J. vet. Pharmacol. Therap. **30**, 305–313, doi: 10.1111/j.1365-2885.2007.00869.x.

Attenuation of acute plasma cortisol response in calves following intravenous sodium salicylate administration prior to castration

J. F. COETZEE*
R. GEHRING*
A. C. BETTENHAUSEN*
B. V. LUBBERS*
S. E. TOERBER†
D. U. THOMSON*
B. KUKANICH‡ &
M. D. APLEY*

**Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan; †PharmCats Bioanalytical Services, Kansas State University, Manhattan; ‡Department of Anatomy and Physiology, Kansas State University, Manhattan, KS, USA*

Coetzee, J. F., Gehring, R., Bettenhausen, A. C., Lubbers, B. V., Toerber, S. E., Thomson, D. U., KuKanich, B., Apley, M. D. Attenuation of acute plasma cortisol response in calves following intravenous sodium salicylate administration prior to castration. *J. vet. Pharmacol. Therap.* **30**, 305–313.

Pain associated with castration in cattle is an animal welfare concern in beef production. This study examined the effect of oral aspirin and intravenous (i.v.) sodium salicylate on acute plasma cortisol response following surgical castration. Twenty bulls, randomly assigned to the following groups, (i) uncastrated, untreated controls, (ii) castrated, untreated controls, (iii) 50 mg/kg sodium salicylate i.v. precastration and (iv) 50 mg/kg aspirin (acetylsalicylic acid) *per os* precastration, were blood sampled at 3, 10, 20, 30, 40, 50 min and 1, 1.5, 2, 4, 6, 8, 10 and 12 h postcastration. Samples were analyzed by competitive chemiluminescent immunoassay and fluorescence polarization immunoassay for cortisol and salicylate, respectively. Data were analyzed using noncompartmental analysis, a simple cosine model, ANOVA and *t*-tests. Intravenous salicylate $V_{d(ss)}$ was 0.18 L/kg, Cl_B was 3.36 mL/min/kg and $t_{1/2\lambda}$ was 0.63 h. Plasma salicylate concentrations above 25 μg/mL coincided with significant attenuation in peak cortisol concentrations ($P = 0.029$). Peak salicylate concentrations following oral aspirin administration was <10 μg/mL and failed to attenuate cortisol response. Once salicylate concentrations decreased below 5 μg/mL, cortisol response in the castrated groups was significantly higher than uncastrated controls ($P = 0.018$). These findings have implications for designing drug regimens to provide analgesia during routine animal husbandry procedures.

(Paper received 17 January 2007; accepted for publication 3 April 2007)

Hans Coetzee, Department of Clinical Sciences, College of Veterinary Medicine, 111B Mosier Hall, Kansas State University, Manhattan, KS 66506-5601, USA. E-mail: jcoetzee@vet.ksu.edu

INTRODUCTION

Pain inflicted by routine animal husbandry procedures, such as castration, is a major animal welfare concern in beef production (Oltjen & Mitloehner, 2003). Pain is defined as an aversive feeling or sensation associated with actual or potential tissue damage, resulting in physiological, neuroendocrine, and behavioral changes that indicate a 'stress' response (Broom, 2000; Anderson & Muir, 2005). Castration of cattle destined for slaughter in the USA is a common practice (Chase *et al.*, 1995). Based on the number of steers weighing >227 kg (500 lbs) reported by the US Department of Agriculture National Agricultural Statistics Service (USDA NASS, 2006), there are approximately 14.9 million bovine castrations performed in the USA annually. The American Veterinary Medical Association (AVMA) considers castration to be one of the most stressful experiences for livestock. Research in developing improved techniques for painless, humane castration is considered a priority (AVMA, 2006).

Plasma cortisol response has been used to assess stress in animals as the magnitude of response (as indicated by peak height), duration and/or integrated response [as indicated by area under the plasma cortisol concentration curve (AUC_{cort})], is reported to correspond with the predicted noxiousness of the procedure (Mellor *et al.*, 2000). Several studies have evaluated acute cortisol response as a method to determine the extent and duration of distress associated with castration in cattle (Chase *et al.*, 1995; Fisher *et al.*, 1997, 2001; Earley & Crowe, 2002; Stafford *et al.*, 2002; Ting *et al.*, 2003). However, the correlation between plasma drug concentration and the associated mitigation in plasma cortisol response has not been concurrently studied following castration.

Salicylic acid derivatives, which include sodium salicylate and acetylsalicylic acid (aspirin), were the first nonsteroidal

anti-inflammatory drugs (NSAIDs) to be used in modern medicine and are still widely used as analgesic, antipyretic and anti-inflammatory agents (Langston, 1993). Although veterinary forms of aspirin are marketed with label indications for pain relief, fever and inflammation, the drug has never been formally approved by the United States Food and Drug Administration Center for Veterinary Medicine (FDA-CVM) for these purposes (USP Veterinary Pharmaceutical Information Monographs, 2004). A dose of 50–100 mg aspirin/kg bodyweight is commonly used to provide analgesia in cattle although the efficacy of this dose has not been conclusively demonstrated in peer-reviewed studies (Gingerich *et al.*, 1975; Jenkins, 1987). The present study was conducted to evaluate plasma salicylate concentrations following oral aspirin or the intravenous (i.v.) sodium salicylate administration and to correlate these with the plasma cortisol response following surgical castration.

MATERIALS AND METHODS

This protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Kansas State University (KSU) (Protocol # 2472).

Experimental cattle

Twenty Angus crossbred bulls aged approximately 4–6 months and weighing between 215 and 275 kg were acquired from a livestock commission company in Kansas in June 2006. Upon arrival the calves received an eight-way clostridial vaccine (Covexin 8; Schering Plough, Union, NJ, USA), a single injection of tulathromycin at 2.5 mg/kg bodyweight (Draxxin; Pfizer, New York, NY, USA), and doramectin administered topically at 500 μg/kg bodyweight (Dectomax Pour-on; Pfizer).

