (P < 0.001), d 1 (P < 0.001), and d 3 (P = 0.003). LF treatment also increased GPT concentrations on d 1 (P = 0.006) and d 3 (P = 0.003). In conclusion, castration of bull calves resulted in time-sequential increases in circulating concentrations of cortisol at h 0.5, SP at h 6, and Hp on d 1. LF treatment did not significantly reduce elevated cortisol and SP, but tended to decrease elevated Hp concentrations in castrated animals. Our study demonstrates that LF treatment is not sufficient for the reduction of the indicators of pain and inflammation in castrated calves, suggesting that additional alleviation methods are required.

1. Introduction

Cattle are often castrated for various management purposes (Stafford and Mellor, 2005) and to improve beef quality grade through increased meat marbling (Park et al., 2002; Bong et al., 2012; Baik et al., 2015). However, castration induces stress, pain, and inflammatory responses in cattle (Warnock et al., 2012; Sutherland et al., 2013; Mintline et al., 2014).

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https://doi.org/10.1016/j.livsci.2018.07.010
Received 15 March 2017; Received in revised form 19 July 2018; Accepted 20 July 2018

Local anesthetic and/or non-steroidal anti-inflammatory drugs (NSAIDs) have been used to reduce stress, pain, and inflammatory responses in cattle after castration (Sutherland et al., 2013; Webster et al., 2013). Combined treatment with both local anesthetic lidocaine and the NSAID flunixin meglumine (FM) has been effective for alleviating cortisol and behavioral responses and maintaining growth performance in surgically castrated dairy calves (Webster et al., 2013). In addition to behavioral responses, the measurement of pain and inflammation indicators may be helpful for monitoring animal welfare. FM treatment has not been effective for mitigating pain indicator substance P (SP) concentrations in cattle (Mintline et al., 2014). Direct effects of lidocaine on SP concentrations in cattle after castration have not been previously reported.

Additionally, the administration of drugs in cattle may affect liver function. However, these effects have not yet been studied. It is postulated that because surgical castration causes bleeding and activates blood coagulation, it may affect blood concentrations of calcium, a major metal involved in blood coagulation.

This study was performed to test the hypothesis that surgical castration increases SP concentrations and that the combined treatment of lidocaine (a local anesthetic) and FM (an NSAID) can effectively reduce SP concentrations in Korean cattle calves. Additionally, this study evaluated the effects of castration and drug treatment on growth, inflammatory mediators, calcium concentrations, and liver function indicators.

2. Materials and methods

All experimental procedures involving animals were approved by the Seoul National University Institutional Animal Care and Use Committees (SNUIACUC), Republic of Korea. The experiments were conducted in accordance with national guidelines provided by SNUIACUC.

2.1. Animals, treatments, and diets

Forty male Korean cattle calves (average 197 ± 2.9 kg and 6.3 ± 0.09 months of age) were used. In Korea, cattle are usually weaned at 3 months of age, transported to a new farm at 5 months of age, and bulls are castrated at 6 months of age. We followed the conventional management system of Korea for selecting the castration age for Korean cattle bulls, which was 6.3 months of age in this study. Calves were weaned at 3 months of age and fed a conventional calf starter concentrate (DM 89%, CP 17%, and TDN 70%) at approximately 1.5% of body weight and timothy hay ad libitum until 5 months of age. From 5 months of age, all calves were acclimated to the new environment and fed an experimental concentrate at approximately 1.5% of body weight and timothy hay ad libitum. Body weight was measured at -d 1 of the experiment. Calves were divided into four groups with both weight and age taken into consideration (10 heads/group) in a 2 × 2 factorial arrangement: no castration with no lidocaine-plus-flunixin injection (NC-NLF); no castration with lidocaine-plus-flunixin injection (NC-LF); castration with no lidocaine-plus-flunixin injection (C-NLF); and castration with lidocaine-plus-flunixin injection (C-LF). Each group was randomly divided into two pens (five heads/pen). All calves were housed in sawdust-bedded pens (5 m × 10 m), indoors, under a roof, and with doors installed at both sides of the barn. Water and mineral blocks were provided beside the pens, so all calves were able to drink water and intake minerals freely.

Timothy hay was provided to calves on a per-pen basis. During the first week, 10 kg (1.0% of body weight as fed base) of timothy/pen/d was provided, and all the roughage given was consumed during the first week. From the second week, 20 kg (2.0% of body weight) of timothy/pen/d was provided. Timothy orts were recorded daily. Timothy intake/pen was measured by subtracting orts from the timothy provided per pen. A concentrate of 3.0 kg/d (1.52% of body weight as fed base) was provided to each individual calf. Calves were fed timothy hay and the concentrate twice daily, at 8:00 am and 5:00 pm. An individual calf was tied in a stanchion during concentrate feeding, and calves consumed all the concentrate given. Calves had ad libitum access to water. Timothy hay consisted of 92.1% DM, 6.2% CP, and 2.1% crude fat; the concentrate consisted of 92.5% DM, 13.7% CP, and 4.8% crude fat. Table 1 lists the ingredients of the concentrate and the chemical composition of the diet. Body weight was measured again at the end of the experiment (d 14).

