



## Plasma ghrelin concentrations of beef cattle consuming a similar amount of dietary energy supplied by different ingredients<sup>1</sup>

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### Summary

Previous research demonstrated that restricting nutrient intake by decreasing DMI of a high-grain diet increased plasma ghrelin concentrations. Objectives of this experiment were to determine 1) whether dietary ingredient composition influenced plasma ghrelin concentrations when energy intake was similar, and 2) whether relationships existed between plasma ghrelin concentrations and plasma insulin, NEFA, and GH concentrations or end-products of carbohydrate fermentation in the rumen. Five steers (1290 ± 39.9 lb) were used in a crossover design with dietary treatments of 50% hay-50% concentrate (**HAY**) offered at an amount that would meet the steer's NEm requirement plus supply an additional 3.5 Mcal of NEg daily, or a diet composed of 10% hay-90% concentrate but limit-fed to achieve an energy intake similar to that of the HAY steers (**LFC**). Feed was offered in equal aliquots twice daily. Period I: on d 21 following initiation of the dietary treatment, serial blood samples were collected via indwelling jugular catheter at 15-min intervals, and rumen fluid samples were collected hourly throughout a 12-h feeding interval. Following period I, steers were weighed, dietary treatments were switched between steer groups, and intake amounts were recalculated on the basis of period I ending BW. Period II adaptation and sampling was repeated as described for period 1. Plasma samples were assayed for ghrelin, insulin, GH, and NEFA concentrations. Rumen fluid was assayed for VFA concentrations and pH. Net energy for gain was similar between treatment groups (3.5 ± 0.04 Mcal NEg/d). However, a higher DMI was required by HAY steers compared with LFC steers (20.7 vs. 15.9 ± 0.13 lb) to achieve the same energy intake. Plasma ghrelin concentrations were similar for HAY and LFC steers (115 vs. 107 ± 3.3 pg/mL) despite differences in DMI and ingredient composition. Plasma GH, NEFA, and insulin concentrations also were similar regardless of dietary ingredient composition. Strong correlations between plasma ghrelin concentrations and other hormones and metabolites or end-products of carbohydrate fermentation did not result. These data are consistent with the hypothesis that ingredient composition and quantity of DMI do not influence plasma ghrelin concentrations of steers when energy intake is similar and steers are in positive energy balance.

### Introduction

Feed intake decreases and composition of gain shifts toward fat deposition, resulting in slower gains and poorer feed efficiencies in cattle approaching market weight. Understanding of factors that regulate feed intake and composition of gain may improve cattle performance. Ghrelin is a peptide hormone that is involved in the central nervous system regulation of feed intake and body composition (Tschöp et al., 2000). The receptor that binds ghrelin is found on the hypothalamus, adipose tissue, skeletal muscle, and liver, and therefore may influence composition of gain (Wang, et al., 2002). Ghrelin has been reported to altered body composition to favor the deposition of fat accretion (Tschöp et al., 2000; Patel et al., 2006). Because body fat content and distribution influence the value of a carcass marketed in a grid-marketing system, and because rumen VFAs are the primary source of energy and glucose in ruminants, this experiment was designed to determine 1) whether dietary ingredient composition influenced plasma ghrelin concentrations when energy intake was similar and 2) whether relationships existed between plasma ghrelin concentrations and plasma insulin, NEFA, and GH concentrations or end-products of carbohydrate fermentation in the rumen.

