2007

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BEEF 2007-03

Summary

Four steers (BW 1281±28.2 lb) were used to determine the relationship of plasma ghrelin concentrations with end-products of carbohydrate fermentation and hormones and metabolites indicative of nutrition status during a 12-h feeding interval. 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)**. At initiation of period I, 2 steers were allowed 2.4xM intake, whereas intake for the remaining 2 steers was restricted to 0.8xM. Equal aliquots of feed were offered at 0800 and at 2000 h. On 7, 14, and 21 d following initiation of intake restriction, serial blood samples were collected via indwelling jugular catheter at 15-min intervals through the 12-h feeding interval. Plasma samples were assayed for ghrelin, GH, insulin **(INS)**, and NEFA concentrations. Rumen fluid samples were collected throughout the feeding interval and processed for subsequent analyses of VFA concentrations. Following period I, steers were weighed, dietary treatments were switched between steer groups, intake amounts were recalculated, and sampling period II then was initiated as described for period I. Regardless of amount of DMI, plasma ghrelin and GH concentrations fluctuated as a result of sampling time relative to feeding. Plasma ghrelin concentrations were elevated prior to feeding at 0800 and 2000 h and reached a nadir from 1 and 3 h post-feeding. Although GH was elevated prior to the 0800 h feeding, it was not elevated at 1800 h despite increasing ghrelin concentrations. A tendency for an interaction of dietary treatment by sampling time relative to feeding which indicated an inverse relationship of plasma INS and ghrelin concentrations for cattle in a positive nutrient balance but no relationship between the two hormones when cattle were in negative energy balance. An interaction of dietary treatment by sampling time relative to feeding also resulted for plasma NEFA concentrations. A positive relationship of NEFA and ghrelin concentrations resulted for cattle when energy and protein intake were below the requirement for maintenance of BW. Ruminal VFA concentrations were weakly correlated to plasma ghrelin concentrations. These data are consistent with the hypothesis that, whereas plasma ghrelin concentrations fluctuate with nutritional status of the ruminant animal, the fluctuation is not completely explained by fluctuations in GH, NEFA, INS or ruminal VFA concentrations.

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). Plasma ghrelin concentrations increased with acute (48 h) complete feed deprivation in mature cattle (Wertz-Lutz et al., 2006. Wertz-Lutz et al. (2006) also demonstrated an increase in time spent feeding and a tendency for increased DMI with pulse doses of ghrelin in cattle. Additionally, in rodents, circulating ghrelin concentrations decreased with re-feeding or infusion of glucose but not water (Tschöp et al., 2000). For ruminants, plasma glucose concentrations are one-half that of monogastric animals, and, because ruminants generate the majority of their glucose from the metabolism of propionate in the liver, plasma

[1] Project funded by National Research Initiative Competitive Research Grant no. 2004-35206-14372.
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glucose fluctuates less relative to meal consumption. Given the differences in gastrointestinal intestinal tract anatomy and glucose metabolism in ruminants, this experiment was designed to evaluate whether a relationship exists between plasma ghrelin concentrations and end-products of carbohydrate fermentation in the rumen during a 12-h feeding interval when DMI of a high-grain diet is restricted.

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 DMI necessary to meet the NEm requirement **(2.4xM)** of a given steer and were calculated by using the equations from the Beef NRC (2000) as described below. To determine the amount of DMI necessary to meet the NEm requirement (Mcal/d) the equation 0.077 x empty BW($kg^{0.75}$) 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 as based on 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 DMI 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 a given amount of feed was determined for each steer, the MP content of the feed was estimated on the basis of degradability of the dietary protein (43.4%) by using the Beef NRC (2000) equation. The amount of dietary MP consumed then was compared with the MP required for BW maintenance as calculated using the equation 3.8 g MP x BW($kg^{0.75}$) (NRC, 2000).

Animals and procedures. Four ruminally-cannulated (3-yr-old) Angus crossbred steers (BW 1281±28.2 lb; 581.4 ± 12.8 kg) steers fitted with an indwelling jugular catheter. 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, DMI of the common high-grain finishing diet (Table 1) was increased during this acclimation period until DMI was 240% greater than the amount required to meet the NEm requirement of each steers. Once all steers had reached 240% of DMI required to meet NEm, the experiment was initiated.

