

# Bovine acute-phase response after different doses of corticotropin-releasing hormone challenge<sup>1,2,3</sup>

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**ABSTRACT.** The objective was to compare the acute-phase response of steers receiving different doses of bovine corticotropin-releasing hormone (CRH). Fourteen weaned Angus steers (BW = 191 ± 2.1 kg, age = 167 ± 4.7 d) fitted with an indwelling jugular catheter and a rectal temperature (RT) monitoring device were assigned to receive 1 of 3 treatments (intravenous infusion): 1) 0.1 µg of CRH/kg of BW (CRH1; n = 5), 2) 0.5 µg of CRH/kg of BW (CRH5; n = 5), and 3) 10 mL of saline (0.9%; n = 4). Blood samples were collected via catheters, relative to treatment infusion (0 h), hourly from -2 to 0 h and 4 to 8 h and every 30 min from 0 to 4 h. Rectal temperatures were recorded every 30 min from -2 to 8 h. Blood samples were also collected via jugular venipuncture and rectal temperatures assessed using a digital thermometer every 6 h from 12 to 72 h and every 24 h from 96 to 168 h. All plasma samples collected during the study were analyzed for concentrations of haptoglobin. All plasma samples collected from -2 to 8 h were analyzed for cortisol concentrations. Serum samples collected hourly from -2 to 8 h were analyzed for concentrations of NEFA, IL-6, tumor necrosis factor (TNF)-α, and interferon-γ. Cortisol peaked at 0.5 h for CRH1 steers but returned to baseline

concentrations at 1 h relative to infusion (time effect;  $P < 0.01$ ). In CRH5 steers, cortisol peaked at 0.5 h and returned to baseline concentrations 3.5 h relative to infusion (time effect;  $P < 0.01$ ). Cortisol concentrations did not change after treatment infusion for saline steers (time effect;  $P = 0.42$ ). In CRH1 steers, NEFA concentrations peaked 5 h after treatment infusion (time effect;  $P = 0.01$ ). Conversely, serum NEFA concentrations did not change for CRH5 and saline steers after treatment infusion (time effect;  $P > 0.37$ ). Mean serum TNF-α concentrations in CRH1 steers after treatment infusion were greater compared with saline ( $P = 0.02$ ), tended to be greater ( $P = 0.08$ ) compared with CRH5, and were similar ( $P = 0.40$ ) between CRH5 and saline steers. Mean RT in CRH1 steers after treatment infusion were greater ( $P < 0.04$ ) compared with saline and CRH5 and similar ( $P = 0.50$ ) between CRH5 and saline steers. Haptoglobin increased and peaked 72 h after treatment infusion for CRH1 steers (time effect;  $P = 0.01$ ) but did not change for CRH5 and saline steers (time effect;  $P > 0.45$ ). In conclusion, the bovine acute-phase response stimulated by CRH infusion is dependent on the CRH dose and the subsequent response in circulating cortisol.

**Key words:** acute-phase proteins, acute-phase response, bovine, corticotropin-releasing hormone, proinflammatory cytokines.

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<sup>2</sup> Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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## INTRODUCTION

The acute-phase response is a key component of the innate immune system but can be detrimental to livestock performance (Carroll and Forsberg, 2007). Circulating concentrations of acute-phase proteins were negatively associated with ADG and DMI in growing cattle (Cooke et al., 2009a; Araujo et al., 2010) and with reproductive performance of beef cows (Cooke et al., 2009b). Hence, strategies to prevent or

alleviate the acute-phase response are beneficial to cattle performance and overall efficiency of beef operations (Duff and Galyean, 2007).

Research demonstrated that the bovine acute-phase response is initiated by infections, trauma, and diseases (Carroll and Forsberg, 2007). Stressful management procedures such as weaning and transport also stimulated an acute-phase reaction in overtly healthy cattle (Arthington et al., 2005; Cooke et al., 2011). To further address this latter subject, our group demonstrated that steers receiving a corticotropin-releasing hormone (CRH; 0.1  $\mu\text{g}/\text{kg}$  of BW) infusion to induce a neuroendocrine stress response experienced an acute-phase reaction characterized by increased rectal temperatures (RT) and plasma concentrations of IL-6 and acute-phase proteins relative to baseline concentrations (Cooke and Bohnert, 2011). We attributed this outcome to the catabolic effects of cortisol on body tissues, including NEFA release from adipose tissues, which can be recognized by the immune system as a disruption in homeostasis (Abbas and Lichtman, 2007). Moreover, Higuchi et al. (1994) reported that synthesis of acute-phase proteins in cultures of bovine liver was dependent on the concentration of glucocorticoid added to the culture media. Therefore, we hypothesized that an increased dose of CRH administered to cattle would mimic a greater stress challenge and alter the magnitude of the resultant acute-phase reaction. On the basis of this rationale, the objective was to compare the acute-phase response of beef steers receiving different doses of CRH infusion.