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## Materials and Methods

**Animals.** Five ruminally-cannulated steers (initial BW 1290 ± 39.9 lb) were used in a crossover design. Animal procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

**Dietary treatments.** Steers were adapted to a climate-controlled facility, their respective diet, and a specific feeding schedule during a 21-d pre-treatment period. Steers were offered feed twice daily (0800 and 2000 h). During the adaptation period, it was established that the steer with the lowest DMI on the hay-based diet was consuming enough feed to meet its NEm requirement plus an additional 3.5 Mcal/d NEg. It was assumed that physical fill was limiting intake of this animal, and that intake was therefore used as the benchmark to calculate a common energy intake for all steers, regardless of dietary ingredient composition. Intake of the remaining steers was adjusted such that all steers consumed 3.5 Mcal NEg in addition to that required to meet their NEm requirement, regardless of diet ingredient composition. Dietary treatments differed in ingredient composition and were 50% hay-50% concentrate (**HAY**) offered at an amount that would meet the steer's NEm requirement plus supply an additional 3.5 Mcal of NEg daily or a diet composed of 10% hay-90% concentrate but limit-fed to achieve a caloric intake similar to that of the HAY steers (**LFC**; Table 1). 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 NEm density of the diet (Mcal/lb) to determine the amount of feed (lb/d) necessary to meet the maintenance requirement of each particular steer based on its own BW. The amount of feed needed to supply the additional 3.5 Mcal/d of NEg was calculated by dividing 3.5 Mcal/d by the NEg density (Mcal/lb) of the diet.

Table 1. Dietary Ingredient Composition

Ingredient	%, Dry Matter Basis	
	HAY	LFC <sup>a</sup>
Grass hay	50.00	10.00
Corn	23.12	65.00
Beet pulp	10.00	10.00
DDGS <sup>b</sup>	11.00	8.00
Soybean meal	5.25	5.67
Limestone	0.50	1.00
Trace mineral salt <sup>c</sup>	0.10	0.30
Rumensin <sup>d</sup>	0.02	0.02
ZnSO <sub>4</sub> <sup>e</sup>	0.007	0.006
Vitamin E <sup>f</sup>	0.005	0.005
Vitamin A <sup>g</sup>	0.0004	0.0004
Vitamin D <sup>h</sup>	0.0001	0.0001
	Calculated Nutrient Composition, % DMB	
CP, %	13.0	12.3
NE <sub>m</sub> , Mcal/lb	0.72	0.91
NE <sub>g</sub> , Mcal/kg	0.44	0.61

<sup>a</sup> Limit-fed concentrate

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

<sup>c</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>d</sup> Formulated to contain 30 g/Ton

<sup>e</sup> 35.54% Zn

<sup>f</sup> 500 IU/g

<sup>g</sup> 30,000 IU/g

<sup>h</sup> 500,000 IU/g

**Sampling period I.** On d 20 following initiation of the dietary treatment, steers were fitted with an indwelling jugular catheter and allowed a minimum of 12 h to recover prior to initiation of the sampling period. On d 21, blood samples were collected via the indwelling jugular catheter at 15-min intervals from 0700 to 1145, 1300 to 1345, 1600 to 1645, and 1800 to 1845 h. At the beginning of each hour of blood sample collection, a rumen fluid sample also was collected via the rumen cannula.

**Crossover and sampling period II.** Following the initial sampling period, steers were weighed and dietary treatments were switched between steer groups. Feed intake required to meet the NEm requirement plus supply an additional 3.5 Mcal/d NEg was re-calculated as described for period I but on the basis of steer BW at the end of sampling period I. Steers were adapted to the change in dietary ingredients for 20 d at which point an indwelling jugular catheter again was inserted, and the sampling period II was conducted as described for period I.

**Rumen fluid data.** Rumen fluid pH was recorded immediately following collection, and an aliquot of rumen fluid was acidified and centrifuged. The acidified supernatant was frozen and subsequently analyzed for molar proportion of VFA using gas chromatography.