Table 1. Ingredient composition of experimental diet

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

[a] Dried distiller's grains with solubles
[b] NaCl 94.0-98.5%, Zn 0.35%, Fe 0.20%, Co 0.005%, Mn 0.20%, Cu 0.30%, I 0.007%.
[c] 30,000 IU/g Vitamin A, 500 IU/g Vitamin E, Vitamin D 500,000 IU/g
[d] 35.54% Zn.
[e] Formulated to contain 32 g/T.

The experiment was conducted as a crossover design with two 21-day treatment periods. During treatment period I, 2 steers were maintained at 240% of intake required to meet the NEm requirement (2.4xM) established during the acclimation period and the remaining 2 steers were limited to 80% of the intake to meet NEm requirement. Serial blood and rumen fluid samples were collected on d 7, 14, and 21 following invocation 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 based-on the BW recorded at the end of sampling period I. A DMI amount of 2.4xM again was established, and a second 21-d treatment and sampling period II 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 to1645, and 1800 to 1845. One 10-mL aliquot of blood was collected into a glass tube containing K_3 EDTA for plasma separation. Aliquots of plasma (1.0 mL each) were processed and stored at –20^{o}C for the subsequent analyses of GH, NEFA, INS, and ghrelin according to procedures outlined by Wertz-Lutz et al. (2006).

Rumen fluid collection. Rumen fluid (50 mL) was collected the beginning of each hour in which blood samples were collected and pH was recorded immediately. A 5-mL aliquot of rumen fluid was acidified, centrifuged at 20,000 x *g,* and frozen at -20 ^{o}C for subsequent quantification of molar proportion of VFA via gas chromatography.

Statistical analyses. To verify that differences in NEm and MP intake resulted from the invoked 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 DMI. Differences in the characterization parameters that resulted from amount of DMI were separated by using a Fisher's t-test. Plasma ghrelin, GH, INS, and NEFA concentrations and ruminal VFA concentrations and pH were analyzed statistically as repeated measures in time by using the MIXED procedure of SAS with independent errors that accounted for error correlation during the sampling times. The model included length of treatment, sampling time relative to feeding, amount of DMI (0.8xM vs. 2.4xM), steer, period, and the interactions of length of treatment, sampling time, and amount of DMI as independent variables. Differences in least squares means for plasma ghrelin, GH, INS, NEFA, and ruminal VFA concentrations and pH were separated by using a Fisher's t-test. The data set then was divided by dietary treatment and Pearson correlation and stepwise regression was performed to characterize the relationship between plasma hormones and metabolites and end-products of ruminal fermentation for steers in different nutritional states.

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 ± 0.4 lb/d, respectively). This DMI restriction resulted in lower ($P \leq 0.001$) energy and MP intake for 0.8xM steers compared with 2.4xM steers. The energy and protein restriction that resulted from restricted DMI decreased ($P \leq 0.001$) BW for 0.8xM (-108.9 lb) steers compared with that for 2.4xM (127.9 lb) steers.

Relationship of plasma hormones and metabolites with end-products of fermentation. For monogastric animals, carbohydrate but not water ingestion decreased plasma ghrelin concentrations elevated by fasting (Tschöp et al., 2000). Monogastric animals traditionally exhibit a transient elevation in plasma glucose subsequent to a carbohydrate-containing meal. In contrast, ruminants generate the majority of their glucose from the metabolism of propionate in the liver, and plasma glucose fluctuates less relative to meal consumption (Fahey and Berger, 1988). Wertz-Lutz et al. (2006) demonstrated no difference in plasma glucose concentrations for mature beef cattle fasted for 48 h, despite differences in plasma ghrelin, INS, and NEFA concentrations. Because the majority of carbohydrate in a ruminant animal diet is fermented by rumen microbes to produce VFA and then converted to glucose, we investigated the relationship of ruminal VFA concentrations and hormones and metabolites indicative of

nutritional status with the fluctuation plasma ghrelin concentrations during a 12-h feeding interval for cattle consuming a high-grain diet.

