



## Prolonged, moderate nutrient restriction in beef cattle results in persistently-elevated plasma ghrelin concentrations<sup>1</sup>

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#### Summary

Four steers (BW 1281±28.2 kg) were used in a crossover design to determine the effects of prolonged, moderate energy and protein restriction on plasma ghrelin and GH concentrations. A common high-energy diet was offered at 240% of the intake necessary for BW maintenance (**2.4xM**) or 80% of the intake necessary for BW maintenance (**0.8xM**). As a common starting point, all steers were adjusted to 2.4xM during a 23-d pre-trial adaptation period. At initiation of period 1, 2 steers remained at 2.4xM, whereas intake for the remaining 2 steers was restricted to 0.8xM. Feed allotments were offered twice daily in equal aliquots at 0800 and at 2000 h. On 7, 14, and 21 d following initiation of restriction, serial blood samples were collected via indwelling jugular catheter at 15-min intervals throughout a 12-h feeding interval. Following period 1, steers were weighed and intake amounts were recalculated. Dietary treatments were switched between steer groups, 2.4xM intake was established, and sampling period II was initiated as described for period I. Plasma samples were assayed for ghrelin, GH, insulin (**INS**), and NEFA concentrations. Subsequent to analyses, hormone data were pooled by hour for statistical analyses. The energy and protein restriction resulted in decreased BW for 0.8xM (-108.9 lb) steers compared with 2.4xM (127.9 lb) steers. Body weight loss along with decreased plasma INS concentrations and elevated plasma NEFA and GH concentrations indicate that these steers were in a catabolic state and mobilizing body tissue stores to meet nutrient requirements not met by dietary intake. Plasma ghrelin concentrations also were elevated for the 0.8xM steers compared with those of 2.4xM steers throughout the 21-d treatment period. These data are consistent with the hypothesis that plasma ghrelin concentrations are elevated in cattle throughout a prolonged, moderate energy and protein restriction that result in a catabolic state.

#### Introduction

Inadequate nutrient intake relative to demand for maintenance and (or) production can result in economic loss from poor production efficiency and metabolic disorders. Therefore, understanding feed intake regulation and energy expenditure in cattle is important. Ghrelin is a peptide hormone synthesized by abomasal and ruminal tissues of cattle (Hayashida et al., 2001; Gentry et al., 2003). In rodents, ghrelin stimulates feed intake through neuropeptides in the hypothalamus (Nakazato et al., 2001) and is reported to influence energy metabolism and body composition (Tschöp et al., 2000). Most research that has been conducted with livestock to evaluate the relationship of plasma ghrelin with feed intake restriction has been done with short-term periods of complete feed deprivation without sufficient length to result in differences in body composition. Wertz-Lutz et al. (2006) observed elevated plasma ghrelin concentrations that persisted for 48 h in mature beef cattle completely deprived of feed. Length and severity of the nutrient restriction may influence plasma ghrelin concentrations. More often than complete feed deprivation, ruminant livestock encounter periods of prolonged, moderate nutrient restriction, whereby nutrient resources are limiting relative to expected production. Currently, research that evaluates the effects of prolonged, moderate nutrient restriction sufficient to result in BW loss on plasma ghrelin concentrations does not exist. The objective of this experiment was to evaluate effects of prolonged,

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moderate energy and protein restriction on plasma ghrelin concentrations and its relationship to other hormones indicative of nutritional status.

## Materials and Methods

**Dietary treatments.** Dietary treatments for this experiment were two different amounts of a common high-energy diet. Feed amounts were 80% of that necessary to meet the NEm requirement (**0.8xM**) or 240% of the feed intake necessary to meet the NEm requirement (**2.4xM**) of a given steer and were calculated as described below. To determine the amount of feed intake necessary to meet the NEm requirement (Mcal/d), the equation  $0.077 \times \text{empty BW}(\text{kg}^{0.75})$  was used (NRC, 2000). This NEm requirement (Mcal/d) then was divided by the energy density of the diet (Mcal/lb) to determine the amount of feed (lb/d) necessary to meet the maintenance requirement of each particular steer on the basis of its own BW. The amount of feed required to meet the NEm requirement then was multiplied by 2.4 to determine the target amount of feed intake for the steers in positive nutrient balance (2.4xM) or multiplied by 0.80 to determine the amount of intake assigned to negative nutrient balance treatment (0.8xM). Once each steer's given amount of feed was determined, the MP content of the feed was estimated based on the degradability of the dietary protein (43.4%; Beef NRC, 2000). The amount of dietary MP consumed then was compared with the MP required for BW maintenance as calculated using the equation  $3.8 \text{ g MP} \times \text{BW}(\text{kg}^{0.75})$  (NRC, 2000).