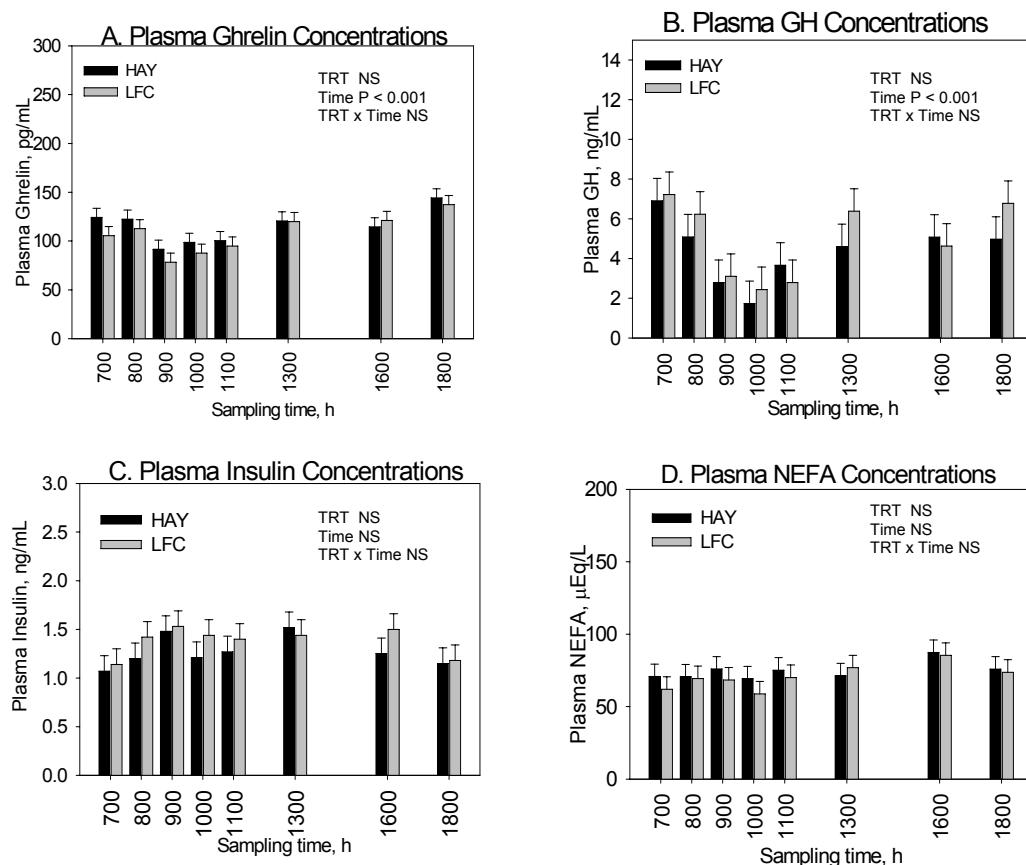
**Hormone concentration data.** Plasma was separated from blood samples by centrifugation, and harvested plasma aliquots were stored at  $-7^{\circ}$  C for subsequent analyses of hormone and metabolite concentrations. Plasma ghrelin and GH are secreted in a pulsatile fashion. Therefore, to minimize variation, ghrelin and GH concentrations were quantified on samples collected at 15-minute intervals and pooled by hour for statistical analyses. Laboratory methodology described by Wertz-Lutz et al. (2006) was used to quantify plasma ghrelin and GH concentrations. Plasma insulin (**INS**) and NEFA concentrations were quantified for samples collected at the beginning of each hour according to the procedures of Wertz-Lutz et al. (2006).

**Statistical analyses.** Plasma ghrelin, GH, INS, and NEFA and ruminal pH and VFA data were analyzed as repeated measures by using the MIXED procedure of SAS. Differences in hormone concentrations that resulted from ingredient composition, sampling time relative to feed offering, or their interaction were separated by using least squares means with the PDIF option of SAS. The data set then was divided by dietary treatment and Pearson correlation and stepwise regression was performed to characterize the relationship between plasma ghrelin concentrations and plasma hormones and metabolites or rumen fermentation characteristics.

## Results and Discussion

**Performance characteristics.** Steers were in positive energy balance throughout the experiment as indicated by their final BW ( $1413 \pm 26.2$  lb) relative to initial BW ( $1290 \pm 39.9$  lb). The net change in BW during the 21-d period was  $128 \pm 44.5$  lb and did not differ as a result of treatment. The NEg intake was similar between treatment groups ( $3.5 \pm 0.04$  Mcal NEg/d). However, a higher DMI ( $P < 0.001$ ) was required by HAY steers compared with LFC steers ( $20.7$  vs.  $15.9 \pm 0.13$  lb/kg) to achieve the same energy intake.