## MATERIALS AND METHODS

The study was conducted in September and October 2010, and the animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

### *Animals*

Fourteen Angus steers were used in the study (d -5 to d 7). All steers were weaned 30 d before the beginning of the study and exposed daily to halter-training techniques to become acclimated to human interaction, thus preventing confounding effects between human handling, weaning, and CRH challenge on the responses measured herein (Arthington et al., 2005; Curley et al., 2008; Cooke et al., 2009a). On d -5, steers were housed in individual pens contained within an enclosed barn and offered free choice water, mineral-vitamin mix, and meadow foxtail (*Alopecurus pratensis* L.) hay. From d -4 to d -2, steers were weighed daily. Average BW and age at the beginning of the study were, respectively,  $191 \pm 2.1$  kg and 167

$\pm 4.7$  d. On d -1, all steers were fitted with an indwelling jugular vein catheter for serial blood collection according to the procedures described by Merrill et al. (2007) and an indwelling RT monitoring device as described by Reuter et al. (2010). During the study, average daily temperature, maximum daily temperature, and minimum daily temperature were, respectively ( $^{\circ}\text{C}$ ),  $14.0 \pm 1.0$ ,  $26.6 \pm 1.5$ , and  $1.2 \pm 0.8$ . Average daily relative humidity (%) during the study was  $45.6 \pm 2.8$ .

### *Treatments and Sample Collection*

On d 0 of the study, steers were ranked by BW and assigned to receive (h 0 of the study) 1 of 3 infusion treatments (intravenous infusion): 1) 0.1  $\mu\text{g}$  of bovine CRH/kg of BW (CRH1;  $n = 5$ ), 2) 0.5  $\mu\text{g}$  of bovine CRH/kg of BW (CRH5;  $n = 5$ ), and 3) 10 mL of physiological saline (0.9%;  $n = 4$ ). Bovine CRH (#34-3-11; American Peptide Co., Inc., Sunnyvale, CA) was dissolved into 10 mL of physiological saline immediately before challenge and administered to steers via the indwelling jugular catheters. Catheters were flushed with additional 10 mL of physiological saline after infusion to ensure that treatments reached the circulation, followed by 5 mL of heparinized saline (20 IU of heparin/mL of saline) to maintain catheter patency.

Blood samples were collected via catheters hourly from -2 to 0 h and 4 to 8 h and every 30 min from 0 to 4 h relative to treatment infusion (0 h). Rectal temperatures were recorded by the indwelling device (Reuter et al., 2010) every 30 min from -2 to 8 h relative to infusion. Immediately after the collection at 8 h relative to infusion, catheters and rectal probes were removed. Blood samples were collected via jugular venipuncture and RT were assessed using a digital thermometer (GLA M750 digital thermometer; GLA Agricultural Electronics, San Luis Obispo, CA) every 6 h from 12 to 72 h and every 24 h from 96 to 168 h (d 7 of the study) relative to treatment infusion.

### *Blood Analysis*

Blood samples that were collected via jugular catheters were immediately transferred into 2 commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ). One sample was transferred into a tube that did not contain additives for the collection of serum. The other sample was transferred into a tube that contained 158 USP units of freeze-dried sodium heparin for the collection of plasma. Samples were placed immediately on ice, centrifuged within 30 min after collection at  $2,500 \times g$  for 30 min at  $4^{\circ}\text{C}$ , and plasma and serum were immediately frozen at  $-80^{\circ}\text{C}$ . Blood samples obtained via jugular venipuncture were collected directly into commercial tubes (Vacutainer,

10 mL; Becton Dickinson) containing 158 USP units of freeze-dried sodium heparin, placed on ice immediately, centrifuged at  $2,500 \times g$  for 30 min at  $4^{\circ}\text{C}$  for plasma harvest, and frozen at  $-80^{\circ}\text{C}$  on the same day of collection. All plasma samples collected during the study were analyzed for concentrations of haptoglobin. Plasma samples collected hourly from  $-2$  to  $0$  h and  $4$  to  $8$  h and every 30 min from  $0$  to  $4$  h relative to treatment infusion were analyzed for cortisol concentrations. Serum samples collected hourly from  $-2$  to  $8$  h relative to treatment infusion were analyzed for concentrations of NEFA, IL-6, tumor necrosis factor (TNF)- $\alpha$ , interferon (IFN)- $\gamma$ .

Concentrations of cortisol were determined using a bovine-specific commercial ELISA kit (Endocrine Technologies Inc., Newark, CA). Concentrations of NEFA were determined using a commercial kit (HR Series NEFA – 2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA) with the modifications described by Pescara et al. (2010). Concentrations of haptoglobin were determined according to procedures described by Makimura and Suzuki (1982). Concentrations of IL-6, IFN- $\gamma$ , and TNF- $\alpha$  were determined by a multiplex bovine-specific ELISA (SearchLight; Aushon Biosystems, Inc., Billerica, MA), previously validated by Carroll et al. (2009). All samples were analyzed in duplicates. The intra- and inter-assay CV were, respectively, 2.5 and 15.5% for haptoglobin, 6.3 and 9.9% for cortisol, 3.3 and 5.3% for NEFA, 4.8 and 9.8% for IL-6, 5.7 and 13.1% for IFN- $\gamma$ , and 12.8 and 13.6% for TNF- $\alpha$ .