Table 1
Ingredients and chemical composition of diets for Korean cattle calves.

Ingredient or chemical composition	Percentage
Concentrate ingredients (DM basis)	
Ground corn	15.82
Ground wheat	18.00
Salt	0.88
Molasses	5.50
Wheat bran	3.00
Corn flour	5.00
Rice bran	3.00
Cottonseed hulls	1.50
Palm kernel meal	10.00
Ammonium chloride	0.15
Rapeseed meal	2.22
Dried distilled grain solubles	9.38
Condensed molasses solubles	1.50
Corn gluten feed	8.50
Limestone	3.30
Copra meal	10.00
Porphyry	2.00
Vitamin/Mineral premix[a]	0.25
Total	100.00
Concentrate composition	
DM	92.5
Crude ash	8.76
CP	13.7
Crude fat	4.78
ADF	12.1
NDF	27.6
ME (Mcal/kg)	2.79
TDN	74.1
Timothy composition	
DM	92.1
Crude ash	6.56
CP	6.16
Crude fat	2.11
Calcium	0.26
Phosphate	0.20
Acid detergent fiber	43.8
Neutral detergent fiber	65.9

[a] Vitamin and mineral premix contained 2,650,000 IU vitamin A, 530,000 IU vitamin D_3, 1,050 IU vitamin E, 10 g Niacin, 4.4 g Mn, 4.4 g Zn, 13.2 g Fe, 2.2 g Cu, 0.44 g I, and 0.44 g Co per kg of additive (Grobic-DC, Bayer Health Care, Leverkusen, Germany).

2.2. Castration and LF injection

During all treatments, the calves were haltered and restrained in a squeeze chute. The no castration groups were not surgically wounded because we wanted to monitor stress indicators of surgical castration. However, the calves in these groups were haltered and restrained in a squeeze chute using the same methods as those used for the castration groups. Calves receiving LF treatment (the NC-LF and C-LF groups) were administered lidocaine hydrochloride (local anesthesia agent) and FM (NSAID drug). Immediately prior to castration, the LF treatment groups received 12 mL of 2% lidocaine hydrochloride (Daihan, Seoul, Korea) via injection through a subcutaneous ring block at the neck of the scrotum, just above the testes. The LF treatment groups also received an intravenous injection of FM (2 mg/kg BW of 50 mg/mL stock solution; Fortis, Dongbang, Seoul, Korea). Calves receiving no drug

treatment (the NC-NLF and C-NLF groups) were treated identically to the LF treatment groups except that they received a 0.9% NaCl placebo solution instead of LF treatment (Hartman, Samyang Anipharm, Seoul, Korea). In the no LF treatment groups, the placebo solution was injected both in the scrotum (12 mL) and the neck intravenously (2 mg/kg BW of 50 mg/mL).

Castration was performed surgically using a Newberry knife and a Henderson castrating tool. After injection of the LF treatment or NaCl solution, the Newberry knife was heated for disinfection purposes. The distal portion of the scrotum was removed using the Newberry knife. The exposed spermatic cord was twisted using a Henderson castrating tool. Immediately following castration, 7 mL of vitamin K_3 (20 mg/mL menadion sodium bisulfite trihydrate, Samyang Anipharm, Seoul, Korea) was injected into the neck muscle to induce hemostasis. The surgical site was coated with aluminum powder (Vetoquinol Korea, Gyeonggi, Korea) to prevent bleeding and potential infection. Vitamin K_3 and aluminum powder were not treated in the no castration groups.

2.3. Rectal temperature and blood collection

Rectal temperatures were measured before blood collection using a thermometer (MT200, Microlife, Widnau, Switzerland) at 10:00 am on d 1, d 3, d 7, and d 14 after castration.

Blood was collected externally from the jugular vein in the neck immediately before castration (h 0) and at h 0.5, h 6, d 1, d 3, d 7, and d 14 after castration. We used the alternative jugular vein for blood collection. Two volumes of blood were collected: i) 15 mL of blood (using a 5 mL and a 10 mL tube) was collected in EDTA vacutainers (K2E, BD, NJ, USA) for plasma preparation; and ii) 20 mL (using two 10 mL tubes) was collected in non-heparinized vacutainers (SST II Advance, BD, NJ, USA) for serum preparation. The non-heparinized vacutainers were stored at room temperature for 30 min and then centrifuged for 15 min at 1800x *g* (4 °C). The 10 mL EDTA tubes were stored on ice and then centrifuged for 15 min at 1800x *g* (4 °C). The serum and plasma were stored at −70 °C for the enzyme-linked immunosorbent assay (ELISA) analyses and metabolite analyses.