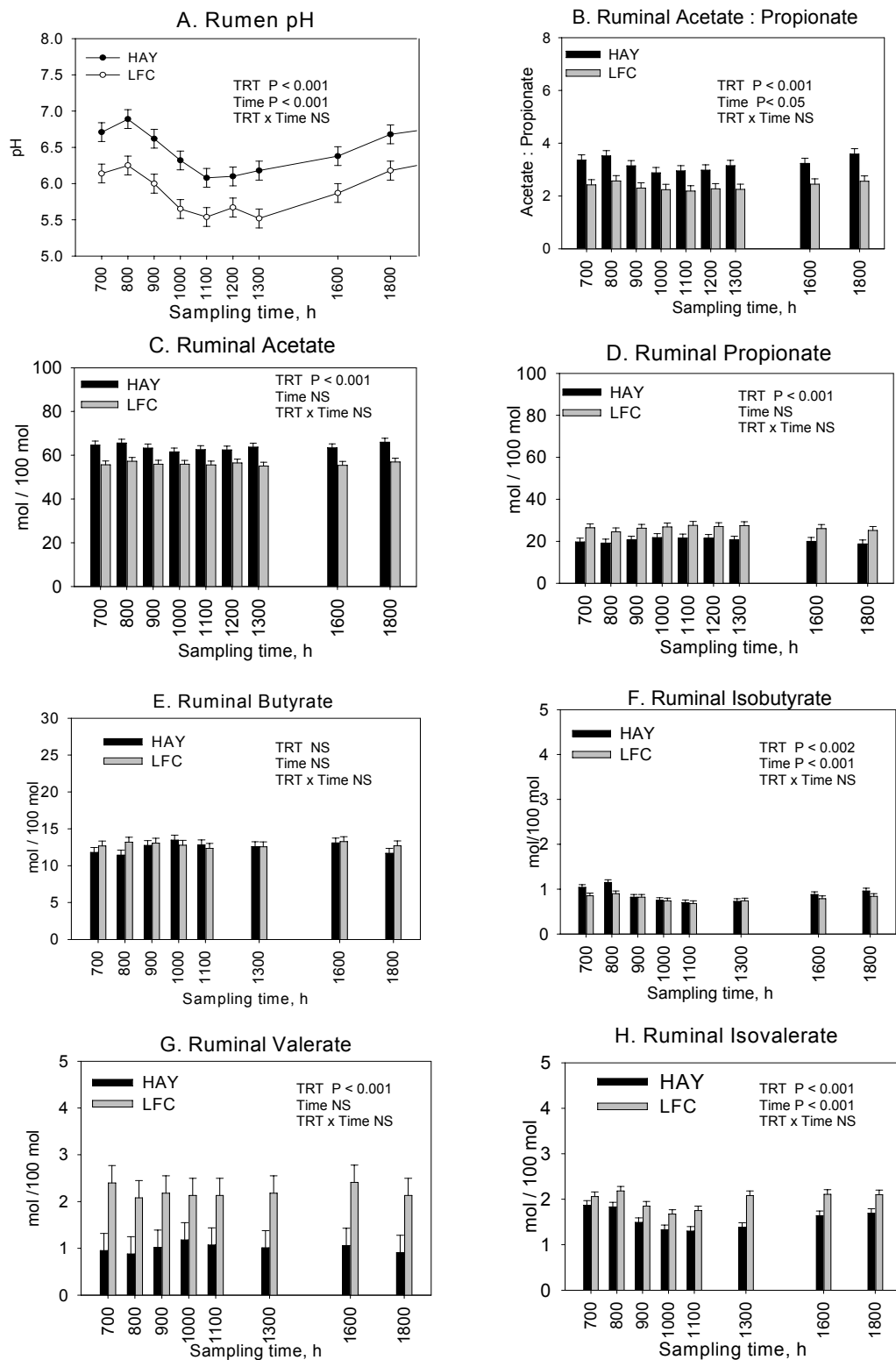
Previous research from our laboratory demonstrated that plasma ghrelin concentrations were elevated when intake of a high-grain diet was restricted to result in a prolonged moderate energy and protein restriction sufficient to result in loss of BW. Because correlated differences in plasma hormone and metabolite concentrations and ruminal VFA concentrations also resulted with restricted intake of the common ingredient diet, it could not be deciphered whether differences in plasma ghrelin concentrations were attributable to energy intake or altered end-products of carbohydrate fermentation. In the current experiment, plasma ghrelin and GH concentrations fluctuated as a result of time relative to feed offering ( $P < 0.001$ ) but did not differ as a result of dietary energy source (HAY vs. LFC; Figure 1A and 1B, respectively). Additionally, plasma INS and NEFA concentrations did not differ as a result of dietary energy source or sampling time relative to feed offering (Figure 1C and 1D, respectively). These data are consistent with the hypothesis that plasma ghrelin concentrations are similar for steers in positive energy balance and similar energy intake regardless of dietary energy source or DMI.