Housing and husbandry

Study animals were acclimated for approximately 2 weeks prior to study commencement. During this time, they were housed in typical dry lot confinement facilities at KSU. Throughout the experiment, the cattle were fed a typical High Plains receiving diet composed of whole corn, wheat middlings, dry distiller's grain, soybean hull pellets, cottonseed hulls, molasses and a protein/vitamin/mineral supplement. Feed and water were offered *ad libitum*. Bulls were moved to individual 13.40 m^2 indoor stalls in the KSU Veterinary Teaching Hospital within 24 h of study commencement. Each stall was fitted with a head gate to allow individual animal restraint. During the individual housing period concentrate feed was withheld prior to study commencement but bulls had free access to water and grass hay.

Jugular catheterization

Bulls were individually restrained in head gates and fitted with rope halters approximately 12 h prior to study commencement. Following restraint, the area over the jugular vein was clipped and disinfected using 70% isopropyl alcohol and povidone iodine swabs. The catheter site was infiltrated with 2% lidocaine injection (Hospira Inc., Lake Forest, IL, USA) prior to performing a small skin incision to facilitate placement of a 14 G × 130 mm MILACATH® extended use catheter (MILA International, Florence, KY, USA) which was sutured to the skin using #3 nylon Suture (Burns Veterinary Supply, Inc., Westbury, NY, USA) for the duration of the study. Catheter patency was maintained using a heparin saline flush containing three USP units heparin sodium/mL saline (Heparin Sodium Injection; Baxter Healthcare, Deerfield, IL, USA).

Group assignment and randomization procedures

The study was conducted in two treatment phases ($n = 10$ bulls per phase) to allow individual animal housing. Study animals were blocked by bodyweight determined within 24 h prior to study commencement. Bulls were randomly assigned to one of four groups ($n = 5$ animals/group) as follows: (i) uncastrated controls (CONT), (ii) castrated without analgesia (CAST), (iii) castration immediately following i.v. administration of sodium salicylate at 50 mg/kg bodyweight (SAL) and (iv) castration immediately following oral administration of acetylsalicylic acid (aspirin) at approximately 50 mg/kg bodyweight (ASP). Designation to treatment within a group occurred by assigning random numbers using computer software (Microsoft Excel® 2003; Microsoft Corporation, Redmond, WA, USA).

Sodium salicylate treatment (SAL)

A 20% w/v solution of sodium salicylate was prepared with sodium salicylate USP standard (Sigma Laboratories, St Louis, MO, USA) that was reconstituted with sterile water for injection (Hospira Inc.) immediately prior to administration. Bodyweights obtained within 24 h prior to castration were used to calculate the administered dose for each animal. Bulls in the SAL group received 50 mg sodium salicylate per kilogram bodyweight as a single i.v. bolus injection immediately (<30 sec) prior to castration. The jugular catheter was flushed with 5 mL of heparinized, physiologic saline after administration.

Acetylsalicylic acid (aspirin) treatment (ASP)

Aspirin tablets (60 grain) (Sparhawk Laboratories, Lenexa, KS, USA) were administered orally to the ASP group within 1 min prior to castration at a nominal dose of 50 mg/kg. A conversion factor of 1 grain equal to 64.8 mg aspirin was used in conjunction with bodyweights obtained within 24 h prior to castration to calculate the required dose for each animal. The required dose was rounded to the nearest whole tablet resulting in actual administered doses ranging from 44.63 to 54.02 mg/kg. Subsequent pharmacokinetic modeling was based on the actual dose administered.

Castration procedure

Both phases of the study commenced at approximately 07:00 h Central Time (US and Canada) with castration or simulated castration occurring at 3 min intervals. The castration procedures during each phase of the study were completed by 07:40 h Central Time. All castrations were performed by the same experienced veterinarian.

Uncastrated controls (CONT)

Prior to simulated castration, bulls in the CONT were restrained in a head gate as conducted during the acclimation period and a blood sample was collected for baseline plasma cortisol determination. Thereafter, the scrotum was washed with dilute chlorhexidine disinfectant and prepared similar to surgical castration. Following manipulation of the testicles, blood samples were collected as described in the Blood sample collection section.

Surgical castration (CAST, ASP and SAL)

Calves in the castration groups were similarly restrained and the scrotum was cleaned in the same way as the uncastrated control calves. Thereafter, the scrotum was incised using a sharp, disinfected Newberry knife. The testes and spermatic cords were then exteriorized by blunt dissection and the scrotal fascia was stripped from each exteriorized testicle. A Henderson castration tool (Stone Manufacturing & Supply Company, Kansas City, MO, USA) attached to a 6.0 V cordless variable-speed hand drill (Black & Decker Inc., Towson, MD, USA) with a 3/8″ chuck was clamped across the entire spermatic cord just proximal to the testicle. The drill was then powered to rotate the clamped spermatic cord at a slow to moderate speed in a clockwise direction with increasing drill speed after the initial five to six turns according to the manufacturer's instructions until the testicle twisted off after approximately 20 turns of the tool. The tightly coiled sealed segment of the cord then retracted into the abdomen. The same procedure was used to remove the second testicle. Following castration, blood samples were collected as detailed in the next section.

Blood sample collection

Blood samples for cortisol and salicylate determination were collected in syringes using the preplaced jugular catheter prior to castration or simulated castration; immediately following castration and again at 10, 20, 30, 40, 50 min and 1, 1.5, 2, 4, 6, 8, 10 and 12 h thereafter. Blood was immediately transferred to 6 mL K_2 EDTA and lithium heparin vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ, USA) and stored on ice prior to centrifugation for 15 min at 1500 ***g*** within 30 min of collection. Plasma was then pipetted to cryovials and frozen at −70 °C prior to analysis.

Sample analysis

Plasma cortisol concentrations were determined using a solid-phase competitive chemiluminescent enzyme immunoassay (Immulite® 1000 Cortisol; DPS, Los Angeles, CA, USA). Sample analysts were masked to the identity of treatment groups. A minimum sample volume of 100 μL was used in each assay well. The calibration range for the assay was 28–1380 nmol/L and the analytical sensitivity was 5.5 nmol/L. The precision of the assay was determined by the manufacturer to be <10% coefficient of variation. The reported accuracy by recovery at concentrations of 109, 215 and 349 μg/dL was between 102% and 117%.