2.4. Plasma ELISA analyses and serum analyses

The analytic reagents for glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), and calcium were obtained from JW Medical (Seoul, Korea). These items were analyzed using an automated chemistry analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). Plasma cortisol was analyzed using a cortisol salivary HS ELISA kit (SLV4635, DRG, Marburg, Germany). Plasma haptoglobin (Hp) was analyzed using a bovine haptoglobin ELISA kit (GWB-A43096, GenWay Biotech, CA, USA). The analytical methods for these substances were verified by previous reports from our laboratory (Kang et al., 2016a, b). Plasma SP was analyzed using a substance P ELISA kit (ADI-901-018, Enzo Life Sciences, NY, US). The coefficients of intra-assay and inter-assay variance of the SP kit were 4.5% and 7.3%, respectively.

2.5. Statistical analyses

Data were assessed for deviations from normal distribution using univariate procedures (SAS, NC, USA). Body weight, average daily gain (ADG), rectal temperature, and the concentrations of serum GOT, GPT, and calcium were distributed normally. However, the concentrations of plasma cortisol, SP, and Hp were not normally distributed due to marked differences among the groups. An individual animal was used as the experimental unit, with the exception of roughage intake, where the pen was used as the experimental unit. Analysis of statistical significance for roughage intake was not possible because the pen number = 2/group. Thus, average roughage intake data are provided in Supplementary Table 1 without statistical analysis.

Normally distributed data were analyzed using the two-way analysis of variance (ANOVA) with the main fixed effects of castration, LF treatment, and the interaction of castration and LF treatment, using the mixed model procedure in SAS. This resulted in the following equation:

$$Y = \beta_0 + \beta_1 \text{Castration} + \beta_2 \text{LF treatment} + \beta_{12} \text{Castration} \times \text{LF treatment} + \varepsilon$$

where Y is the data variable, β_0 is the intercept, β_1 is the coefficient for the castration effect, β_2 is the coefficient for the LF treatment effect, β_{12} is the coefficient for castration × LF treatment interaction effect, and ε is random error. Abnormally distributed data were log-transformed, and the log-transformed data were also analyzed using two-way ANOVA, as described above. Significance was indicated by $P < 0.05$, and a tendency was indicated by $0.05 < P < 0.10$.

Blood cortisol, SP, Hp, GOT, GPT, and calcium data were also analyzed using repeated measures of ANOVA and the Tukey-Kramer post-test to examine changes over time within the same groups.

3. Results and discussion

3.1. Growth performance and rectal temperature

Castration tended ($P = 0.07$) to decrease ADG (Table 2), and LF treatment did not affect ADG. Several previous studies have reported that castration decreased ADG (Warnock et al., 2012; Repenning et al.,

Table 2
Effects of castration and a lidocaine-plus-flunixin (LF) treatment on growth performance and rectal temperature in Korean cattle calves[a,b].

Item	No castration		Castration			*P*-value		
	No LF	LF	No LF	LF	SEM	Castration	LF	Interaction
Body weight, kg								
-d 1	197.1	196.8	197.0	197.0	2.94	–	–	–
d 14	207.6	208.5	204.7	205.4	3.02	0.64	0.90	0.99
Average daily gain, kg/d from -d 1 to d 14	0.70	0.78	0.51	0.56	0.054	0.07	0.56	0.88
Rectal temperature, °C								
d 1	37.68bc	37.56c	38.48a	38.26ab	0.101	<0.001	0.31	0.76
d 3	38.29	38.34	38.1	38.36	0.060	0.48	0.20	0.38
d 7	38.59	38.74	38.51	38.87	0.052	0.80	0.014	0.30
d 14	37.90ab	38.30a	37.93ab	37.71b	0.079	0.07	0.57	0.04
Daily concentrate intake,[c] kg DM/d	2.78	2.78	2.78	2.78		–	–	–

$N = 10$/group.

[a] A 2 × 2 factorial design was used with main effects (castration and LF treatment) and their interaction. The LF treatment comprised lidocaine hydrochloride and a flunixin meglumine injection immediately prior to castration.

[b] Degree of freedom: castration = 1; LF treatment = 1; interaction = 1; residuals = 36.

[c] Daily concentration intake was all the same during two weeks in all groups.

2013a). Calves consumed all of the concentrate provided, and thus daily concentrate intake was the same (2.78 kg/d) among all treatments throughout the duration of the experiment. Supplementary Table 1 lists daily roughage intake per pen. Numerical values of roughage intake per pen were similar among treatments. Our study reveals that castration slightly decreased ADG and that LF treatment did not improve ADG. Previously, meloxicam NSAID oral treatment was not found to be effective for preventing decreases in ADG due to castration (Repenning et al., 2013b; Brown et al., 2015). LF treatment also does not appear to alleviate the negative effects of castration on calf growth.

Castration increased ($P < 0.001$) rectal temperature by 0.8 °C on d 1 after castration, but did not affect rectal temperature on d 3, d 7, or d 14 (Table 2). LF treatment increased ($P = 0.014$) rectal temperature on d 7 post-castration, but did not affect rectal temperature on d 1, d 3, or d 14. Other studies have also reported that castration increased rectal temperature (Brown et al., 2015; Repenning et al., 2013a). Previously, pain mitigation treatment with a multi-alleviation approach (ketamine stun, lidocaine block, and FM) did not affect the rectal temperatures of castrated calves (Repenning et al., 2013a). However, one study reported that the oral administration of meloxicam induced a delayed increase in the rectal temperatures of castrated calves between h 40 to h 48 (Roberts et al., 2015). It has been suggested that an inflammatory response to surgical site tissue injury might be responsible for the elevated temperatures observed after surgical castration (Kahn and Line, 2010). Collectively, the findings from our study and others suggest that a castration-induced inflammatory response temporarily increases body temperature, and that LF treatment is not effective at reducing the elevated body temperature response.