**Animals and procedures.** Four ruminally-cannulated (3-yr-old) Angus crossbred steers (BW  $1281 \pm 28.2$  lb;  $581.4 \pm 12.8$  kg) steers fitted with an indwelling jugular catheter were used in a crossover design to evaluate the effects of prolonged, moderate protein and energy restriction on plasma hormone and metabolite concentrations. This experiment was conducted in a climate-controlled metabolism facility at South Dakota State University, and all animal procedures were approved by the Institutional Animal Care and Use Committee.

During a 23-d pre-experiment adaptation period, steers were acclimated to the climate-controlled facility. Equal aliquots of feed were offered twice daily at 0800 and 2000 h, and this 12-h feeding interval was maintained throughout the trial. To establish a common starting point, feed intake of the common high-grain finishing diet (Table 1) was increased during this acclimation period until feed intake was 240% greater than the amount required to meet the NEm requirement of each steers.

Table 1. Ingredient composition of experimental diet

Ingredient	%, DM Basis
Beet pulp	20.00
Corn	65.00
Soybean meal	5.67
DDGS <sup>a</sup>	8.00
Limestone	1.00
TM salt <sup>b</sup>	0.30
Vitamin A D E <sup>c</sup>	0.0055
Zinc sulfate <sup>d</sup>	0.0056
Rumensin <sup>e</sup>	0.02
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Calculated Nutrient Composition	DM Basis
CP, %	12.5
Degradable intake protein, %	43.4
NEm, Mcal/lb	0.95

<sup>a</sup> Dried distiller's grains with solubles

<sup>b</sup> NaCl 94.0-98.5%, Zn 0.35%, Fe 0.20%, Co 0.005%, Mn 0.20%, Cu 0.30%, I 0.007%.

<sup>c</sup> 30,000 IU/g Vitamin A, 500 IU/g Vitamin E, Vitamin D<sub>3</sub> 500,000 IU/g

<sup>d</sup> 35.54% Zn.

<sup>e</sup> Formulated to contain 32 g/T.

Once all steers had reached 240% of feed intake required to meet NEm, the experiment was initiated. The experiment was conducted as a crossover design with two, 21-d treatment periods. During treatment period I, 2 steers were maintained at 240% of the intake required to the NEm requirement (2.4xM) established during the acclimation period and the remaining 2 steers were limited to 80% of the intake required to meet NEm requirement. Serial blood and rumen fluid samples were collected on d 7, 14, and 21 following initiation of the restriction. Following period I, dietary treatments were switched between steer groups, steers were weighed, and feed amounts were re-calculated as described for period I on the basis of the BW recorded at the end of sampling period I. Feed intake gradually was increased until steers that had been fed 0.8xM reached 2.4xM intake. A second 21-d treatment and sampling period then was conducted as described for period I.

**Blood collection.** During each 21-d treatment period, blood and rumen fluid samples were collected at 7, 14, and 21 d after the intake restriction was invoked. On each sampling day, blood samples were collected at 15-min intervals from 0700 and 1145, 1300 to 1345, 1600 to 1645, and 1800 to 1845 h. Aliquots of plasma (1.0 mL each) were processed and stored at  $-20^{\circ}\text{C}$  for the subsequent analyses of GH, INS, NEFA and ghrelin according to procedures outlined by Wertz-Lutz et al. (2006).