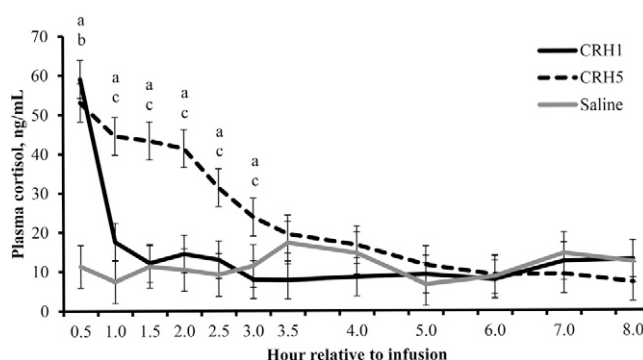
### Statistical Analysis

For all analyses, significance was set at  $P \leq 0.05$ , and tendencies were determined if  $P > 0.05$  and  $\leq 0.10$ . All data were initially tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Only cytokine data were not normally distributed ( $W \leq 0.46$ ); therefore, data were log-transformed to achieve normality ( $W \geq 0.90$ ). All data were analyzed using the PROC MIXED procedure of SAS and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. Steer was considered the experimental unit. The model statements contained the effects of treatment, time, and the resultant interaction. Data obtained before treatment application ( $-2$ ,  $-1$ , and  $0$  h relative to infusion) were averaged and used as covariate, and steer(treatment) was used as random variable. The specified term for the repeated statement was time, and steer(treatment) was included as subject. The covariance structure utilized was autoregressive, which provided the lowest Akaike information criterion and therefore the best fit. Results are reported as covariately adjusted least square means if the covariate was significant ( $P < 0.05$ ) and were separated

by PDIFF. Serum concentrations of proinflammatory cytokines were also analyzed, with the MIXED procedure of SAS and Satterthwaite approximation, but without log-transformation as percentage increase from average pre-infusion values (set as 100%), which yielded normal distribution according to the Shapiro-Wilk test ( $W = 0.93$ ). This model statement contained the effects of treatment, time, and the resultant interaction. Data were analyzed using steer(treatment) as random variable, the specified term for the repeated statement was time, and steer(treatment) was included as subject. The covariance structure utilized was also autoregressive on the basis of the Akaike criterion. Results are reported according to treatment effects if no interactions were significant or according to the highest order interaction detected.

## RESULTS AND DISCUSSION

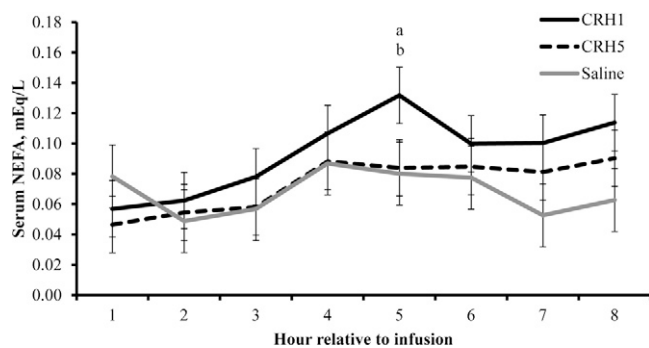
A treatment  $\times$  time interaction was detected ( $P < 0.01$ ) for plasma cortisol (Figure 1). Cortisol concentrations before treatment infusion were significant covariates ( $P < 0.01$ ) but did not differ ( $P = 0.68$ ; data not shown) among treatments (16.3, 12.9, and 10.8 ng/mL for saline, CRH1, and CRH5, respectively; SEM = 4.4). After treatment infusion, plasma cortisol peaked at 0.5 h for CRH1 and returned to baseline concentrations at 1 h relative to infusion (time effect;  $P < 0.01$ ). In CRH5 steers, plasma cortisol peaked at 0.5 h and returned to baseline concentrations 3.5 h relative infusion (time effect;  $P < 0.01$ ). Plasma cortisol concentrations did not change after infusion for saline steers (time effect;  $P = 0.42$ ). Hence, plasma cortisol concentrations were simi-



**Figure 1.** Plasma cortisol concentrations ( $\pm$  SEM) of steers receiving an intravenous infusion containing 10 mL of physiological saline (0.9%;  $n = 4$ ), 0.1  $\mu\text{g}/\text{kg}$  of BW of corticotropin-releasing hormone (CRH1;  $n = 5$ ), or 0.5  $\mu\text{g}/\text{kg}$  of BW of corticotropin-releasing hormone (CRH5;  $n = 5$ ). Corticotropin-releasing hormone was dissolved into 10 mL of physiological saline (0.9%) immediately before infusion. Values obtained from samples collected before treatment infusion ( $-2$ ,  $-1$ , and  $0$  h relative to infusion) served as covariate ( $P < 0.01$ ), but did not differ ( $P = 0.68$ ) among treatments (16.3, 12.9, and 10.8 ng/mL for saline, CRH1, and CRH5, respectively; SEM = 4.4). Therefore, results reported are covariately adjusted least squares means. A treatment  $\times$  time interaction was detected ( $P < 0.01$ ). Within hour, letters indicate the following treatment comparison ( $P < 0.05$ ): a = CRH5 vs. saline, b = CRH1 vs. saline, and c = CRH5 vs. CRH1.