3.2. Plasma cortisol concentrations

Cortisol is well known to be a stress marker (Abelson et al., 2007). Immediately following castration (h 0.5), cortisol concentrations were profoundly elevated in castrated groups (C-NLF [101 ng/mL; $P = 0.046$] and C-LF [103 ng/mL; $P = 0.04$]) compared to non-castrated groups (NC-NLF [67.4 ng/mL] and NC-LF [29.87 ng/mL]) (Fig. 1). However, castration did not affect cortisol concentrations at other times (h 6, d 1, d 3, d 7, and d 14. Several studies have reported that castration acutely increased circulating cortisol concentrations within a short time period (at around h 0.5 or h 1 after castration) (Coetzee et al., 2008; Sutherland et al., 2013; Webster et al., 2013). We also observed an acute increase in cortisol at h 0.5 after castration.

Cortisol concentrations were also elevated ($P < 0.05$) in the non-castrated groups (NC-NLF and NC-LF) at h 0.5 compared to h 0, even though these calves were not subjected to a painful treatment. Our results suggest that handling procedures such as tying the animals up and collecting blood might themselves induce minor stress, elevating cortisol concentrations. Previous research has suggested that the magnitude of cortisol responses following castration may not be specifically associated with nociception in castrated calves (Coetzee et al., 2008). Cortisol concentrations may be useful as a biomarker for general stress.

LF treatment did not significantly reduce cortisol concentrations at h 0.5 in castrated animals. In a previous study, meloxicam administration temporarily decreased cortisol concentrations at h 2, h 2.5, and h 3, but not at h 0.5, h 1, h 1.5, or from h 4 to h 72 after castration (Roberts et al., 2015).

We observed an unexplained interaction ($P < 0.001$) between castration and LF treatment at h 0 (before castration). Our data revealed that cortisol concentrations at h 0 of the C-NLF group were lower than those of the NC-NLF and C-LF groups (Fig. 1). Basal levels of cortisol concentrations of each group appeared to be different, resulting in a significant interaction, and limiting interpretation of the cortisol levels at h 0.

3.3. Plasma SP concentrations

At h 0.5, neither castration nor LF treatment affected SP concentrations (Fig. 1). However, at h 6 following castration, these were markedly elevated (4.2-fold increase; $P = 0.03$) in the C−NLF group (3.09 ng/mL) compared to the control NC-NLF group (0.74 ng/mL). These then returned to the control concentrations at d 1, and remained at basal concentrations thereafter. In this study, SP concentrations on d 14 were not measured because other studies have suggested that SP concentrations did not change on d 14 (Coetzee et al., 2008; Mintline et al., 2014). Substance P is a neuropeptide in more than 50 neuroactive molecules, including calcitonin gene-related peptide, neuropeptide Y, and endothelin (Onuoha and Alpar, 1999; Carrasco and Van de Kar, 2003). Substance P is known to regulate the excitability of dorsal horn nociceptive neurons and can be detected in areas of the neuroaxis involved with the integration of pain, stress, and anxiety (Devane, 2001). In previous studies, blood SP concentrations were found to be elevated in beef calves after castration (Coetzee et al., 2008) and after dehorning in Holstein calves (Coetzee et al., 2012). Collectively, the findings of our study and others demonstrate that painful procedures caused by castration or dehorning temporarily increase SP concentrations. These results suggest that SP concentrations could be a useful biomarker for pain detection in calves subjected to painful procedures, such as castration and dehorning.