There was no three-way interaction of dietary treatment by length of treatment by sampling time relative to feeding for any of the measured variables. For this reason, data were pooled for d 7, 14, and 21 and are reported as the interaction of dietary treatment by sampling time relative to feeding in Figure 1. Whereas differences in plasma ghrelin and GH concentrations resulted from the main effects of dietary treatment and sampling time relative to feeding, there was not a significant interaction of dietary treatment by sampling time for these parameters. Plasma ghrelin concentrations were elevated ($P < 0.001$) for 0.8xM compared with those for 2.4xM steers (181.8 and 86.0 ± 4.8 pg/mL, respectively) throughout the 12-h feeding interval. Plasma GH concentrations also were elevated ($P < 0.001$) for 0.8xM compared with those for 2.4xM steers (15.0 and 9.4 ± 0.45 ng/mL, respectively) throughout the 12-h feeding interval. Regardless of dietary treatment, plasma ghrelin and GH concentrations fluctuated as a result of sampling time relative to feeding ($P < 0.003$). Plasma ghrelin concentrations were elevated ($P \leq 0.05$) prior to feeding at 0800 and 2000 h, reached a nadir from 1 and 3 h post-feeding, and then began to increase as time progressed toward the next feeding (Figure 1A). Average plasma GH concentration the hour before the 0800 h feeding was higher than plasma GH concentrations throughout the remainder of the sampling period (Figure 1B). Although GH was elevated at the 0800 h feeding along with ghrelin, plasma GH was not elevated at 1800 h prior to the evening feeding despite increasing ghrelin concentrations.