lar between CRH1 and CRH5 ( $P = 0.38$ ) and greater ( $P < 0.01$ ) for both treatments compared with saline cohorts at 0.5 h relative to challenge. From 1 to 3 h relative to treatment infusion, plasma cortisol concentrations were similar between CRH1 and saline ( $P > 0.17$ ) and reduced ( $P < 0.05$ ) for both treatments compared with CRH5 cohorts (Figure 1). Supporting our results, Veissier et al. (1999) and Gupta et al. (2004) reported that Holstein cattle receiving an intravenous infusion of CRH of 0.1  $\mu\text{g}/\text{kg}$  of BW had similar peak concentrations but reduced persistence in the CRH-induced increase in plasma cortisol concentrations compared with cohorts receiving greater CRH doses. The maximum concentration of cortisol detected herein in CRH1 and CRH5 steers was similar compared with circulating concentrations of cortisol reported in stressed cattle (approximately 50 ng/mL; Crookshank et al., 1979, Arthington et al., 2003; Cooke et al., 2011) but greater compared with our previous research effort (13.9 ng/mL; Cooke and Bohnert, 2011). However, in the present study, plasma cortisol was assessed 0.5 h relative to CRH infusion, whereas in Cooke and Bohnert (2011) the first assessment was 1 h after infusion when plasma cortisol concentrations in steers receiving CRH of 0.1  $\mu\text{g}/\text{kg}$  of BW were likely returning to baseline concentrations.

A tendency for a treatment  $\times$  time interaction was detected ( $P = 0.09$ ) for serum NEFA (Figure 2). Mean NEFA concentration before treatment infusion were not significant covariates ( $P = 0.23$ ) and hence similar ( $P = 0.55$ ; data not shown) among treatments (0.072, 0.069, and 0.061 for saline, CRH1, and CRH5, respectively; SEM = 0.007). In CRH1 steers, serum NEFA concentra-



**Figure 2.** Serum NEFA concentrations ( $\pm$  SEM) of steers receiving an intravenous infusion containing 10 mL of physiological saline (0.9%;  $n = 4$ ), 0.1  $\mu\text{g}/\text{kg}$  of BW of corticotropin-releasing hormone (CRH1;  $n = 5$ ), or 0.5  $\mu\text{g}/\text{kg}$  of BW of corticotropin-releasing hormone (CRH5;  $n = 5$ ). Corticotropin-releasing hormone was dissolved into 10 mL of physiological saline (0.9%) immediately before infusion. Values obtained from samples collected before treatment infusion ( $-2$ ,  $-1$ , and 0 h relative to infusion) were not significant covariates ( $P = 0.23$ ) and hence similar ( $P = 0.55$ ) among treatments (0.072, 0.069, and 0.061 for saline, CRH1, and CRH5, respectively; SEM = 0.007). A tendency for a treatment  $\times$  time interaction was detected ( $P = 0.09$ ). A time effect was detected for serum NEFA concentrations in CRH1 steers ( $P = 0.01$ ), but not for CRH5 and saline steers ( $P > 0.37$ ). Within hour, letters indicate the following treatment comparison ( $P < 0.05$ ): a = CRH1 vs. saline, b = CRH1 vs. CRH5.

tions increased and peaked 5 h after treatment infusion (time effect;  $P = 0.01$ ). Conversely, serum NEFA concentrations did not change for CRH5 and saline steers after treatment infusion (time effect;  $P > 0.37$ ). Accordingly, serum NEFA concentrations were greater ( $P = 0.05$ ) for CRH1 steers compared with CRH5 and saline cohorts 5 h relative to treatment infusion (Figure 2). One of the physiological effects of cortisol during a stress response is to degrade body tissues, such as hepatic, adipose, and muscle cells (Nelson and Cox, 2005) to increase the availability of circulating nutrients for the animal to cope with the stressor and restore homeostasis (Carroll and Forsberg, 2007). As expected, serum NEFA concentrations increased after infusion and were greater for CRH1 steers compared with cohorts receiving saline, indicating that the CRH-induced increase in plasma cortisol stimulated lipolysis in CRH1 steers. The same outcome, however, was not detected for CRH5 steers. Khani and Tayek (2001) reported that human subjects infused intravenously with hydrocortisone at 1.8  $\mu\text{g}/\text{min}/\text{kg}$  of BW for 4 h had increased plasma cortisol (approximately 700 nmol/L) and NEFA concentrations compared with control subjects. Conversely, Samra et al. (1998) reported that human subjects infused intravenously with hydrocortisone for 11 h to increase plasma cortisol concentrations to approximately 1,600 nmol/L had reduced NEFA release and consequent veno-arterialized NEFA concentrations in adipose tissues compared with non-infused cohorts. Samra et al. (1998) concluded that severe hypercortisolemia decreased the rate of intracellular lipolysis and thus the efflux of NEFA from adipose depots to the local circulation. These findings may suggest that in the present study, the brief CRH-induced increase in plasma cortisol concentrations in CRH1 did not halt lipolysis and stimulated NEFA release from fat depots. In contrast, the prolonged CRH-induced increase in plasma cortisol concentrations in CRH5 may have prevented significant NEFA release into the circulation by lessening the rate of lipolysis in adipose tissues.