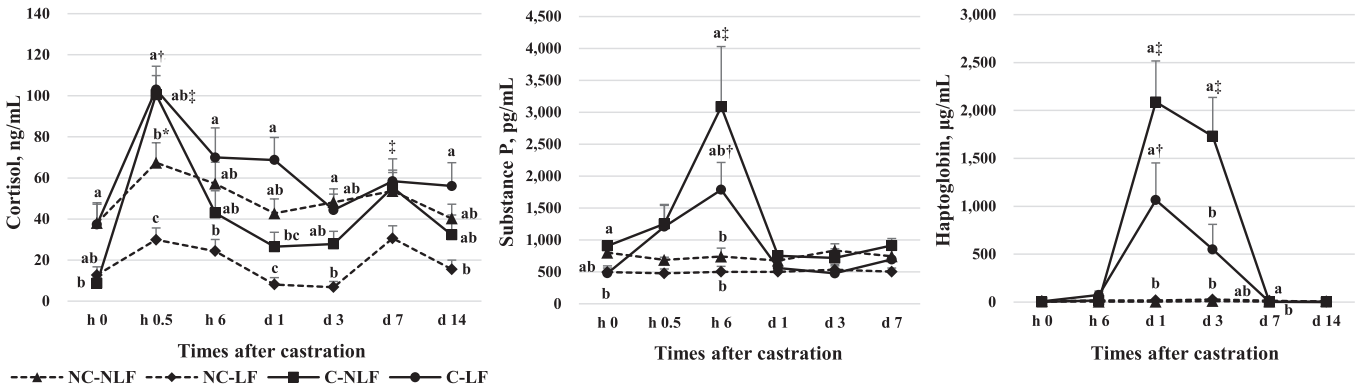

Fig. 1. Effects of castration and lidocaine-plus-flunixin (LF) treatment on concentrations of plasma cortisol, substance P, and haptoglobin in Korean cattle calves ($n = 10$/group). The calves were allocated into groups based on a 2 × 2 factorial design: NC-NLF = no castration with no LF injection; NC-LF = no castration with LF injection; C-NLF = castration with no LF injection; and C-LF = castration with LF injection. Blood was collected immediately before castration (h 0), and at h 0.5, h 6, d 1, d 3, d 7, and d 14 after castration. LF treatment comprised lidocaine hydrochloride and a flunixin meglumine (FM) injection immediately prior to castration. [a-c]At each time point, means with different superscripts differ at $P < 0.05$. An asterisk (*), a double cross (‡), and a single cross (†) indicate that NC-NLF, C-NLF, and C-LF values, respectively, at each time point differ from those at h 0 ($P < 0.05$) within the same group. In the NC-LF group, no significant differences were detected over time. An interaction between castration and LF treatment for cortisol concentrations occurred at h 0 ($P < 0.05$).

LF treatment did not significantly reduce elevated SP concentrations at h 6, although it numerically lowered SP concentrations in the C-LF group (1.79 ng/mL) compared to the C-NLF group (3.09 ng/mL).

3.4. Plasma Hp concentrations

At h 6, neither castration nor LF treatment affected plasma Hp concentrations (Fig. 1). However, on d 1 and d 3 after castration, Hp concentrations were markedly elevated (more than a 200-fold increase; $P < 0.001$) in the C-NLF group (2.09 mg/mL and 1.73 mg/mL on d 1 and d 3, respectively) compared to the NC-NLF group (0.008 mg/mL). Hp concentrations in the C-NLF group returned to control concentrations on d 7 and remained at basal levels thereafter. Other studies have also reported peak Hp concentrations between d 1 to d 3 after castration (Faulkner et al., 1992; Warnock et al., 2012; Ballou et al., 2013; Brown et al., 2015). In our study, Hp concentrations at h 0.5 were not measured, as these were not expected to change at h 0.5.

In the castrated groups, LF treatment tended ($P = 0.09$) to reduce Hp on d 1 and decreased ($P = 0.02$) Hp on d 3; Hp concentrations were lower in the C-LF group (1.07 and 0.55 mg/mL on d 1 and d 3, respectively) compared to the C-NLF group (2.09 and 1.73 mg/mL on d 1 and d 3, respectively), revealing that Hp concentrations were reduced by 49% and 68% on d 1 and d 3 by LF treatment, respectively. In the castrated groups (C-NLF and C-LF), Hp concentrations on d 1 were higher ($P < 0.001$) than those at h 0 within the same group, and concentrations were higher ($P < 0.001$) on d 3 than at h 0 in the C-NLF group, but not in the C-LF group.

Hp is an acute-phase protein that induces anti-inflammatory processes; it is mainly synthesized from the liver (Murata et al., 2004). Hp participates in defense mechanisms against acute changes such as infection, stress, damage, or injury by binding to hemoglobin (Jain et al., 2011). It has been suggested that Hp can be used as a clinical parameter to determine the occurrence and severity of inflammatory responses in cattle (Makimura and Suzuki, 1982). Hp concentrations on d 1 after castration were elevated in castrated calves (Ballou et al., 2013). Increased plasma Hp concentrations were also reported following both surgical (Ting et al., 2003) and non-surgical castration (Ting et al., 2005). Our study demonstrates that LF treatment tended ($P = 0.09$) to decrease Hp concentrations on d 1 and decreased ($P = 0.02$) Hp concentrations on d 3 after castration. Inflammation often results in decreased feedlot performance (Araujo et al., 2010; Warnock et al., 2012). Therefore, treatment with anti-inflammatory agents is recommended to reduce inflammation during stressful events such as castration and dehorning.

In this study, LF treatment included a combination of lidocaine hydrochloride and FM. Ballou et al. (2013) demonstrated that combined treatment with lidocaine hydrochloride and FM was effective for reducing Hp concentrations on d 1, but not on d 3, after castration and dehorning. In contrast, lidocaine hydrochloride treatment alone did not reduce Hp concentrations in castrated beef cattle (Ting et al., 2003) and bull calves (Earley and Crowe, 2002). Our findings suggest that FM treatment may be more effective for reducing Hp concentrations than lidocaine hydrochloride treatment, as FM has anti-inflammatory and analgesic effects (Smith et al., 2008), whereas lidocaine hydrochloride primarily serves an anesthetic function.