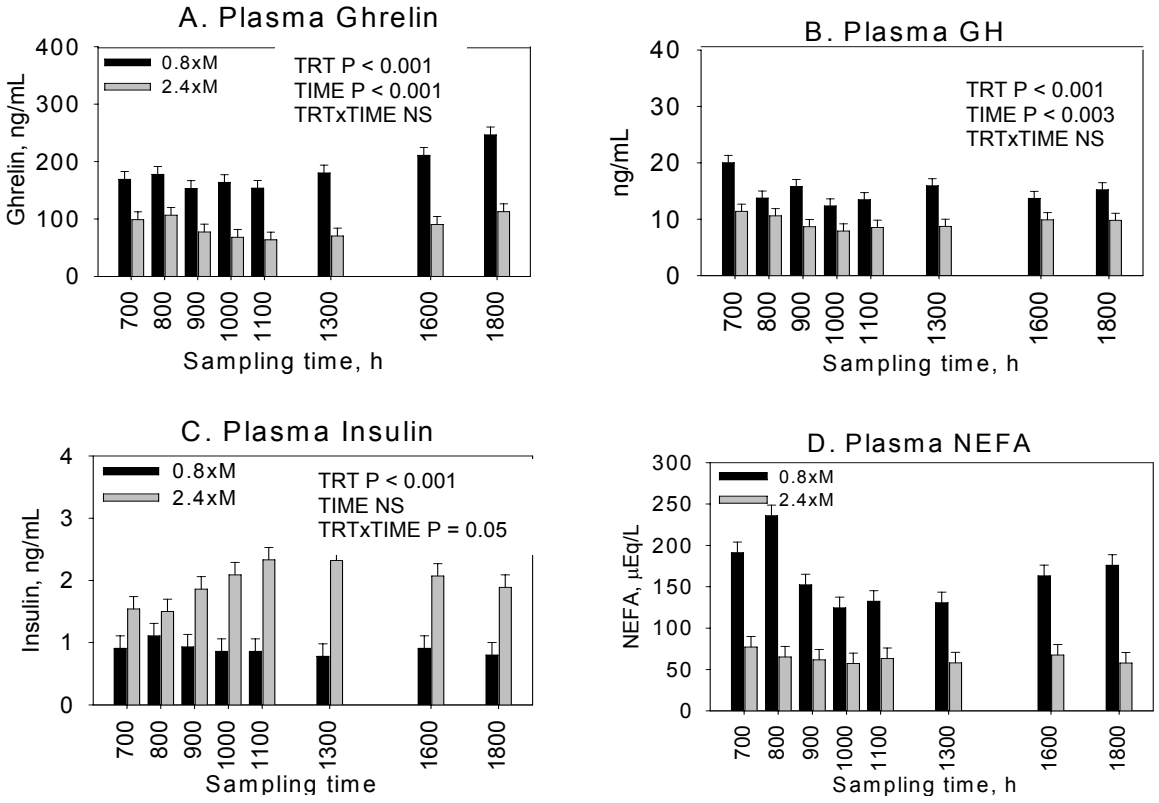

Figure 1. Relationship of plasma ghrelin with hormones and metabolites during a 12-h feeding interval for cattle consuming energy and protein above or below that necessary for maintenance of BW. TRT = 0.8xM – 80% of the DMI needed to meet the energy requirement for maintenance; 2.4xM - 240% of the DMI needed to meet the requirement for maintenance. TIME = sampling time relative to feeding times of 0800 and 2000h. TRTxTIME= interaction of the main effects.

Plasma INS concentrations were lower ($P < 0.001$) for 0.8xM steers compared with 2.4xM steers (0.9 and 1.9 ± 0.07 ng/mL, respectively). There was an interaction of dietary treatment by sampling time relative to feeding ($P \leq 0.05$) where plasma INS concentrations did not vary relative to feeding time for 0.8xM cattle but increased through 5 h post-feeding for 2.4xM steers and then began to decline (Figure 1C). Plasma NEFA concentrations were elevated for 0.8xM steers compared with those of 2.4xM. An interaction of dietary treatment by sampling time relative to feeding also resulted for plasma NEFA concentrations ($P < 0.001$). Plasma NEFA concentrations were elevated 0.8xM steers prior to both the 0800 and the 2000 h feeding and reached a nadir between the two feedings (Figure 1D), whereas a strong relationship between plasma NEFA and ghrelin concentrations did not exist for 2.4xM steers.

Fluctuation of ruminal fermentation characteristics relative to feeding. There was not an interaction of dietary treatment by sampling time relative to feeding for ruminal pH or VFAs. These data therefore, have been reported on the basis of main effects of dietary treatment and sampling time relative to feeding (Figure2 A-H). Ruminal pH was lower ($P < 0.001$) for 2.4xM steers compared with 0.8xM steers. Ruminal acetate (61.7 and 50.8 ± 0.39 molar%) and butyrate (14.3 and 7.7 ± 0.28 molar%) concentrations were higher ($P < 0.001$) for 0.8xM steers compared with 2.4xM steers. However, both acetate and butyrate concentrations were similar regardless of sampling time relative to feeding. In contrast, ruminal valerate (1.2 and 2.1 ± 0.07 molar%) and propionate (19.4 and 37.9 ± 0.42 molar%) concentrations were lower ($P < 0.001$) for 0.8xM steers compared with 2.4xM steers. Ruminal valerate concentrations were similar regardless of sampling time relative to feeding; however, ruminal propionate concentrations were higher ($P \leq 0.05$) at 3 and 5 h post-feeding compared with pre-feeding propionate concentrations. Ruminal isovalerate (2.3 and 0.9 ± 0.07 molar%) and isobutyrate (1.2 and 0.6 ± 0.02 molar%) concentrations were higher ($P < 0.001$) for 0.8xM compared with 2.4xM steers. For both isovalerate and isobutyrate, concentrations in the rumen were elevated ($P \leq 0.05$) at sampling times prior to both the 0800 and 2000 h feedings and reached a nadir from 1 to 5 h post-feeding (Figure 2G and 2H). Acetate : propionate was higher ($P < 0.001$) for 0.8xM steers compared with 2.4xM steers, (3.3 and 1.4 ± 0.05, respectively). Acetate : propionate was higher ($P \leq 0.05$) before the 0800 h feeding compared with the remainder of the sampling times relative to feeding. Differences in acetate : propionate predominantly were the result of differences in molar proportions of propionate, as acetate concentrations did not fluctuate with DMI, which suggest a shift in the availability of glucogenic (propionate) versus lipogenic (acetate) precursors.