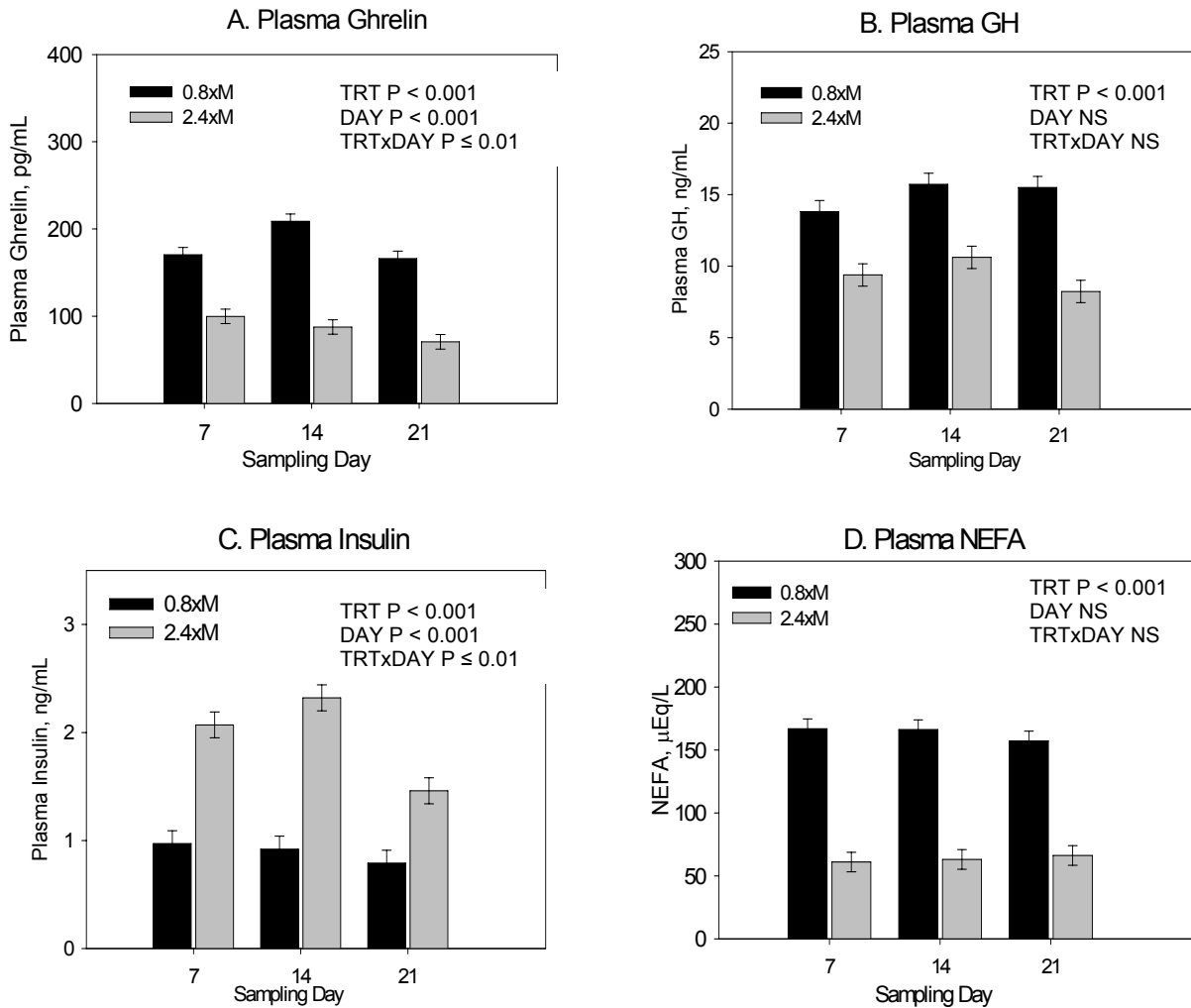
**Statistical analyses.** To verify that differences in NEm and MP intake resulted from the planned differences in DMI, these characteristics and BW change were analyzed statistically as a crossover design with a model that accounted for variation from sampling period, steer, and amount of feed intake. Differences in the characterization parameters that resulted from amount of feed intake were separated using a Fisher's t-test. Plasma ghrelin, GH, INS, and NEFA concentrations were analyzed statistically as repeated measures in time by using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with independent errors that accounted for error correlation of sampling times. The model included length of treatment (7, 14, and 21 d), amount of feed intake (0.8xM vs. 2.4xM), steer, sampling period (I or II), and the interactions of length of treatment and amount of feed intake as independent variables. Differences in least squares means for plasma ghrelin, GH, INS, and NEFA were separated by using a Fisher's t-test.

## Results and Discussion

**Nutritional state of steers.** Imposed dietary treatments resulted in less ( $P \leq 0.002$ ) DMI of the common compositional diet for steers assigned to the 0.8xM compared with the 2.4xM steers (8.6 and  $22.9 \pm 0.40$  lb/d, respectively). This feed intake restriction resulted a net energy intake that was below the NEm requirement for 0.8xM (-1.0 Mcal/d) and a net energy intake that was lower ( $P \leq 0.001$ ) than that of the steers assigned to the 2.4xM treatment (9.0 Mcal/d). The feed intake restriction also resulted in MP intake below that required to meet the maintenance requirement for the 0.8xM steers (-107 g/d relative to maintenance requirement). Metabolizable protein intake also was lower ( $P \leq 0.001$ ) for 0.8xM steers compared with 2.4xM steers (500 g/d in excess of maintenance requirement). The energy and protein restriction resulted in decreased ( $P \leq 0.001$ ) BW for 0.8xM (-108.9 lb) steers compared with 2.4xM (127.9 lb) steers.