It has been suggested that Hp may be a more specific inflammatory indicator and that cortisol may be a whole-body stress indicator (Faulkner et al., 1992). Our study also confirms that Hp is a more specific inflammatory indicator, as evidenced by a decrease in Hp concentrations following LF treatment, which contains an anti-inflammatory drug.

3.5. Serum GOT and GPT concentrations

Fig. 2 presents the concentrations of serum GOT and GPT (liver function indicators). Castration did not affect GOT and GPT concentrations. LF treatment increased GOT and GPT concentrations at several time points: it increased GOT at h 6 ($P < 0.001$), d 1 ($P < 0.001$), and d 3 ($P = 0.003$), and increased GPT on d 1 ($P = 0.006$) and d 3 ($P = 0.003$). Elevated concentrations of GOT and GPT returned to baseline levels on d 7, indicating that the increases had reflected an acute response. When body tissue or an organ such as the liver or heart is diseased or damaged, GOT and GPT concentrations subsequently rise (Huang et al., 2006). However, increases in GOT and GPT concentrations by LF treatment did not appear to be linked with tissue damage, as neither lidocaine hydrochloride nor FM are associated with tissue damage. Increases in GOT and GPT concentrations by LF treatment could have been a result of hyper-activated liver function for the active degradation of lidocaine and/or FM. Normal reference ranges in cattle have been previously reported for GOT (88–132 U/L) and GPT (11–40 U/L) (Jackson and Cockcroft, 2002). In our study, GOT levels (165 and 159 U/L at h 6 and d 1 in the CM group, respectively) and GPT levels (42.4 U/L on d 1 in the CM group) were slightly higher than the upper limit of normal ranges reported for enzymes. Collectively, liver function did not appear worse in the LF treatment groups, although hyper-activation of the liver may temporally occur for drug degradation.

3.6. Serum calcium concentrations

Castration tended ($P = 0.08$) to decrease serum calcium concentrations at h 0.5, and decreased them at h 6 ($P = 0.006$) and d 1 ($P < 0.001$) after castration (Fig. 2), but not thereafter. LF treatment did not affect calcium concentrations in the castrated groups. Calcium is the major mineral involved in blood coagulation (Quick and Stefanini, 1948). In our study, surgical castration resulted in bleeding at the surgical site. Therefore, calcium utilization for activating blood coagulation after castration likely decreased serum calcium concentrations in the castrated groups. Vitamin K facilitates blood coagulation (Davie and Fujikawa, 1975). In this study, vitamin K was administered only to castrated animals, but not to non-castrated animals. Thus, vitamin K treatment may facilitate blood coagulation in castrated animals, indirectly contributing to the reduction of blood calcium concentration. Our study demonstrates that castration temporarily decreases serum calcium concentrations.

4. Conclusions

In this study, surgical castration was associated with a minor decrease in ADG, and temporarily increased stress (cortisol), pain (SP), and inflammation (Hp) indicators in a time sequential manner at h 0.5, h 6, and d 1 and d 3, respectively, after castration. LF treatment (the combination of lidocaine hydrochloride and FM) did not significantly reduce elevated SP, but tended to decrease elevated Hp concentrations in castrated animals. Our study suggests that additional treatment, in addition to LF treatment, is required for the alleviation of pain and inflammation in castrated calves.

Conflict of interest statement

We declare that we have no relevant financial or personal relationships with people or organizations that would create a conflict of interest.

Acknowledgement

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (IPET) through Advanced Production Technology Development Program, funded by Ministry of Agriculture, Food, and Rural Affairs (MAFRA) (118016-03-1-SB010).