Plasma salicylate concentrations were determined using a fluorescence polarization immunoassay kit (TDx®/TDxFLx®; Abbott Laboratories, Abbott Park, IL, USA). A minimum sample volume of 50 μL was used in each assay well. The TDx® and TDxFLx® software calculated a calibration curve using a best-fit curve equation determined using six calibration points with a detection concentration range between 5 and 800 μg/mL. The concentration of salicylate in unknown samples was calculated from this curve using polarization values generated for each sample. The sensitivity of the assay is defined by the manufacturer as the lowest measurable concentration which can be distinguished from zero with 95% confidence, was 5 μg/mL. The precision of the assay was determined by the manufacturer to be <8% coefficient of variation. The reported accuracy by recovery at concentrations of 50, 100, 200, 400 and 800 μg/mL was between 98.3% and 102.5%.

Data analysis and statistics

Data were entered into a spreadsheet (Microsoft Excel® 2003; Microsoft Corporation) for subsequent calculation and manipulation. The mean ± SEM (standard error of the mean) were calculated for cortisol and salicylate concentrations at each time point. Salicylate and cortisol plasma concentrations were also modeled mathematically as detailed below. Hypothesis tests were conducted using JMP analytical software (SAS Institute Inc., Cary, NC, USA). The analysis of variance approach to repeated measures data (MANOVA) was used to analyze plasma cortisol levels based on a review by Everitt (1995). Within group interactions and evidence of time × group interactions were evaluated using the Wilk's lambda test. This test uses a likelihood ratio statistic for testing that a multivariate contrast is zero, assuming multivariate normality and further assuming the equality of covariance matrices across groups (Everitt & Dunn, 2001).

Group differences between animals were analyzed using ANOVA and Student's *t*-tests for normally distributed data and Kruskal–Wallis ANOVA on ranks for data that were not normally distributed. Statistical significance was designated *a priori* as a *P*-value ≤ 0.05. Model parameter values were summarized as mean ± standard error (normally distributed) or median and range (not normally distributed).

Salicylate pharmacokinetic analysis

Noncompartmental analysis (based on statistical moment theory) of the i.v. salicylate time–concentration data was performed

using the commercially available software program (WinNonlin®; Pharsight Corporation, Cary, NC, USA). This program estimates the initial concentration (C_0) for an i.v. bolus dose by back-extrapolating from the first two time–concentration points to the *y*-axis. The extrapolated data point is then used to calculate the area under the time–concentration curve (*AUC*) by the trapezoidal rule. The *AUC* is then extrapolated to infinity using Eqn 1.

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_{\text{last}}}{\lambda_z}, \quad (1)$$

where C_{last} is the last observed plasma concentration; AUC_{0-t} and $AUC_{0-\infty}$ are the area under the time–concentration curve up to the last sampling point and extrapolated to infinity, respectively; and λ_z is the first-order rate constant associated with the terminal (log-linear) portion of the curve that is estimated by linear regression of the semi-logarithmic time–concentration curve. Data points were weighted by the inverse of their value squared (i.e. $1/y^2$) to improve the fit of the extrapolated line.

Other calculated parameters are the mean residence time (*MRT*), apparent volume of distribution at steady-state ($V_{d(ss)}$) and total body clearance (Cl_B) (Eqns 2 – 5).

$$MRT_{0-t} = \frac{AUMC}{AUC_{0-t}}, \quad (2)$$

where *AUMC* is the area under the first moment curve.

$$MRT_{0-\infty} = MRT_{0-t} + \frac{t_{\text{last}} \times C_{\text{last}}}{\lambda_z} + \frac{C_{\text{last}}}{\lambda_z^2}, \quad (3)$$

where t_{last} is the time of the last observation.

$$Cl_B = \frac{\text{Dose}}{AUC_{0-\infty}}. \quad (4)$$

$$V_{d(ss)} = MRT_{0-\infty} \times Cl_B. \quad (5)$$

Cortisol model

To account for the circadian variation in plasma cortisol concentrations, a model incorporating a simple cosine function was fitted to the plasma cortisol concentration–time data (Kong *et al.*, 1989) (Eqns 6 and 7).

$$R_{\text{cort}} = R_m + R_b \times \cos(T) \quad (6)$$

$$T = (t + X)\left(\frac{2\pi}{24}\right) \quad (7)$$

where R_{cort} is the circadian concentration of cortisol, R_m is the mean cortisol concentration, R_b is the amplitude of the variation of cortisol concentration, t is the clock time within the 24-h cycle, T is the time converted to radians by the numeric ratio in Eqn 7 and X is the peak time of the circadian function. This analysis was implemented using the commercially available software program (WinNonlin®; Pharsight Corporation). Parameter values for each individual animal were estimated by fitting the model to the data using the Gauss–Newton algorithm. Assessment of the goodness-of-fit was based on the variability of the parameter estimates, graphs of predicted vs. observed plasma cortisol concentrations and residual plots.

The maximum plasma cortisol concentration (C_{cortmax}) and the time at which this occurred (t_{cortmax}) were observed directly from the data. The area under the plasma cortisol concentration–time curve (AUC_{cort}) was calculated using the trapezoidal method.

RESULTS

Salicylate pharmacokinetic analysis

Following i.v. sodium salicylate administration, plasma salicylate concentrations declined rapidly to levels below the limit of quantification (LOQ) of the assay (5 μg/mL) by 240 min (4 h) postadministration. The $V_{d(ss)}$ was 0.18 L/kg, Cl_B was 3.36 mL/min/kg and elimination half-life ($t_{1/2\lambda}$) was 0.63 h. The pharmacokinetic parameters for salicylate following i.v. administration are summarized in Table 1 and the average plasma time–concentration curve is illustrated in Fig. 1. Salicylate concentrations above the LOQ of the assay were evident in only three of the five animals receiving oral aspirin. For one animal this occurred between 1.5 and 3 h and for the other two animals this occurred at 2 h postadministration. In all cases, plasma salicylate concentrations following oral aspirin administration remained below 10 μg/mL for the duration of the study.

