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# Intravenous Ghrelin Infusion Affects Plasma Growth Hormone Concentrations, Dry Matter Disappearance, and Length of Time Spent Feeding[1]

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### Summary

Six steers (915 ± 37.8 kg) were used in a crossover design to determine the effects of intravenous infusion of bovine ghrelin (BGR) on plasma growth hormone (GH) concentrations, length of time spent feeding, and dry matter disappearance per unit of metabolic weight. Steers were fed individually once daily (0800 h) and allowed to consume ad libitum until 2000 h when feed was removed. Daily feed allotment was sufficient to result in ≥ 10% feed refusal. Serial blood samples were collected from steers fitted with an indwelling jugular catheter at 15-min intervals from 0600 h through 1800 h. Harvested plasma was assayed for ghrelin and GH concentrations. Saline (SAL) or BGR was infused via jugular catheter at 1200 h and 1400 h. Treatment infusion times were selected on the basis of the observation that steers did not consistently feed at these times. Exogenous BGR was infused to achieve a plasma concentration of 1000 pg/mL. This dosage was chosen on the basis of previous research that indicated a peak ghrelin concentration of 1000 pg/mL for fasting steers. Steers were allowed 5 d to adjust between treatment periods and then treatments were switched between steer groups and the sampling period repeated. Compared to SAL steers, average plasma ghrelin concentration was elevated ($P \leq 0.0001$) at the first post-infusion sampling for BGR steers at both infusion. Bovine ghrelin infusion resulted in elevated ($P \leq 0.005$) plasma GH concentrations compared to SAL steers after the first infusion. The second infusion of BGR resulted in numerically higher GH concentrations, but this difference was not statistically different from SAL steers or baseline concentrations. Both plasma ghrelin and GH concentrations returned to baseline 30 min post-BGR infusion. Length of time spent feeding ($P = 0.03$) and dry matter disappearance per unit of metabolic body weight ($P = 0.05$) for the combined infusion times were increased for steers infused with BGR. Bovine ghrelin is a compound that has the potential to elevate plasma GH concentrations and to increase length of time spent feeding and dry matter disappearance per unit of metabolic body weight.

### Introduction

Feeding cattle properly is an intricate part of any successful beef cattle operation. Feed costs account for 40 to 50 percent of the total on-farm costs of beef production, and Miller et al. (2001) reported that 50 percent of the herd-to-herd variation in profitability among beef cow/calf operations can be attributed to variation in feed costs. Additionally, dramatic fluctuations in feed intake can cause metabolic acidosis or inefficient production of meat, whereas inadequate feed intake can result in ketosis (Baile and Della-Fera, 1981). Voluntary feed intake is often compromised during stress associated with stage of production and temperature extremes (Baile and Della-Fera, 1981). In rodents, ghrelin has been reported to influence energy metabolism, increase growth hormone (GH) secretion, and stimulate feed intake, all of which contribute to the growth and the nutritional status of an animal (Tshöp et al. 2000). Ghrelin is a peptide hormone synthesized by the abomasumal and ruminal tissues of cattle (Hayashida et al., 2001 and Gentry et al., 2003, respectively). Ghrelin has been reported to stimulate feed intake through neuropeptides found in the appetite center of the hypothalamus (Tshöp et al. 2000; Inui, 2001; Shintani et al., 2001; and Nakazato et al., 2001). As feed intake contributes greatly to the nutritional status of cattle and therefore animal well-being and economic viability of an operation, this experiment was designed to

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study the influence of ghrelin on plasma GH concentrations, length of time spent feeding, and dry matter disappearance (as a indicator of feed intake) in beef cattle. The long-term goal of this research is to understand more thoroughly the regulation of feed intake to minimize economic loss and maximize animal well-being during nutritionally critical stages of production such as weaning, parturition, lactation, or temperature extremes.