**Figure 1.** Effects of ingredient composition on plasma hormone concentrations for steers fed similar amounts of energy. HAY (50% hay, 50% concentrate) was fed to meet NEm requirement plus 3.5 Mcal/d NEg as calculated based on individual steer weight by using NRC (2000) equations. LFC (10% hay 90% concentrate) was limit-fed to meet NEm requirement plus 3.5 Mcal/d NEg as calculated based on individual steer weight by using NRC (2000) equations. Statistical effects are reported as TRT – effects of HAY vs. LFC; TIME effect of sampling time relative to feeding; TRTxTIME is the interaction of the main effects.

We speculated, on the basis of previous research in our lab, that differences in plasma ghrelin concentrations for steers fed different amounts of a common high-grain diet could have resulted from energy intake or could be attributed to differences in rumen distention as the vagus nerve has been implicated as responsive to ghrelin and involved in its release in various animal species (Sugino et al., 2003; Arnold et al., 2006). In the current experiment, plasma ghrelin concentrations did not differ when energy intake was similar but DMI and dietary ingredient composition differed. These data further support the hypothesis that differences in plasma ghrelin concentrations observed for cattle experiencing prolonged nutrient restriction were the result of differences in nutrient intake and not rumen fill.

Ruminal pH differed as a result of time relative to feeding ( $P < 0.001$ ) and dietary energy source ( $P < 0.001$ ) (Figure 2A). Ruminal pH was lower ( $P < 0.001$ ) for LFC steers compared with HAY steers, regardless of sampling time relative to feed offering. Additionally, rumen pH decreased following feed offering and reached a nadir 3 to 4 h post feed consumption and then began to rise regardless of ingredient composition. Dietary energy source influenced ruminal VFA concentrations ( $P < 0.002$ ) (Figure 2B – 2H). Molar concentrations of acetate were higher ( $P < 0.001$ ) for HAY cattle at all sampling times. In contrast, propionate, valerate, and isovalerate concentrations were higher for LFC steers ( $P < 0.001$ ). Ruminal acetate : propionate was higher ( $P < 0.001$ ) for HAY steers compared with LFC. Other researchers have demonstrated that the increased acetate : propionate that occurs with greater dietary forage content is the result of decreased propionate production (Bauman et al., 1971), as acetate production is constant with a wide variety of diets (Esdale, et al., 1968 and Davis, 1967).