No treatment effects were detected ( $P > 0.33$ ; Table 1) for serum concentrations of IFN- $\gamma$  (1.35, 1.39, and 1.41 log pg/mL for saline, CRH1, and CRH5 steers, respectively; SEM = 0.03) and serum concentrations of IL-6 (0.71, 0.55, and 0.77 log pg/mL for saline, CRH1, and CRH5 steers, respectively; SEM = 0.20). However, a treatment effect was detected ( $P = 0.05$ ) for serum concentrations of TNF- $\alpha$  (Table 1). Mean serum TNF- $\alpha$  concentrations before treatment infusion were significant covariates ( $P < 0.01$ ) but did not differ ( $P = 0.58$ ; data not shown) among treatments (1.68, 2.43, and 1.96 log pg/mL for saline, CRH1, and CRH5 steers, respectively; SEM = 0.49). After treatment infusion, mean serum TNF- $\alpha$  concentrations in CRH1 steers were greater compared with saline ( $P = 0.02$ ), tended to be greater ( $P =$

**Table 1.** Physiological responses of steers receiving an intravenous infusion containing 10 mL of physiological saline (0.9%; n = 4), 0.1 µg/kg of BW of corticotropin-releasing hormone (CRH1; n = 5), or 0.5 µg/kg of BW of corticotropin-releasing hormone (CRH5; n = 5)<sup>1</sup>

| Item  | Treatment |       |       |      | P-values          |                              |                              |                            |
|---|-----------|-------|-------|------|-------------------|------------------------------|------------------------------|----------------------------|
|   | Saline    | CRH1  | CRH5  | SEM  | Main <sup>2</sup> | Saline vs. CRH1 <sup>3</sup> | Saline vs. CRH5 <sup>3</sup> | CRH1 vs. CRH5 <sup>3</sup> |
| Interferon-γ, pg/mL (log) <sup>2</sup>            | 1.35      | 1.39  | 1.41  | 0.03 | 0.33              | 0.29                         | 0.17                         | 0.71                       |
| IL-6, pg/mL (log) <sup>2</sup>                    | 0.71      | 0.55  | 0.77  | 0.20 | 0.72              | 0.61                         | 0.84                         | 0.43                       |
| Tumor necrosis factor-α, pg/mL (log) <sup>4</sup> | 2.23      | 2.40  | 2.28  | 0.04 | 0.05              | 0.02                         | 0.40                         | 0.08                       |
| Tumor necrosis factor-α, % increase <sup>5</sup>  | 158.9     | 202.5 | 180.7 | 12.5 | 0.09              | 0.03                         | 0.26                         | 0.23                       |
| Rectal temperature, °C <sup>4</sup>               | 39.03     | 39.26 | 39.09 | 0.05 | 0.03              | 0.01                         | 0.50                         | 0.04                       |

<sup>1</sup> Corticotropin-releasing hormone was dissolved into 10 mL of physiological saline (0.9%) immediately before infusion.

<sup>2</sup> Main treatment effect.

<sup>3</sup> Calculated using PDIFF for means separation.

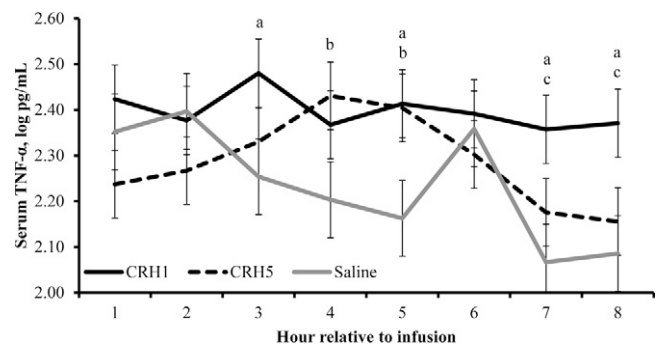
<sup>4</sup> Serum samples were collected hourly, whereas rectal temperature was assessed every 30 min from -2 to 8 h relative to treatment infusion (0 h). Values obtained before treatment infusion served as covariate; therefore, results are reported as covariately adjusted least square means.