## Materials and Methods

*Animals and treatments.* Six steers (915 ± 37.8 lb) were used in a crossover design to determine the effects of intravenous infusion of bovine ghrelin (BGR) on plasma growth hormone (GH) concentrations, length of time spent feeding, and dry matter (DM) disappearance. Steers were acclimated to a climate-controlled facility and a specific feeding schedule during a 10-d pre-treatment adaptation period. Steers were fed individually once daily (0800 h) and allowed to consume ad libitum until 2000 h when feed was removed. Prior to entering the climate-controlled facility, steers were acclimated to a common finishing diet that was fed throughout the experiment (Table 1). Once in the climate-controlled facility, daily feed allotment for each steer was sufficient for ≥ 10% feed refusal to result. Each feeding apparatus was attached to a digital load cell capable of relaying weight differences to a computer. Feeder weight data were logged at 20-sec intervals. Volatility of logged weights indicated that an animal was feeding, whereas a consistently stable weight indicated that the animal was not feeding. The difference between a stable weight prior to and following a volatile weight period was used to calculate DM disappearance during a feeding period. Dry matter disappearance was recorded two days prior to treatment, the day of treatment, and one day following treatment. These data were used to calculate length of time spent feeding and dry matter disappearance per unit of metabolic body weight.

*Plasma sample collection.* Steers were fitted with an indwelling jugular catheter following the adaptation period. Steers were allowed a minimum of 12 h to recover between catheterization and initiation of the sampling / treatment period. Surgical procedures for this experiment were approved by the South Dakota State University Institutional Animal Care and Use Committee prior to the initiation of this experiment. Serial blood samples were collected from indwelling jugular catheters at 15-min intervals from 0600 h through 1800 h, on treatment day. Plasma was assayed for ghrelin and GH using radioimmunoassay procedures. During the sampling period, saline (SAL) or BGR was infused via jugular catheter at 1200 h and 1400 h. The catheter was then flushed with 5 mL of saline to ensure that BGR had been flushed from the catheter. Treatment infusion times were selected based on the observation that steers did not consistently consume feed during this time period. Therefore, steers were in a satiated state when BGR was administered. Exogenous BGR was infused to achieve a plasma concentration of 1000 pg/mL. This dosage was chosen on the basis of previous research that indicated a peak BGR concentration of 1000 pg/mL for fasting steers (Wertz et al., 2004). This model allowed for determining if the fasting concentration of ghrelin was adequate to stimulate feeding in a satiated steer. Steers were allowed a 5-d rest between the first and second treatment periods, and then treatments were switched between steer groups and the sampling / treatment period was repeated.

*Statistical Analyses.* Two steers were removed from the experiment because of catheter malfunction during a treatment period, therefore statistical analyses were performed on data from four steers that completed both treatment periods. Plasma ghrelin and GH concentrations were analyzed statistically as repeated measures in time using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Differences in plasma ghrelin and GH concentrations that resulted from treatment at specific time points were separated using least squares means. Dry matter disappearance and length of feeding period data were analyzed as crossover design using the MIXED procedure of SAS. Differences in DM disappearance or length of time spent feeding that resulted from treatment were separated using least squares means.

## Results

The first post-infusion blood sample was collected 15 min after treatment infusion or at approximately the first half-life for ghrelin. Compared to SAL steers, average plasma ghrelin concentration was elevated ($P \leq 0.0001$) at the first post-infusion sampling for BGR steers after both the 1200 and 1400 h infusion times

(Figure 1). The time by treatment interaction tended ($P$ = 0.12) to be significant for the effects of ghrelin infusion on GH concentrations. Bovine ghrelin infusion resulted in elevated ($P \leq 0.005$) plasma GH concentrations at the initial sampling after the first infusion time (1200 h) compared to SAL steers (Figure 2). The second infusion of BGR resulted in numerically higher GH concentrations, but concentrations were not statistically different from SAL steers. The magnitude of GH elevation following the second BGR infusion (1400 h) was less ($P \leq 0.0001$) when compared to the first infusion time. A clear explanation for this response cannot be established from data collected from this trial however, the attenuated GH response to BGR infusion may suggest a feedback mechanism or mechanisms in response to repeated BGR surges. Both plasma ghrelin and GH concentrations returned to concentrations similar to baseline by 30 min post-BGR infusion and were not different from that of SAL steers.