Although isobutyrate and isovalerate compose relatively small proportion of the total VFA profile of rumen fluid, the fluctuation in their proportions relative to the other VFAs mimics the pattern of plasma ghrelin concentrations more so than do the other VFAs. Previous research, however, demonstrated no relationship between supplementation of isovalerate and isobutyrate and voluntary forage intake (Gunter et al., 1990). Although acetate, propionate, and butyrate have been implicated as potential regulators of DMI, their affects on DMI have been varied. Sheperd and Combs (1998) reported that intraruminal propionate infusion decreased DMI to a greater extent than did acetate in dairy cows, whereas Bahttacharya and Alulu (1975) demonstrated that acetate was more efficacious than propionate in decreasing DMI. In contrast, Quigley et al. (1991) demonstrated no effects of propionate infusion into the portal vein on DMI regardless of whether lambs were in positive or negative energy balance. It is important to note that infusion of VFAs does not completely suppress DMI. Reported suppression of DMI as a result of VFA infusion ranges from 3 to 58% (Bahttacharya and Alulu, 1975; Combs, 1998). Incomplete suppression of DMI with VFA infusion suggests that factors other than VFAs are involved in the regulation of DMI in ruminants.

Pearson correlation and stepwise regression established that significant relationships existed between ghrelin and end-products of ruminal fermentation or hormones and metabolites indicative nutritional status but that the relationships were moderate to weak. Pearson correlations indicated that for 2.4xM steers, plasma GH concentrations (Pearson coefficient = 0.40) and ruminal acetate concentrations (Pearson coefficient = 0.27) were correlated positively ($P \leq 0.01$) to plasma ghrelin concentration, whereas plasma NEFA (Pearson coefficient = -0.23) and ruminal propionate (Pearson coefficient = -0.24) concentrations were correlated negatively ($P \leq 0.05$) to plasma ghrelin concentrations. Stepwise regression indicated that the fluctuation in ghrelin for 2.4xM steers was explained by ($P \leq 0.01$) plasma