Table 1. The pharmacokinetic parameters for salicylate based on noncompartmental analysis of the salicylate time–concentration following intravenous administration of sodium salicylate (20% w/v) at 50 mg/kg

		Animal							
Parameter	Units	12	13	34	36	40	Mean	SD	SE
AUC_{inf}	h·μg/mL	286.80	216.26	217.33	195.94	180.18	219.30	40.76	18.23
$AUMC_{\text{inf}}$	h·h·μg/mL	318.92	187.83	184.91	150.06	154.61	199.27	69.05	30.88
Cl	mL/min/kg	2.51	3.32	3.31	3.70	3.99	3.36	0.55	0.25
$t_{1/2\lambda}$	h	0.79	0.57	0.60	0.54	0.61	0.63	0.10	0.04
λ_z	Per hour	0.87	1.16	1.15	1.28	1.14	1.12	0.15	0.07
MRT_{inf}	h	1.11	0.87	0.85	0.77	0.86	0.89	0.13	0.06
$V_{d(ss)}$	L/kg	0.17	0.17	0.17	0.17	0.21	0.18	0.02	0.01

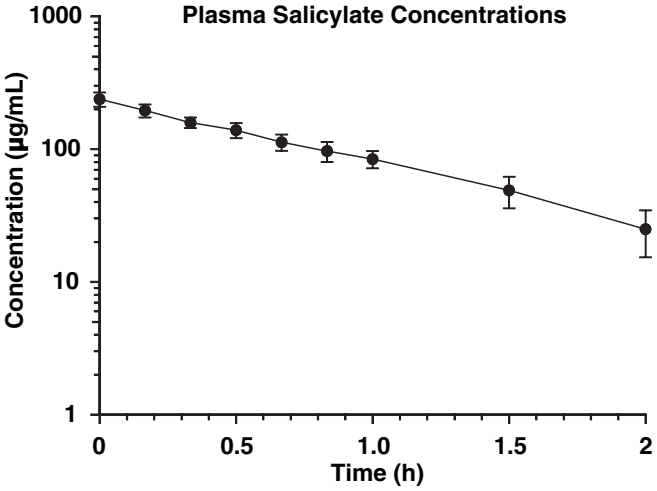

Fig. 1. Semilogarithmic plot of plasma salicylate concentrations following intravenous administration of sodium salicylate (20% w/v) at 50 mg/kg prior to castration.

Observed plasma cortisol concentrations

In all analyses, the Wilk's lambda test was not significant ($P = 0.24$). This suggests that cortisol response following castration was essentially parallel over time. Prior to castration, the baseline mean plasma cortisol concentration (±SEM) for the different treatment groups ranged from 137.60 ± 15.38 to 145.64 ± 25.74 nmol/L (Fig. 2). Immediately following castration, mean plasma cortisol response increased in all groups except the SAL group where there was a decrease to 131.64 ± 24.30 nmol/L. An observed peak mean cortisol concentration of 227.80 ± 17.40 nmol/L in the ASP group and 188.60 ± 26.27 nmol/L in the CAST group occurred at 20 min postcastration. At this time point, the mean plasma cortisol concentration in the SAL group was similar to baseline levels and significantly lower than the ASP group ($P < 0.016$). Statistically significant differences between the SAL and ASP groups were also evident at 40 and 90 min postcastration ($P < 0.05$). Observed mean peak cortisol responses of 157.96 ± 26.45 nmol/L in the SAL group and 182.60 ± 10.41 nmol/L in the CONT group were recorded at 30 min postcastration.

Cortisol concentrations decreased from peak levels to precastration levels in the SAL, CONT and CAST groups within 60 min postcastration. However, the mean cortisol concentration in the ASP group remained above baseline levels for 120 min after castration. From 240 min postcastration to the end of the study plasma cortisol levels in the three castration groups were similar. However, animals in the CONT group had lower mean cortisol responses that were significantly less than castrated cattle at 360 min after castration ($P = 0.018$).

Cortisol model

The calculated cortisol parameters are summarized in Table 2. There were no statistically significant differences between treatment groups based on ANOVA of the cortisol parameters calculated using the noncompartmental model. However, the mean (±SEM) AUC_{cort} ranged from 42.75 ± 4.14 to 63.97 ± 4.86 µmol·min/L in the CONT and ASP groups, respectively, which was significantly different based on Student's *t*-tests ($P = 0.026$). Furthermore, the mean (±SEM) $C_{cortmax}$ ranged from 168 ± 22.61 nmol/L in the SAL group to 235 ± 18.01 nmol/L in the ASP group which was also significantly different based on *t*-tests ($P = 0.029$). The $t_{cortmax}$ ranged from 20 min (range 10–130 min) in the CONT group to 40 min (range 0–70 min) in the SAL group.