Dry matter disappearance and length time spent feeding were quantified for the first hour following each infusion (Infusion 1: 1200 to 1300 or Infusion 2: 1400 to 1500) because this time period corresponded with the time that ghrelin was elevated in the plasma (Table 2). Data for the two post-infusion periods were combined so that DM disappearance and length time spent feeding could be evaluated for the entire treatment period. Dry matter disappearance and length time spent feeding also were calculated for the entire day of treatment. Numerically, DM disappearance and time spent feeding were greater BGR steers ($P \leq 0.47$), however, the increase was not significantly different than SAL steers except for the combined post-infusion periods. For the combined post-infusion periods, BGR steers spent an average of 11.7 min more time feeding ($P$ = 0.03), and DM disappearance was an average of 9.4 g/kg of metabolic body weight greater ($P$ = 0.05) compared to SAL steers. Because DM disappearance was not significantly different following a single BGR infusion but was significantly increased when data for the individual infusions were combined, a single BGR infusion may not be sufficient to alter feeding but multiple or perhaps sustained elevation of ghrelin may be necessary to attain an increase in DM disappearance.

Exogenous BGR administered intravenously to finishing steers results in a transient increase in plasma GH concentration. Additionally, these data suggest that when plasma ghrelin concentrations are elevated that steers spend more time feeding and consume more feed. However, the effects on feed intake are not sustained beyond the treatment period.

## Implications

These data indicate that bovine ghrelin can stimulate steers to consume feed. During critical production situations when feed intake can be compromised, ghrelin treatment may be a means of stimulating feed intake to offset poor performance and animal well-being associated with compromised feed intake. However, these data indicate that increased dry matter disappearance is detectable only during the ghrelin infusion, and therefore may require multiple intravenous infusions or an alternate means of sustained release to be efficacious. More research is needed to evaluate the effects of sustained ghrelin administration on dry matter disappearance, animal production, and animal well-being.

## Acknowledgement

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## Tables

Table 1. Experimental diet composition

| Ingredient | %, Dry Matter Basis |
|---|---|
| Corn | 75.0 |
| Grass hay | 11.0 |
| Wheat midds | 7.52 |
| Soybean meal | 4.66 |
| Urea | 0.42 |
| Limestone | 1.23 |
| Vitamin A [a] | 0.007 |
| Vitamin E [b] | 0.005 |
| Trace mineral salts [c] | 0.100 |
| $ZnSO_4$ [d] | 0.006 |
| Rumensin [e] | 0.019 |
| | |
| Calculated Nutrient Composition | |
| NEm, Mcals/lb | 0.92 |
| NEg, Mcals/lb | 0.62 |
| CP, % | 13.0 |

[a] 30,000 IU/g.
[b] 500 IU/g.
[c] NaCl 94.0-98.5%, Zn 0.35%, Fe 0.20%, Co 0.005%, Mn 0.20%, Cu 0.30%, I 0.007%.
[d] 35.54% Zn.
[e] Formulated to contain 30 g/Ton

Table 2. Effects of intravenous ghrelin or saline injection on length of time spent feeding and dry matter disappearance for finishing beef steers

| | Ghrelin | Saline | SE | $P \leq$ |
|---|---|---|---|---|
| Number of Animals | 4 | 4 | ---- | ---- |
| After first infusion, 1200 to 1300 | | | | |
| Time spent feeding, min | 10.0 | 3.8 | 3.5 | 0.33 |
| Dry matter disappearance, g/kg MBW [a] | 6.9 | 2.2 | 2.3 | 0.29 |
| After second infusion, 1400 to 1500 | | | | |
| Time spent feeding, min | 19.0 | 13.5 | 4.0 | 0.44 |
| Dry matter disappearance, g/kg MBW | 13.8 | 9.3 | 3.6 | 0.47 |
| Combined post-infusion | | | | |
| Time spent feeding, min | 29.0 | 17.3 | 1.4 | 0.03 |
| Dry matter disappearance, g/kg MBW | 20.7 | 11.3 | 1.6 | 0.05 |
| Entire treatment day | | | | |
| Time spent feeding, min | 136 | 124 | 5.9 | 0.28 |
| Dry matter disappearance, g/kg MBW | 93.6 | 84.4 | 5.4 | 0.37 |