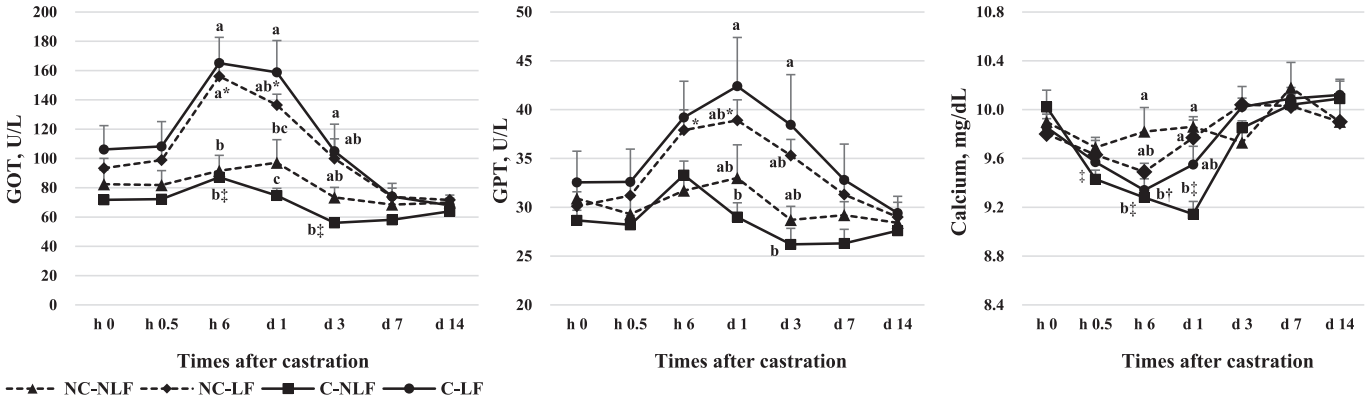

Fig. 2. Effects of castration and lidocaine-plus-flunixin (LF) treatment on the concentrations of serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), and calcium in Korean cattle calves (n = 10/group). Calves were allocated into groups based on a 2 × 2 factorial design: NC-NLF = no castration with no LF injection; NC-LF = no castration with LF injection; C-NLF = castration with no LF injection; and C-LF = castration with LF injection. Blood was collected immediately before castration (h 0), and at h 0.5, h 6, d 1, d 3, d 7, and d 14 after castration. LF treatment comprised lidocaine hydrochloride and a flunixin meglumine (FM) injection immediately prior to castration. [a-c]At each time point, means with different superscripts differ at $P < 0.05$. An asterisk (*), a double cross (‡), and a single cross (†) indicate that NC-LF, C-NLF, and C-LF values, respectively, at each time point differ from h 0 ($P < 0.05$) within the same groups. In the NC-NLF group, no significant differences were detected for items over time. An interaction between castration and LF treatment for calcium concentrations occurred on d 1 ($P < 0.05$).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2018.07.010.

References

Abelson, J.L., Khan, S., Liberzon, I., Young, E.A., 2007. HPA axis activity in patients with panic disorder: review and synthesis of four studies. Depress. Anxiety 24, 66–76.

Araujo, D.B., Cooke, R.F., Hansen, G.R., Staples, C.R., Arthington, J.D., 2010. Effects of rumen-protected polyunsaturated fatty acid supplementation on performance and physiological responses of growing cattle after transportation and feedlot entry. J. Anim. Sci. 88, 4120–4132.

Baik, M., Nquyen, T.H., Jeong, J.Y., Piao, M.Y., Kang, H.J., 2015. Effects of castration on expression of lipid metabolism genes in the liver of Korean cattle. Asian-Australas. J. Anim. Sci. 28, 127–134.

Ballou, M.A., Sutherland, M.A., Brooks, T.A., Hulbert, L.E., Davis, B.L., Cobb, C.J., 2013. Administration of anesthetic and analgesic to prevent the suppression of many leukocyte responses following surgical castration and physical dehorning. Vet. Immunol. Immunopathol. 151, 285–293.

Bong, J.J., Jeong, J.Y., Rajasekar, P., Cho, Y.M., Kwon, E.G., Kim, H.C., Paek, B.H., Baik, M., 2012. Differential expression of genes associated with lipid metabolism in longissimus dorsi of Korean bulls and steers. Meat Sci 91, 284–293.

Brown, A.C., Powell, J.G., Kegley, E.B., Gadberry, M.S., Reynolds, J.L., Hughes, H.D., Carroll, J.A., Burdick Sanchez, N.C., Thaxton, Y.V., Backes, E.A., Richeson, J.T., 2015. Effect of castration timing and oral meloxicam administration on growth performance, inflammation, behavior, and carcass quality of beef calves. J. Anim. Sci. 93, 2460–2470.

Carrasco, G.A., Van de Kar, L.D., 2003. Neuroendocrine pharmacology of stress. Eur. J. Pharmacol 463, 235–272.

Coetzee, J.F., Lubbers, B.V., Toerber, S.E., Gehring, R., Thomson, D.U., White, B.J., Apley, M.D., 2008. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. Am. J. Vet. Res. 69, 751–762.

Coetzee, J.F., Mosher, R.A., KuKanich, B., Gehring, R., Robert, B., Reinbold, J.B., White, B.J., 2012. Pharmacokinetics and effect of intravenous meloxicam in weaned Holstein calves following scoop dehorning without local anesthesia. BMC Vet. Res. 8, 153.

Davie, E.W., Fujikawa, K., 1975. Basic mechanisms in blood coagulation. Annu. Rev. Biochem. 44, 799–829.

DeVane, C.L., 2001. Substance P: a new era, a new role. Pharmacotherapy 21, 1061–1069.

Earley, B., Crowe, M.A., 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. J. Anim. Sci. 80, 1044–1052.

Faulkner, D.B., Eurell, T., Tranquilli, W.J., Ott, R.S., Ohl, M.W., Cmarik, G.F., Zinn, G., 1992. Performance and health of weanling bulls after butorphanol and xylazine administration at castration. J. Anim. Sci. 70, 2970–2974.

Huang, X.J., Choi, Y.K., Im, H.S., Yarimaga, O., Yoon, E., Kim, H.S., 2006. Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. Sensors 6, 756–782.

Jackson, P.G.G., Cockcroft, P.D., 2002. Clinical Examination of Farm Animals. Blackwell Science, Inc., Malden, MA, USA.

Jain, S., Gautam, V., Naseem, S., 2011. Acute-phase proteins: as diagnostic tool. J. Pharm. Bioallied Sci 3, 118–127.