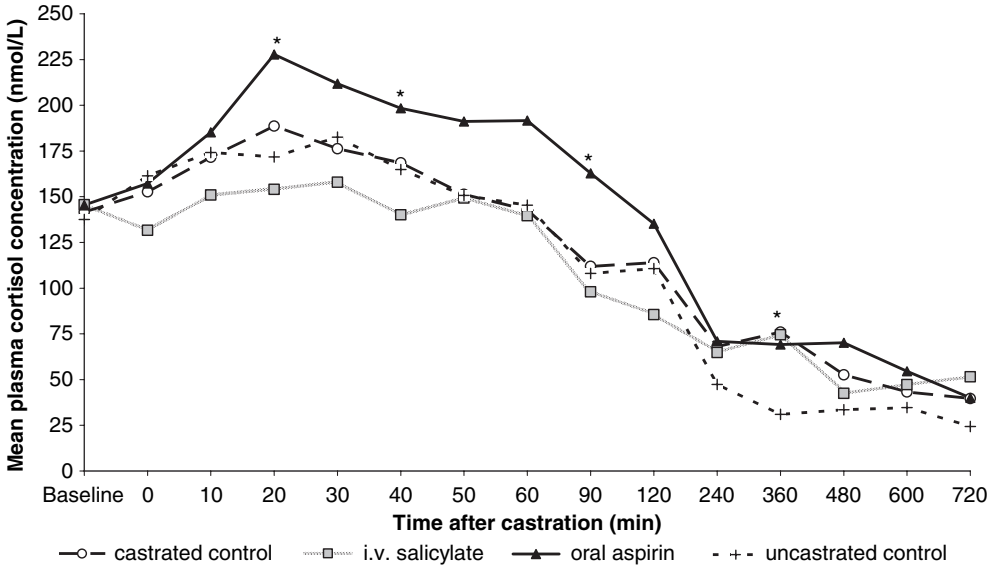

Fig. 2. Mean observed plasma cortisol concentrations over time in bulls randomly assigned to be uncastrated controls, castrated without analgesia or to receive intravenous sodium salicylate or oral aspirin administered at 50 mg/kg prior to castration. *Student's *t*-test $P < 0.05$.

Table 2. Plasma cortisol concentrations based on noncompartmental analysis of cortisol time–concentration measurements

		Castrated control		Intravenous salicylate		Oral aspirin		Uncastrated control	
Parameter	Units	Mean	SEM or range	Mean	SEM or range	Mean	SEM or range	Mean	SEM or range
AUC_{cort}	μmol·min/L	54.34	8.22	50.90	6.58	63.97[b]	4.86	42.75[a]	4.14
$C_{cortmax}$	nmol/L	190.60	24.88	168.80[a]	22.61	235.00[b]	18.01	192.00	8.69
$t_{cortmax}$	min	30	20–40	40	0–70	30	30–50	20	10–130

Entries with different superscript letters are significantly different based on Student's *t*-test ($P < 0.05$).
AUC_{cort}, area under the plasma cortisol concentration–time curve; $C_{cortmax}$, maximum plasma cortisol concentration; $t_{cortmax}$, time at which this occurred.

Table 3. Optimized parameter estimates for the plasma cortisol model based on a simple cosine function to approximate the temporal profile of plasma cortisol concentrations

		Castrated control		Intravenous salicylate		Oral aspirin		Uncastrated control	
Parameter	Units	Mean	SE	Mean	SE	Mean	SE	Mean	SE
R_m	nmol/L	109.03	14.21	104.59	17.86	124.00	8.49	105.76	4.89
R_b	nmol/L	74.82	9.07	69.05	19.53	87.32	10.66	94.79	5.05
X	h	−3.04	0.40	−2.69	0.68	−2.10	0.65	−3.26	0.30

R_m, mean cortisol concentration; R_b, amplitude of the variation of cortisol concentration; X, peak time of the circadian function.

The optimized parameter estimates for the plasma cortisol model are summarized in Table 3 and the average simulated time–concentration curves for the different study groups are compared with the experimental data in Fig. 3. There were no statistically significant group differences between treatments for mean plasma cortisol levels (R_m), amplitude of the variation of cortisol concentration (R_b) and peak time of the cortisol circadian function (X) based on ANOVA. The R_m ranged from 104.59 ± 17.86 nmol/L in animals receiving i.v. salicylate to 123.76 ± 8.49 nmol/L in the group receiving oral aspirin. The R_b ranged from 69.05 ± 19.53 nmol/L in the SAL group to 94.80 ± 5.06 nmol/L in the uncastrated control animals. Finally, the peak time of X ranged from −2.10 ± 0.65 h in the group receiving oral aspirin to −3.26 ± 0.30 h in CONT.

DISCUSSION

The results of the present study demonstrate that i.v. sodium salicylate administered at 50 mg/kg immediately prior to surgical

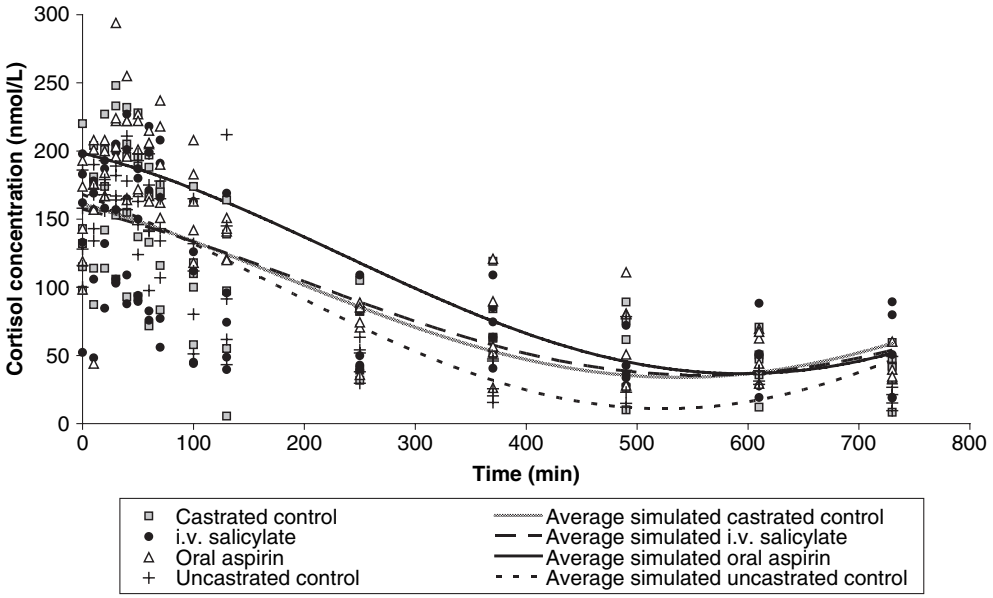

Fig. 3. Observed plasma cortisol concentrations over time fitted to a cosine model to simulate average circadian cortisol response in castrated, uncastrated and salicylate-treated cattle.