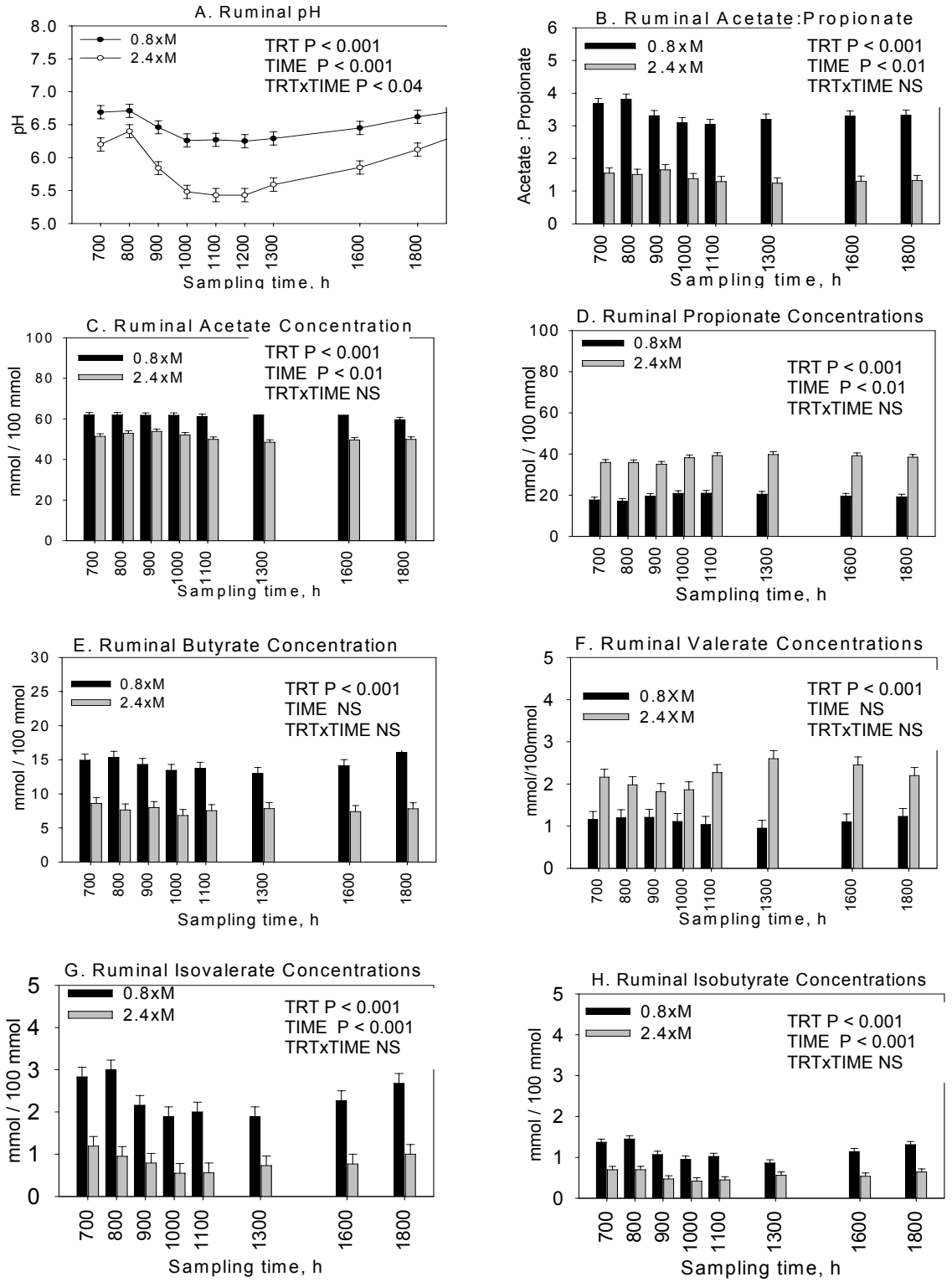

Figure 2. Ruminal fermentation characteristics during a 12-h feeding interval for cattle consuming energy and protein above or below that necessary for maintenance of BW. TRT = 0.8xM – 80% of the DMI needed to meet the energy requirement for maintenance; 2.4xM - 240% of the DMI needed to meet the requirement for maintenance. TIME = sampling time relative to feeding times of 0800 and 2000h. TRTxTIME= interaction of the main effects.

GH concentrations (partial R^2 = 0.15), and to a lesser extent by ruminal acetate concentrations (partial R^2 = 0.07) and plasma NEFA concentrations (partial R^2 = 0.06). In contrast, the fluctuation in plasma ghrelin concentrations for 0.8xM steers was correlated positively ($P \leq 0.05$) to plasma NEFA concentrations (Pearson coefficient = 0.20), and ruminal isovlaerate (Pearson coefficient = 0.25), and valerate (Pearson coefficient = 0.21), but negatively correlated ($P \leq 0.05$) to ruminal acetate concentrations (Pearson coefficient = -0.23), acetate : propionate (Pearson coefficient = -0.23) and plasma INS concentrations (Pearson coefficient = -0.53). Stepwise regression indicated that the fluctuation in plasma ghrelin concentration was explained ($P \leq 0.01$) by plasma INS concentrations (partial R^2 = 0.28) and to a lesser extent by ruminal acetate concentrations (partial R^2 = 0.03).

If indeed ghrelin is an orexigenic peptide in ruminant animals, it does not have a strong correlation with end-products of carbohydrate fermentation in the rumen or other hormones and metabolites indicative of nutritional status in ruminants. Data from this experiment are consistent with the hypothesis that, whereas plasma ghrelin concentrations fluctuate with nutritional status of the ruminant animal, fluctuations in plasma ghrelin concentrations within a 12-h feeding interval are not explained completely by fluctuations in plasma GH, NEFA, and INS concentrations or end-products of carbohydrate fermentation in the rumen.

Implications

Although ghrelin is responsive to acute and prolonged nutrient restriction, fluctuation in plasma ghrelin concentrations within a 12-h feeding interval is not explained completely by other hormones or metabolites indicative of nutritional status or end-products of carbohydrate fermentation in the rumen. These data imply that ghrelin may serve as a signal for long-term nutrient status in the ruminants.

Acknowledgement

This project was supported by National Research Initiative Competitive Research Grant no. 2004-35206-14372 from the USDA Cooperative State Research, Education and Extension Service. Additionally, the authors thank Heather Werner, Anna Taylor, and Jessie Liebenstein for their help with animal care and sample collection.

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