<sup>5</sup> Calculated as percentage increase from average pre-infusion values (set as 100%).

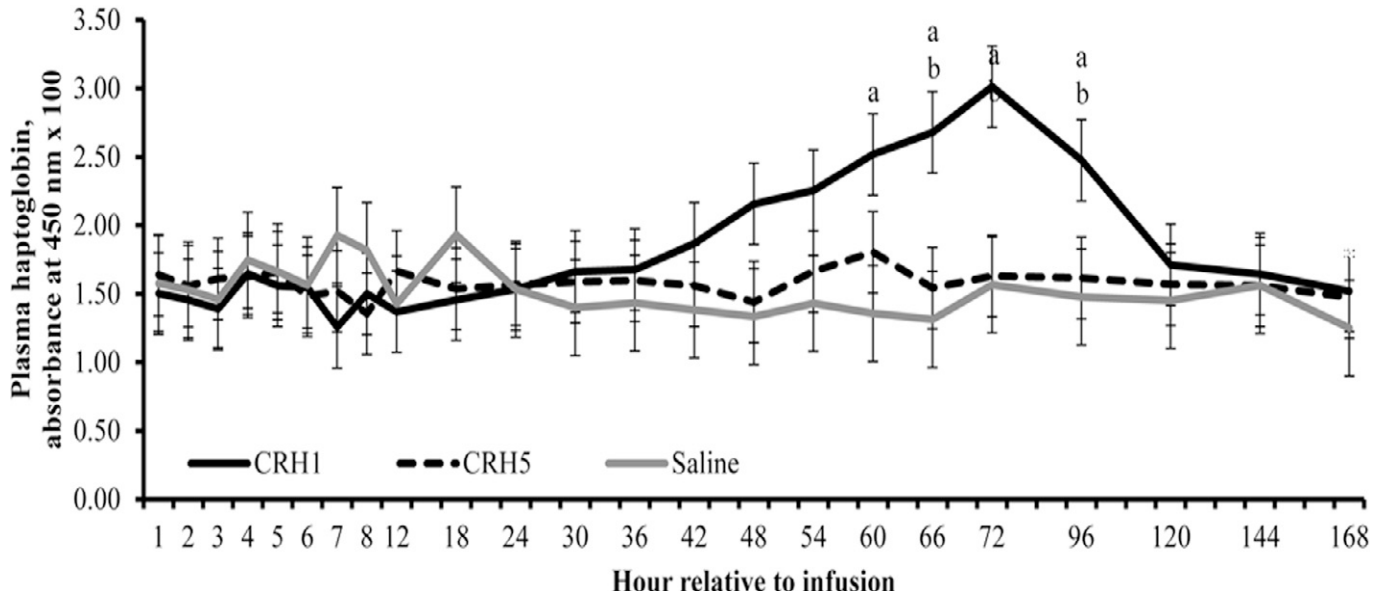
0.08) compared with CRH5, and were similar ( $P = 0.40$ ) between CRH5 and saline steers (2.23, 2.40, and 2.28 log pg/mL for saline, CRH1, and CRH5 steers, respectively; SEM = 0.04). Further, a tendency for a treatment × time interaction was detected ( $P = 0.08$ ) for serum TNF-α (Figure 3) because TNF-α concentrations were greater ( $P < 0.05$ ) for CRH1 compared with saline steers at 3, 5, 7, and 8 h, greater ( $P < 0.05$ ) for CRH5 compared with saline steers at 4 and 5 h, and greater ( $P < 0.05$ ) for CRH1 compared with CRH5 steers at 7 and 8 h relative to treatment infusion. Similarly to Cooke and Bohnert (2011), CRH infusion did not induce an increase in serum IFN-γ, not even when administered at 0.5 µg/kg of BW, suggesting that this proinflammatory cytokine may not be stimulated by the neuroendocrine stress response (Paik et al, 2000). Conversely, in our previous research effort (Cooke and Bohnert, 2011), plasma IL-6 were increased, whereas plasma TNF-α did not change compared with baseline concentrations after CRH infusion of 0.1 µg/kg of BW. The reason for this inconsistency in CRH-induced proinflammatory cytokine response between studies is unknown. The biological activity of proinflammatory cytokines is highly pleiotropic, redundant, overlapping (Ozaki and Leonard, 2002), and still not completely understood in beef cattle (Cooke et al., 2011). In fact, numerous research studies evaluating the interactions among inflammatory and neuroendocrine stress responses in animals yielded contradictory results with respect to synthesis and activity of proinflammatory cytokines (Turnbull and Rivier, 1999). Given the substantial role of proinflammatory cytokines on cattle health and performance (Klasing and Korver, 1997), additional research is warranted to fully understand the bovine proinflammatory cytokine response induced by stress.