Kahn, C.M., Line, S., 2010. The Merck Veterinary Manual, tenth ed. Merck Sharp & Dohme Corp., a Subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA.

Kang, H.J., Lee, I.K., Piao, M.Y., Gu, M.J., Yun, C.H., Kim, H.J., Kim, K.H., Baik, M., 2016a. Effects of ambient temperature on growth performance, blood metabolites, and immune cell populations in Korean cattle steers. Asian-Australas. J. Anim. Sci. 29, 436–443.

Kang, H.J., Lee, I.K., Piao, M.Y., Kwak, C.W., Gu, M.J., Yun, C.H., Kim, H.J., Ahn, H.J., Kim, H.B., Kim, G.H., Kim, S.K., Ko, J.Y., Ha, J.K., Baik, M., 2016b. Effects of road transportation on metabolic and immunological responses in Holstein heifers. Anim. Sci. J. 88, 140–148.

Makimura, S., Suzuki, N., 1982. Quantitative determination of bovine serum haptoglobin and its elevation in some inflammatory diseases. Jpn. J. Vet. Sci. 44, 15–21.

Mintline, E.M., Varga, A., Banuelos, J., Walker, K.A., Hoar, B., Drake, D., Weary, D.M., Coetzee, J.F., Stock, M.L., Tucker, C.B., 2014. Healing of surgical castration wounds: a description and an evaluation of flunixin. J. Anim. Sci. 92, 5659–5665.

Murata, H., Shimada, N., Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. Vet. J. 168, 28–40.

Onuoha, G.N., Alpar, E.K., 1999. Calcitonin gene-related peptide and other neuropeptides in the plasma of patients with soft tissue injury. Life Sci 65, 1351–1358.

Park, G.B., Moon, S.S., Ko, Y.D., Ha, J.K., Lee, J.G., Chang, H.H., Joo, S.T., 2002. Influence of slaughter weight and sex on yield and quality grades of Hanwoo (Korean native cattle) carcasses. J. Anim. Sci. 80, 129–136.

Quick, A.J., Stefanini, M., 1948. The chemical state of the calcium reacting in the coagulation of blood. J. Gen. Physiol. 32, 191–202.

Repenning, P.E., Ahola, J.K., Callan, R.J., Fox, J.T., French, J.T., Giles, R.L., Peel, R.K., Whittier, J.C., Engle, T.E., 2013a. Effects of pain mitigation and method of castration on behavior and feedlot performance in cull beef bulls. J. Anim. Sci. 91, 4975–4983.

Repenning, P.E., Ahola, J.K., Callan, R.J., French, J.T., Giles, R.L., Bigler, B.J., Coetzee, J.F., Wulf, L.W., Peel, R.K., Whittier, J.C., Fox, J.T., Engle, T.E., 2013b. Impact of oral meloxicam administration before and after band castration on feedlot performance and behavioral response in weanling beef bulls. J. Anim. Sci. 91, 4965–4974.

Roberts, S.L., Hughes, H.D., Burdick Sanchez, N.C., Carroll, J.A., Powell, J.G., Hubbell, D.S., Richeson, J.T., 2015. Effect of surgical castration with or without oral meloxicam on the acute inflammatory response in yearling beef bulls. J. Anim. Sci. 93, 4123–4131.

Smith, G.W., Davis, J.L., Tell, L.A., Webb, A.I., Riviere, J.E., 2008. Extralabel use of nonsteroidal anti-inflammatory drugs in cattle. J. Am. Vet. Med. Assoc. 232, 697–701.

Stafford, K.J., Mellor, D.J., 2005. The welfare significance of the castration of cattle: a review. N. Z. Vet. J. 53, 271–278.

Sutherland, M.A., Ballou, M.A., Davis, B.L., Brooks, T.A., 2013. Effect of castration and dehorning singularly or combined on the behavior and physiology of Holstein calves. J. Anim. Sci. 91, 935–942.

Ting, S.T., Earley, B., Crowe, M.A., 2003. Effect of repeated ketoprofen administration during surgical castration of bulls on cortisol, immunological function, feed intake, growth, and behavior. J. Anim. Sci. 81, 1253–1264.

Ting, S.T.L., Earley, B., Veissier, I., Gupta, S., Crowe, M.A., 2005. Effects of age of Holstein-Friesian calves on plasma cortisol, acute-phase proteins, immunological function, scrotal measurements and growth in response to Burdizzo castration. Anim. Sci. 80, 377–386.

Warnock, T.M., Thrift, T.A., Irsik, M., Hersom, M.J., Yelich, J.V., Maddock, T.D., Lamb, G.C., Arthington, J.D., 2012. Effect of castration technique on beef calf performance, feed efficiency, and inflammatory response. J. Anim. Sci. 90, 2345–2352.

Webster, H.B., Morin, D., Jarrell, V., Shipley, C., Brown, L., Green, A., Wallace, R., Constable, P.D., 2013. Effects of local anesthesia and flunixin meglumine on the acute cortisol response, behavior, and performance of young dairy calves undergoing surgical castration. J. Dairy Sci. 96, 6285–6300.