castration attenuates acute plasma cortisol response in cattle following castration. To the best of our knowledge, this is the first study demonstrating this effect while concurrently evaluating plasma cortisol and drug concentrations postcastration. Our study also found that aspirin administered *per os* at 50 mg/kg failed to achieve plasma salicylate concentrations above 10 μg/mL and failed to mitigate an acute effect on acute cortisol response. In fact, plasma cortisol concentrations in the ASP group were significantly higher than the SAL group at 20, 40 and 90 min postcastration. It is also noteworthy that once plasma salicylate concentrations fell below the LOQ at 4 h post i.v. administration, plasma cortisol concentrations in the SAL group were similar to the other castrated animals and significantly higher than the CONT group at the next sampling time point. These findings have important implications for designing effective analgesic regimens to aid in alleviating the stress response associated with a noxious stimulus during routine animal husbandry procedures.

Cortisol is widely used to quantify a stress response associated with nociception as its response magnitude, duration and/or integrated response is reported to correspond with the predicted noxiousness of different animal husbandry procedures (Chase *et al.*, 1995; Fisher *et al.*, 1997; Mellor *et al.*, 2000; Fisher *et al.*, 2001; Earley & Crowe, 2002; Stafford *et al.*, 2002; Ting *et al.*, 2003). The results of the present study concur with studies reviewed by Stafford and Mellor (2005) that showed that peak cortisol concentrations occur within 30 min of surgical castration. However, peak and baseline cortisol responses in previous reports were much lower and remained above baseline concentrations for longer than reported herein (Stafford & Mellor, 2005). Our study also found considerable variation in cortisol response between individual animals. This is consistent with earlier reports that found plasma cortisol concentrations vary greatly between animals in terms of the extent to which a particular stressor elicits a sympatho-adrenal response (Ingram *et al.*, 1980; Stafford *et al.*, 2002). This variability reduced the power of the statistical tests we performed and contributed to a lack of statistically significant difference between treatment groups.

The disparity between our results and those reported previously is most likely attributable to the amount of time cattle spent in the research facilities prior to study commencement. Previous researchers typically examined changes in cortisol response in bulls that were acclimated in the research facilities for a period of at least 7 days. In contrast, animals in the present study were moved to the research facilities within 24 h prior to study commencement in an effort to simulate the conditions encountered in typical production settings. This abbreviated acclimation period could explain the higher overall plasma cortisol concentrations measured in both castrated and uncastrated cattle as a result of handling and restraint. It is conceivable that the higher precastration cortisol levels in the present study could mask subtle differences in cortisol response following castration. This is supported by our observation that the mean plasma cortisol in the castrated and uncastrated control groups was essentially identical up to 240 min postcastration. It is also noteworthy that the cortisol response in the ASP group was significantly higher than the SAL group at 20, 40 and 90 min postcastration and remained above baseline nearly twice as long as the other groups. We attribute this to additional stress associated with oral dosing. Taken together, these results suggest that cortisol measurement in conditions equating to a typical practice environment is not a specific indicator of pain associated with castration in cattle.

Although peak cortisol response is reported to correlate with the noxiousness of a procedure, interpretation at each end of the response range are less predictive (Mellor *et al.*, 2000; Toscano *et al.*, 2003). At the lower end of the cortisol response, studies have shown that tail docking with a ring and tail docking with a docking iron cause similar cortisol responses to control handling in older lambs (Lester *et al.*, 1991, 1996). At the upper end of the range, there are several studies, including the present report, that fail to demonstrate an increase in cortisol response that is proportional to the severity of a procedure as might be expected (Lester *et al.*, 1991; Molony & Kent, 1997). This is especially evident when baseline cortisol levels are high which suggest a 'ceiling effect' on plasma cortisol responses that may be reached through the stress of handling alone and not necessarily castration (Molony & Kent, 1997). This phenomenon may also limit the utility of plasma cortisol as a pharmacodynamic indicator of a pain response.

Despite these limitations, Fisher *et al.* (1996) used reduction in acute cortisol response to demonstrate an analgesic effect of lidocaine local anesthesia administered prior to surgical or burdizzo castration in calves. Cortisol suppression lasted 90 min which coincides with the period of local anesthesia (Lemke & Dawson, 2000; Spoormakers *et al.*, 2004). It has also been shown that ketoprofen reduces the acute plasma cortisol response in cattle when administered at the time of castration (Earley & Crowe, 2002; Stafford *et al.*, 2002; Ting *et al.*, 2003). In fact, ketoprofen more effectively attenuated plasma cortisol response following burdizzo castration than a local anesthetic or an epidural (Ting *et al.*, 2003). These data support the use of plasma cortisol as a pharmacodynamic indicator of stress augmented by pain associated with castration (Stafford & Mellor, 2005).

The pharmacodynamic analysis of plasma cortisol concentrations as a marker of response to stress can be confounded by the diurnal rhythm of cortisol release (Mellor *et al.*, 2000). To overcome this effect, we used a simple cosine function to approximate the temporal profile of plasma cortisol concentrations (Kong *et al.*, 1989). When applied to this model, mean plasma cortisol levels (R_m) in animals receiving oral aspirin were higher than the R_m in the SAL group. This finding supports our earlier observation that cortisol response in the ASP group was higher presumably due to additional handling and unmitigated as plasma salicylate concentrations remained below 10 μg/mL throughout the study.

Similarly differences in R_b indicate that the amplitude of the variation of cortisol concentration was greater in the CONT group than the SAL group. This could be attributed to cortisol concentrations in the SAL group remaining similar to baseline

levels during the period in which salicylate concentrations were above 25 μg/mL after which they adopted the same profile as animals in other castration groups. In contrast, the cortisol response profile in the castrated group resembled the uncastrated group during the first 120 min after which levels in the control group were significantly lower resulting in greater amplitude of variation. Although variations in peak time of the cortisol circadian function (*X*) were observed using this model, the significance of these differences is not known. We believe that *X* may provide information regarding the profile of the cortisol response although further studies to collect data at later time points are required to make this model more robust.