Nevertheless, treatment effects detected for serum TNF-α demonstrate that CRH infusion and the conse-

quent increase in plasma cortisol concentrations triggered, at least partially, a proinflammatory cytokine response in beef steers. This response, however, was more prominent in CRH1 compared with CRH5 steers. In fact, when serum TNF-α concentrations are analyzed as percentage increase from pre-infusion values (treatment effect,  $P = 0.09$ ; Table 1), the mean increase in TNF-α concentrations was greater ( $P = 0.03$ ) for CRH1 compared with saline steers but similar ( $P > 0.23$ ) between CRH1 and CRH5 and between CRH5 and saline steers (158.9, 202.5, and 180.7% increase for saline, CRH1, and CRH5, respectively; SEM = 12.5). Upon synthesis and release into the circulation, proinflammatory cytokines elicit 2 major acute-phase responses: 1) increased body temperature and 2) altered liver metabolism



**Figure 3.** Serum tumor necrosis factor-α (TNF-α) concentrations (± SEM) of steers receiving an intravenous infusion containing 10 mL of physiological saline (0.9%; n = 4), 0.1 µg/kg of BW of corticotropin-releasing hormone (CRH1; n = 5), or 0.5 µg/kg of BW of corticotropin-releasing hormone (CRH5; n = 5). Corticotropin-releasing hormone was dissolved into 10 mL of physiological saline (0.9%) immediately before infusion. Values obtained from samples collected before treatment infusion (-2, -1, and 0 h relative to infusion) were significant covariates ( $P < 0.01$ ), although did not differ ( $P = 0.58$ ) among treatments (1.68, 2.43, and 1.95 log pg/mL for saline, CRH1, and CRH5, respectively; SEM = 0.49). Therefore, results reported are covariately adjusted least squares means. A tendency for a treatment × time interaction was detected ( $P = 0.08$ ). Within hour, letters indicate the following treatment comparison ( $P < 0.05$ ): a = CRH1 vs. saline, b = CRH5 vs. saline, and c = CRH1 vs. CRH5.



**Figure 4.** Plasma haptoglobin concentrations ( $\pm$  SEM) of steers receiving an intravenous infusion containing 10 mL of physiological saline (0.9%;  $n = 4$ ), 0.1  $\mu\text{g}/\text{kg}$  of BW of corticotropin-releasing hormone (CRH1;  $n = 5$ ), or 0.5  $\mu\text{g}/\text{kg}$  of BW of corticotropin-releasing hormone (CRH5;  $n = 5$ ). Corticotropin-releasing hormone was dissolved into 10 mL of physiological saline (0.9%) immediately before infusion. Values obtained from samples collected before treatment infusion ( $-2$ ,  $-1$ , and  $0$  h relative to infusion) were significant covariates ( $P = 0.03$ ), although did not differ ( $P = 0.93$ ) among treatments (1.50, 1.49, and 1.46 absorbance at  $450 \text{ nm} \times 100$  for saline, CRH1, and CRH5, respectively; SEM = 0.07). Therefore, results reported are covariately adjusted least squares means. A treatment  $\times$  time interaction was detected ( $P = 0.04$ ). Within hour, letters indicate the following treatment comparison ( $P < 0.05$ ): a = CRH1 vs. saline, and b = CRH1 vs. CRH5.

and gene regulation, favoring hepatic synthesis of the acute phase proteins (Carroll and Forsberg, 2007). Accordingly, a treatment effect was detected ( $P = 0.03$ ; Table 1) for RT. Mean RT before treatment infusion were significant covariates ( $P < 0.01$ ) but similar ( $P = 0.20$ ; data not shown) among treatments (39.18, 39.16, and 39.17 for saline, CRH1, and CRH5, respectively; SEM = 0.01). After treatment infusion, mean RT in CRH1 steers were greater ( $P < 0.04$ ) compared with saline and CRH5 steers (39.26, 39.03, and 39.09°C, respectively; SEM = 0.05) and similar ( $P = 0.50$ ) between CRH5 and saline. In addition, during the initial 8 h after treatment infusion (data not shown) when RT were expected to be mostly affected by treatments (Carroll et al., 2009; Cooke and Bohnert, 2011), mean RT were greater for CRH1 compared with saline steers ( $P = 0.03$ ) but similar ( $P > 0.16$ ) between CRH1 vs. CRH5 and saline vs. CRH5 steers (39.07, 39.21, and 39.29°C for saline, CRH1, and CRH5, respectively; SEM = 0.06). Supporting our previous research (Cooke and Bohnert, 2011), steers receiving CRH infusion at 0.1  $\mu\text{g}/\text{kg}$  of BW had increased RT compared with saline cohorts. However, the same outcome was not detected for steers receiving CRH at 0.5  $\mu\text{g}/\text{kg}$  of BW.