[a] MBW = metabolic body weight = body weight ($kg^{0.75}$)

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**Figures**

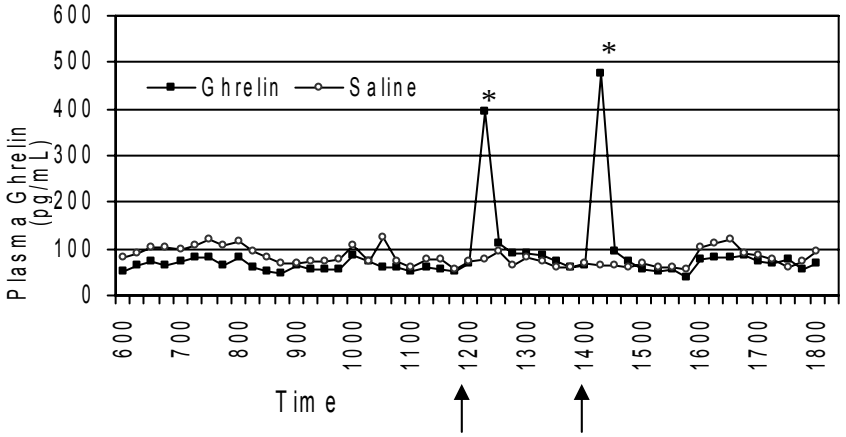


Figure 1. Plasma ghrelin concentrations for beef steers intravenously infused with ghrelin or saline. Arrows indicate infusion times. * Plasma ghrelin concentration was elevated ($P \leq 0.0001$) following intravenous infusion of ghrelin. Plasma ghrelin concentrations returned to baseline concentrations and similar to saline-treated steers by 30 min. post infusion.

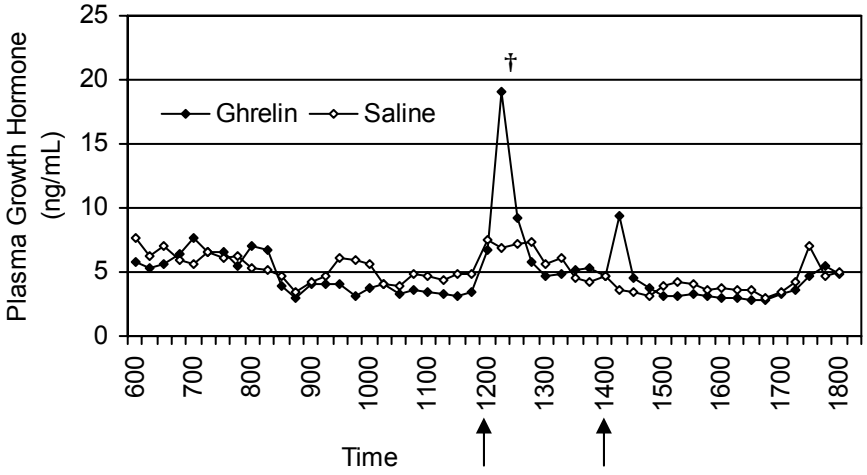


Figure 2. Plasma growth hormone concentrations for beef steers intravenously infused with ghrelin or saline. Arrows indicate infusion times. † Intravenous ghrelin infusion resulted in elevated ($P \leq 0.005$) plasma growth hormone (GH) concentrations following the first infusion. Plasma GH was not significantly different than baseline concentrations or saline-treated steers after second infusion.