The pharmacokinetics and dosage of aspirin in adult dairy cows has been described by Gingerich *et al.* (1975). These authors reported a mean elimination half-life of 0.54 h following i.v. administration of a 20% w/v solution of sodium salicylate at 50 mg/kg which is similar to the 0.63 h reported herein. However, the apparent specific volume of distribution of 0.24 L/kg reported previously is slightly higher than the $V_{d(ss)}$ of 0.18 L/kg reported here. This could be attributed to differences in the method of calculation or possibly due to high protein binding. Plasma clearance of salicylate in the present study was 3.36 mL/min/kg, which is less than 5.13 mL/min/kg reported previously. Inconsistencies between these two reports could be attributed to differences in analytical technique, method of calculation, drug formulation and physiological maturity or rumen development between beef calves and adult dairy cows. This earlier report also examined the pharmacokinetics of aspirin administered orally to adult dairy cows at 50 and 100 mg/kg. The peak plasma concentration achieved with the 50 mg/kg oral dose was approximately 20 μg/mL and the bioavailability of salicylate was reported to be 70%. These data conflict with our findings which demonstrate plasma salicylate concentrations below 10 μg/mL for the duration of the study. This observation requires further evaluation but suggests that oral aspirin may have a lower bioavailability than previously believed.

It is noteworthy that mean plasma cortisol concentrations in the animals receiving i.v. sodium salicylate were lower than other groups for at least 120 min postcastration although this difference was not statistically significant at every time-point. Attenuation of cortisol response in this group coincided with plasma salicylate concentrations above 25 μg/mL. By 240 min postcastration, plasma salicylate concentrations were below the LOQ of 5 μg/mL. This corresponded with higher mean plasma cortisol concentrations in the castrated groups than uncastrated controls. At the next time point, plasma cortisol concentrations in the SAL, CAST and ASP groups were significantly higher than the CONT group. For the ASP group, plasma salicylate concentrations above 10 μg/mL were not observed and acute cortisol response following castration was not attenuated. These results support the conclusion that attenuation of peak cortisol response was related to plasma salicylate concentrations and that the maximal effect was observed when concentrations remained above 25 μg/mL. These results also provide support to previous studies that have suggested a serum salicylate concentration of 30 μg/mL as an effective therapeutic goal in cattle based on the therapeutic minimum concentration in humans (Gingerich *et al.*, 1975).

The reason why mean plasma cortisol response in the SAL group remained below the CONT group is not known. Aspirin inhibits prostaglandin (PG) synthesis by irreversibly blocking both cyclooxygenase (COX) 1 and 2 isoforms. Turnbull and Rivier (1996) determined the plasma adrenocorticotropic hormone (ACTH) and corticosterone responses of rats to acute local inflammation induced by intramuscular injection of turpentine. These researchers concluded that ACTH response and the associated rise in plasma cortisol were mediated by PG and that inhibition of PG synthesis with an NSAID attenuated this response. If restraint of the uncastrated animals resulted in PG release, this might offer an explanation why the cortisol response in the SAL group was lower than the uncastrated control animals for the first 120 min. However, research examining cortisol responses in uncastrated animals receiving i.v. salicylate are required to investigate this explanation.

In conclusion, the results of the present study provide evidence that sodium salicylate administered at 50 mg/kg i.v. at the time of castration (SAL) attenuates peak cortisol response while plasma drug concentrations remain above 25 μg/mL. In contrast, oral aspirin administered at 50 mg/kg (ASP) was poorly absorbed as evidenced by plasma salicylate concentrations that remained below 10 μg/mL for the duration of the study. Plasma cortisol concentrations in the ASP group were higher than untreated castrated and uncastrated animals presumably due to the additional stress of handling associated with oral drug administration. These findings suggest that oral aspirin doses in excess of 50 mg/kg would be required to attenuate the cortisol response associated with castration in bulls weighing over 200 kg. Furthermore, salicylic acid derivatives may need to be administered more frequently than every 12 h as currently recommended. Further research using larger groups of animals, higher doses of aspirin and more sampling time-points around the time where plasma drug concentration are decreasing, are required to elucidate this effect further.

ACKNOWLEDGMENTS

This research was funded in part by a Career MAPS award administered under the KSU ADVANCE Institutional Transformation Program and by the KSU College of Veterinary Medicine. Ms Bettenhausen was supported by the KSU Veterinary Research Scholars program funded by the National Center for Research Resources of the National Institutes of Health (NCRR T35RR007064). The authors wish to acknowledge the invaluable contributions of the following KSU faculty members, staff and students who assisted with sample collection, processing and care of the study animals: Dr Jepkoech Tarus, Dr David Anderson, Erin Evanson, Dan Linden, Paul Wagoner, Elliot Stevens, Keith DeDonder, Shauna England, Kellie Triplett, Bryan Kerling, Lindsay Waechter-Mead, Sarah Weber and Sara McReynolds. This study also benefited from technical laboratory

assistance provided by Kara Smith and Rita Doyle from the KSU-Veterinary Clinical Sciences laboratory. The authors also wish to thank Shirley Arck, PharmD and David Conner, RPh from the KSU-Veterinary Teaching Hospital for preparing the sodium salicylate and aspirin treatments.

REFERENCES

American Veterinary Medical Association (2006) *AVMA Policy Statements; Castration and Dehorning of Cattle*. Available at: http://www.avma.org/issues/policy/animal_welfare/dehorning_cattle.asp (accessed 9 March 2007).

Anderson, D.E. & Muir, W.W. (2005) Pain management in cattle. *Veterinary Clinics of North America. Food Animal Practice*, **21,** 623–635.

Broom, D.M. (2000) The evolution of pain. In *Pain: Its Nature and Management in Man and Animals*. Eds Soulsby, E.J.L., Morton, D., pp. 17–25. The Royal Society of Medicine Press, London, UK.