**Length of restriction on hormone and metabolite profiles.** Plasma hormone and metabolite profiles are reported in Figure 1. Plasma GH and NEFA concentrations were elevated ( $P \leq 0.001$ ) for 0.8xM steers throughout the 21-d treatment sampling period (Figures 1B and 1D, respectively). In contrast, plasma INS concentrations were lower ( $P \leq 0.001$ ) for 0.8xM steers throughout the 21-d treatment period (Figure 1C). A dietary treatment by length of treatment interaction resulted for plasma INS concentrations ( $P \leq 0.01$ ). Plasma INS concentrations were similar across the 21-d sampling period for 0.8xM steers, whereas plasma INS concentrations were higher at d 7 and 14 compared with d 21 for 2.4xM steers. Plasma GH, NEFA, and INS concentrations indicate that 0.8xM steers were in catabolic state throughout the 21-d treatment period. Body weight loss along with decreased plasma INS concentrations and elevated plasma NEFA and GH concentrations indicate that these steers were mobilizing body tissue stores to meet nutrient requirements not met by dietary intake. Plasma ghrelin concentrations were elevated ( $P \leq 0.001$ ) for the 0.8xM steers compared with 2.4xM steers throughout the 21-d treatment period (Figure 1A). There was an interaction of dietary treatment by length of treatment ( $P \leq 0.01$ ) for plasma ghrelin concentrations. Plasma ghrelin concentrations for 2.4xM steers were similar regardless of length of treatment, whereas plasma ghrelin concentrations for 0.8xM steers were higher at d 14

compared with d 7 and 21. These data are consistent with the hypothesis that plasma ghrelin concentrations remain elevated through a prolonged, moderate energy and protein restriction that result in a catabolic state in cattle.



**Figure 1.** Effects of prolonged energy and protein restriction sufficient to result in body weight loss on plasma hormone and metabolite concentrations in beef cattle. TRT = 0.8xM – 80% of the feed intake needed to meet the energy requirement for maintenance; 2.4xM - 240% of the feed intake needed to meet the requirement for maintenance. DAY = sampling day relative to the invoked DMI restriction. TRTxDAY = interaction of the main effects.

Rumen distention must be considered as a plausible explanation of the interaction of treatment with length of treatment for plasma ghrelin concentrations. Sugino et al. (2003) reported that cholinergic activity of the vagus nerve suppressed ghrelin secretion, suggesting that distention of the rumen may regulate ghrelin secretion. However, Arnold et al. (2006) acknowledged that phasic increases in ghrelin influence activity of some load-sensing vagal afferents, but concluded that the acute eating-stimulatory effect of ghrelin did not require vagal afferent signaling. By design, steers in the current experiment were adapted to an intake amount that would supply 240% of the NEm requirement as a means of standardizing the group before the restriction was invoked. Intake did differ for the two treatment groups throughout the treatment period such that the amount of feed consumed was greater for 2.4xM steers (22.9 lb) compared with 0.8xM steers (8.6 lb), and therefore distention and suppression of ghrelin secretion would be expected to be greater for the 2.4xM steers. In the current experiment, no objective measure of rumen distention was used and this observations warrants further investigation before a definitive conclusion can be drawn. However, adaptation of the rumen to the quantity of feed provided

may explain partially the fluctuation in plasma ghrelin concentrations that was observed during the 21-d sampling period for 0.8xM steers but not 2.4xM steers. The relationship of ghrelin and the vagal nerve only partially explains the relationship of ghrelin and feed intake and has not need demonstrated as a link between ghrelin and body composition.

The observation that plasma ghrelin concentrations differ between cattle in a positive nutritional state compared with a negative nutritional also warrants further investigation. A link between ghrelin and adiposity has been established. Patel et al. (2006) reported that ghrelin stimulated deoxyglucose uptake by rodent epididymal adipocytes in the presences but not in the absence of insulin. Patel et al. (2006) also reported differential expression of the receptor to which ghrelin binds (GHS-R) in various adipose depots. The GHS-R was expressed in adipocytes that responded to ghrelin stimulation and not expressed in adipocytes that did not respond to ghrelin stimulation. Although completed with rodents, these data suggest a complex interaction of nutritional state, form of ghrelin, and adipose depot, whereby investigating the role of ghrelin in altering composition of gain in cattle is warranted.

### **Implications**

Data from the current experiment and other experiments in our laboratory indicate that plasma ghrelin concentrations fluctuate both with acute feed deprivation and prolonged moderate nutrient intake restriction. The fluctuation of ghrelin with other hormones and metabolites indicative of nutritional status may influence the ability of ghrelin to communicate nutritional status, as evidence that ghrelin alters glucose uptake by adipocytes and altered use of metabolic fuels suggest that ghrelin also has the potential to influence nutrient expenditure. As a result, the potential role of ghrelin in regulating composition of gain and (or) feed efficiency warrants further exploration.

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