**Figure 2.** Effects of ingredient composition on characteristics of rumen function for steers similar amounts of energy. HAY (50% hay, 50% concentrate) was fed to meet NEM requirement plus 3.5 Mcal/d NE<sub>g</sub> as calculated based on individual steer weight by using NRC (2000) equations. LFC (10% hay 90% concentrate) was limit-fed to meet NEM requirement plus 3.5 Mcal/d NE<sub>g</sub> as calculated based on individual steer weight by using NRC (2000) equations.

Ruminants generate the majority of their glucose from the metabolism of propionate in the liver, for this reason, plasma glucose concentrations are lower than those of monogastric animals and fluctuates less relative to meal. In rodents, circulating ghrelin concentrations decreased with re-feeding or infusion of glucose but not water (Tschöp et al., 2000). Glucose infusion, however, did not decrease plasma ghrelin concentrations when gastric emptying was prevented, which suggests that glucose absorption is necessary to influence plasma ghrelin concentrations (Williams et al., 2003). Little carbohydrate that can be converted to glucose for absorption reaches the small intestine of the ruminant. Therefore little glucose is absorbed from the intestine of ruminants. If however, propionate in ruminants is analogous to glucose in monogastric animals, we hypothesized that ghrelin concentrations should be lower when propionate concentrations are high (acetate : propionate is low). Because decreased acetate : propionate occurs with a high-grain diet as a result of increased propionate production (Bauman et al., 1971), greater glucogenic precursor is available when a high-grain diet is fed to steers. Despite a lower acetate : propionate for LFC compared with HAY steers in the current experiment, plasma ghrelin concentrations did not differ, suggesting that the ratio of glucogenic VFA to lipogenic VFA does not influence plasma ghrelin concentrations when steers are in positive energy balance and energy intake is similar.

Pearson correlation was used to determine whether significant relationships existed between plasma ghrelin concentrations and other hormones and metabolites indicative of nutritional status or end-products of carbohydrate fermentation. For LFC steers, acetate (Pearson coefficient = 0.29) and isovalerate (Pearson coefficient = 0.44) were correlated positively ( $P \leq 0.02$ ) to plasma ghrelin concentrations, whereas ruminal propionate (Pearson coefficient = -0.32) and ruminal valerate concentrations (Pearson coefficient = -0.41) were correlated negatively ( $P \leq 0.05$ ) to plasma ghrelin concentrations. Stepwise regression revealed that, although significant ( $P \leq 0.05$ ), isovalerate (partial  $R^2 = 0.19$ ), valerate (partial  $R^2 = 0.16$ ), butyrate (partial  $R^2 = 0.11$ ) and isobutyrate (partial  $R^2 = 0.09$ ) explained a small amount of the variation in plasma ghrelin concentrations. For HAY cattle, INS (Pearson coefficient = -0.72) was correlated negatively ( $P \leq 0.001$ ) and ruminal butyrate concentrations (Pearson coefficient = -0.27) tended to be negatively correlated ( $P = 0.09$ ) to plasma ghrelin concentrations. Stepwise regression indicated that plasma INS concentrations explained half (partial  $R^2 = 0.51$ ) of the variation in plasma ghrelin concentrations and ruminal butyrate concentrations explained an additional 10 percent (partial  $R^2 = 0.10$ ) ( $P \leq 0.001$ ).

These data indicate that plasma ghrelin concentrations are similar when dietary energy intake is similar for cattle in positive energy balance, regardless of ingredient composition or DMI. For steers in positive energy balance, fluctuations in plasma ghrelin concentrations relative to feeding time are not explained completely by differences in plasma GH, INS, or NEFA concentrations or by fluctuations in end-products of carbohydrate fermentation.

### **Implications**

Similar plasma ghrelin concentrations for cattle with similar energy intake regardless of differences in ingredient composition, resulting differences in VFA or hormone profiles, or amount of DMI implies the fluctuation of plasma ghrelin concentrations are the result of differences in nutrient intake and not the result of end-products of carbohydrate fermentation or rumen fill. Further research is warranted to investigate the role of ghrelin communicating nutrient status in the animal and its potential influence on composition of gain and efficiency of nutrient utilization.

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