Chase, C.C. Jr, Larsen, R.E., Randel, R.D., Hammond, A.C. & Adams, E.L. (1995) Plasma cortisol and white blood cell responses in different breeds of bulls: a comparison of two methods of castration. *Journal of Animal Science*, **73,** 975–980.

Earley, B. & Crowe, M.A. (2002) Effects of ketoprofen alone or in combination with local anesthesia during castration of bull calves on plasma cortisol, immunological, and inflammatory responses. *Journal of Animal Science*, **80,** 1044–1052.

Everitt, B.S. (1995) The analysis of repeated measures: a practical review with examples. *The Statistician*, **44,** 113–135.

Everitt, B.S. & Dunn, G., 2001. *Applied Multivariate Data Analysis*, p. 223. Arnold, London.

Fisher, A.D., Crows, M.A., Alonso de la Varga, M.E. & Enright, W.J. (1996) Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. *Journal of Animal Science*, **74,** 2336–2343.

Fisher, A.D., Crowe, M.A., O'Naullain, E.M., Monaghan, M.L., Larkin, J.A., O'Kiely, P. & Enright, W.J. (1997) Effects of cortisol on in vitro interferon-γ production, acute-phase proteins, growth and feed intake in a calf castration model. *Journal of Animal Science*, **75,** 1041–1047.

Fisher, A.D., Knight, T.W., Cosgrove, G.P., Death, A.F., Anderson, C.B., Duganzich, D.M. & Matthews, L.R. (2001) Effects of surgical or banding castration on stress responses and behavior of bulls. *Australian Veterinary Journal*, **79,** 279–284.

Gingerich, D.A., Baggot, J.D. & Yeary, R.A. (1975) Pharmacokinetics and dosage of aspirin in cattle. *Journal of the American Veterinary Medical Association*, **167,** 945–948.

Ingram, D.L., Daucey, M.J., Barrand, M.A. & Callingham, B.A. (1980) Variations in plasma catecholamines in the young pig in response to extremes of ambient temperature compared with exercise and feeding. In *Catecholamines and Stress: Recent Advances*. Eds Usdin, E., Kvetnansky, R. & Kopin, I., pp. 273–278. Elsevier North Holland Inc., New York.

Jenkins, W.J. (1987) Pharmacological aspects of analgesic drugs in animals: an overview. *Journal of the American Veterinary Medical Association*, **191,** 1231–1240.

Kong, A.N., Ludwig, E.A., Slaughter, R.L., DiStefano, P.M., DeMasi, J., Middleton, E. Jr & Jusko, W.J. (1989) Pharmacokinetics and pharmacodynamic modeling of direct suppression effects of methylprednisolone on serum cortisol and blood histamine in human subjects. *Clinical Pharmacology and Therapeutics*, **46,** 616–628.

Langston, V.C. (1993) Therapeutic management of inflammation. In *Current Veterinary Therapy 4: Food Animal Practice*. Eds Howard J.L. & Smith R.A., pp. 7–12. W.B. Saunders, Philadelphia, PA.

Lemke, K.A. & Dawson, S.D. (2000). Local and regional anesthesia. *Veterinary Clinics of North America. Small Animal Practice*, **30,** 839–857.

Lester, S.J., Mellor, D.J. & Ward, R.N. (1991) Effects of repeated handling on the cortisol responses of young lambs castrated and tailed surgically. *New Zealand Veterinary Journal*, **39,** 147–149.

Lester, S.J., Mellor, D.J., Holmes, R.J., Ward, R.N. & Stafford, K.J. (1996) Behavioural and cortisol responses of lambs to castration and tailing using different methods. *New Zealand Veterinary Journal*, **44,** 45–54.

Mellor, D.J., Cook, C.J. & Stafford, K.J. (2000) Quantifying some responses to pain as a stressor. In *The Biology of Animal Stress: Basic Principals and Implications for Animal Welfare*. Eds Moberg, G.P. & Mench, J.A., pp. 171–198. CABI publishing, New York, USA.

Molony, V. & Kent, J.E. (1997) Assessment of acute pain in farm animals using behavioral and physiological measurements. *Journal of Animal Science*, **75,** 266–272.

Oltjen, J.W. & Mitloehner, F.M. (2003) *An Overview of Current Beef Welfare Concerns*. Available at: http://www.nal.usda.gov/awic/pubs/Beef/overview.htm (accessed 9 March 2007).

Spoormakers, T.J., Donker, S.H. & Ensink, J.M. (2004) Diagnostic anaesthesia of the equine lower limb: a comparison of lidocaine and lidocaine with epinephrine. *Tijdschr Diergeneeskd*, **129,** 548–551.

Stafford, K.J. & Mellor, D.J. (2005) The welfare significance of the castration of cattle: a review. *New Zealand Veterinary Journal*, **53,** 271–278.

Stafford, K.J., Mellor, D.J., Todd, S.E., Bruce, R.A. & Ward, R.N. (2002) Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. *Research in Veterinary Science*, **73,** 61–70.

Ting, S.T., Earley, B., Hughes, J.M. & Crowe, M.A. (2003) Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. *Journal of Animal Science*, **81,** 1281–1293.

Toscano, M.F., Lay, D.C. & Wilson, M.E. (2003) Physiological indicators of stress. In *The Science and Ethics behind Animal Well-Being Assessment*. Ed. Reynnells, R., pp. 10–13. USDA, Washington, DC.

Turnbull, A.V. & Rivier, C. (1996) Corticotropin-releasing factor, vasopressin, and prostaglandins mediate, and nitric oxide restrains, the hypothalamic-pituitary-adrenal response to acute inflammation in the rat. *Endocrinology*, **137,** 455–463.

U.S. Department of Agriculture National Agricultural Statistics Service (2006) *Agricultural Statistics 2006*. Available at: http://usda.mannlib.cornell.edu/usda/current/Catt/Catt-07-21-2006.pdf.

USP Veterinary Pharmaceutical Information Monographs (2004) Anti-inflammatories: aspirin. *Journal of Veterinary Pharmacology and Therapeutics*, **27** (Suppl. 1), 4–14.