In agreement with treatment effects detected for serum TNF- $\alpha$  and RT, a treatment  $\times$  time interaction was detected ( $P = 0.04$ ) for plasma haptoglobin (Figure 4). Mean haptoglobin concentration before treatment infusion were significant covariates ( $P = 0.03$ ) but similar ( $P = 0.93$ ; data not shown) among treatments (1.50, 1.49, and 1.46 absorbance at  $450 \text{ nm} \times 100$  for saline, CRH1, and CRH5, respectively; SEM = 0.07). In CRH1 steers, plasma haptoglobin

increased and peaked 72 h after treatment infusion (time effect;  $P = 0.01$ ). Conversely, plasma haptoglobin did not change for CRH5 and saline steers after treatment infusion (time effect;  $P > 0.45$ ). Hence, plasma haptoglobin concentrations were greater ( $P < 0.05$ ) for CRH1 compared with saline steers at 60, 66, 72, and 96 h and greater ( $P < 0.05$ ) for CRH1 compared with CRH5 steers at 66, 72, and 96 h relative to treatment infusion (Figure 4). Increased RT and plasma haptoglobin concentration in CRH1 steers can be associated with the TNF- $\alpha$  responses detected herein, given that this cytokine has been shown to increase body temperature (Flores et al., 1989) and stimulate hepatic synthesis of acute-phase proteins (Yoshioka et al., 2002). Supporting the delayed haptoglobin response relative to treatment infusion in CRH1 steers, Gabay and Kushner (1999) demonstrated that significant haptoglobin increases are only detected several hours after an inflammatory stimulus, whereas peak haptoglobin concentrations are typically observed between 24 h and 192 h after a stress challenge (Arthington et al., 2008; Araujo et al., 2010; Cooke et al., 2011). Indeed, plasma haptoglobin concentrations peaked at 66 h after a CRH infusion of 0.1  $\mu\text{g}/\text{kg}$  of BW in our previous study (Cooke and Bohnert, 2011).

The main hypothesis of the present study, based on our previous results (Cooke and Bohnert, 2011), was that CRH5 steers would experience a greater neuroendocrine stress response and thus altered CRH-induced acute-phase reaction compared with CRH1 and saline steers. Higuchi et al. (1994) isolated parenchymal cells from the liver of male calves, and the monolayer cultures formed were treated

with different doses of dexamethasone. These authors reported that addition of dexamethasone from  $10^{-12}$  to  $10^{-4}$  M stimulated haptoglobin release into the medium, whereas maximal release was observed at intermediate dexamethasone concentrations ( $10^{-8}$  to  $10^{-6}$  M). In the present study, steers receiving a CRH infusion of  $0.1 \mu\text{g}/\text{kg}$  of BW experienced an acute-phase response characterized by increased RT, serum concentrations of TNF- $\alpha$ , and plasma concentrations of haptoglobin. This outcome can be attributed, at least partially, to increased plasma cortisol concentrations that promoted tissue degradation, as reflected by the increase in serum NEFA concentrations. Tissue degradation can be recognized by the innate immune system as a disruption in homeostasis (Abbas and Lichtman, 2007). In turn, leukocytes are activated and synthesize proinflammatory cytokines (Gabay and Kushner, 1999) triggering the acute-phase and other immune responses to eliminate damaged cells (Carroll and Forsberg, 2007). Conversely, steers receiving a CRH infusion of  $0.5 \mu\text{g}/\text{kg}$  of BW experienced a marginal increase in serum TNF- $\alpha$  but not in RT and haptoglobin compared with saline cohorts, which agrees with reduced haptoglobin release in liver cultures receiving the greater doses of glucocorticoid reported by Higuchi et al. (1994). It is also important to note that results reported by Higuchi et al. (1994) suggest a direct effect of glucocorticoids on hepatic synthesis of haptoglobin because of the potential lack of leukocytes in *in vitro* cultures, which may also have contributed to treatment differences detected herein for plasma haptoglobin. The lack of a substantial acute-phase reaction in CRH5 steers can be attributed, at least partially, to the lack of a NEFA increase after CRH infusion, suggesting that prolonged CRH-induced increase in plasma cortisol did not effectively stimulate tissue degradation and consequent activation of the innate immune system. In addition, sustained increases in circulating cortisol are known to promote anti-inflammatory and immunosuppressive responses, mainly by decreasing synthesis of proinflammatory cytokines by leukocytes (Kelley, 1988). In the present study, however, an anti-inflammatory reaction was not characterized in CRH5 steers because RT and concentrations of proinflammatory cytokines and haptoglobin were not reduced compared with saline steers. Therefore, it could be speculated that the prolonged CRH-induced increase in plasma cortisol of CRH5 steers was not appropriate to either stimulate or suppress acute-phase and inflammatory reactions.

In conclusion, beef steers receiving an intravenous CRH challenge of  $0.1$  or  $0.5 \mu\text{g}/\text{kg}$  of BW experienced, although in different patterns, increased concentrations of plasma cortisol and TNF- $\alpha$  compared with cohorts receiving saline. However, tissue mobilization (characterized by serum NEFA) and increases in RT and plasma haptoglobin were only detected in steers receiving  $0.1 \mu\text{g}$  of CRH per kg of BW. Therefore, the bovine acute-

phase response stimulated by CRH infusion appears to be dependent on the dose of CRH applied and the subsequent response in circulating cortisol.

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