

# Effect of Feed Intake on Plasma Ghrelin Concentration in Beef Cattle

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### Summary and Implications

Two experiments were conducted to determine if ghrelin mRNA existed in ruminant digestive tract tissues and to establish the fluctuation in plasma ghrelin concentrations for fed and fasting steers. Tissues collected from the ruminant digestive tract indicate detectable ghrelin mRNA in the upper, middle, and lower portion of the abomasum and in the small intestine but no detectable ghrelin mRNA in the reticulum, omasum, and rumen. Plasma ghrelin concentrations are elevated by a fasting period that is as short as 22 h. Additionally, plasma ghrelin remain elevated throughout a 48-h fasting period. The magnitude of difference in plasma ghrelin concentration between the fed and fasting states warrants further investigation as to the impact that this fluctuation in hormone concentration has on feed intake, composition of gain, and energy expenditure in cattle.

### Introduction

Ghrelin is a hormone secreted from the digestive tract of many animals. For sheep, plasma ghrelin concentrations are associated with fluctuation in feed intake, peaking just before feeding time and reaching a nadir 2 to 4 h post feeding. For Japanese Black cattle, plasma ghrelin concentrations at 4 h post-feeding were intermediate to plasma ghrelin concentrations pre-feeding and 1h post-feeding. These data suggest that ghrelin acts as a signal of hunger and therefore may stimulate feed intake. Feed intake is important to beef cattle because it impacts efficiency of gain and therefore cost of production. Additionally, inconsistent feed intake also can result in metabolic disorders such as ketosis and acidosis, impacting both animal welfare and economic returns. Because feed costs account for 40 – 70% of the total on-farm costs of beef production, a greater understanding of regulation of feed intake by cattle and the potential to control feed intake would have a major impact on the efficiency and therefore the profitability of beef production.

Additionally, research with rodents suggests that, during times of inadequate nutrition, elevated plasma ghrelin concentrations may signal a need for more energy-

efficient metabolism, resulting in positive energy balance. No data substantiating this effect have been reported for cattle, but, if similarities exist between species, ghrelin may influence (1) composition of gain for compensating calves or (2) energy expenditure of cows experiencing periods of inadequate nutrition.

The mechanism by which ghrelin stimulates feed intake has been studied in detail. However, few research trials designed to investigate the role of ghrelin in ruminant species have been reported, and to date the relationship of ghrelin to feed intake and nutritional status of cattle has not been established. By studying these relationships, we can establish a foundation on which to anchor future research aimed at developing more accurate feeding that will minimize production loss and maximize animal well-being during critical stages of production.

We hypothesize that ghrelin mRNA exists in the digestive tract tissues of cattle as has been reported in monogastric species. Furthermore, we hypothesize that plasma ghrelin concentrations fluctuate relative to feed intake. Two experiments were conducted with the following objectives 1) to determine if ghrelin mRNA exists in tissues of the ruminant digestive tract and 2) to establish the fluctuation of plasma ghrelin concentration for cattle in a fed and fasted state.

### Materials and Methods

#### *Experiment 1. Determining relative ghrelin mRNA concentration in digestive tract tissues*

Digestive tract tissues were collected from four cattle that were slaughtered at the Iowa State University Meats Laboratory. Cattle were not selected by age, gender, or previous diet and, as a result, included mature cows, heifers, and steers from a variety of feeding backgrounds. Tissue samples were collected from the lining of the reticulum, omasum, three sites in the rumen, upper, middle, and lower sections of the abomasum, and the small intestine. Samples were obtained within 45 min of slaughter and were snap frozen in liquid nitrogen and stored at -80° C prior to RNA extraction and analysis.

Total RNA was isolated from the tissues and quantified by using a UV spectrophotometer. The RNA (20 µg per lane) was separated by using a denaturing formaldehyde-containing agarose gel. The gels were photographed before capillary transfer to charged nylon membranes. The membranes were subjected to typical northern hybridization conditions and probed with a randomly labeled 488 base-pair PCR product resulting from primers designed on the basis of the published bovine ghrelin preprotein mRNA. Detection was achieved by using typical x-ray film.

### *Experiment 2. Determining fluctuation of plasma ghrelin concentration*

Four crossbred feeder steers (initial weight  $450 \pm 28.5$  kg) were used in a crossover design to determine the fluctuation in plasma ghrelin concentrations of cattle in the fed and fasted state. Steers used in this experiment were cared for by adhering to the guidelines set forth by the Iowa State University Committee on Animal Care. Steers were adapted to a climate-controlled facility over an 11-d period. During the adaptation period, lights were turned on at 06:00 and shut off at 22:00. Temperature and humidity were maintained at 19° C and 89%, respectively. A finishing diet composed of 83% grain, 5% alfalfa hay, and 12% corn silage was fed throughout the adaptation and sampling periods. During the adaptation period, all steers were offered feed once daily at 08:00 and allowed to consume ad libitum until 20:00 each day. Feed not consumed by 20:00 each evening was weighed back and ad libitum intake was established on the basis of a minimum of 10% feed weigh-back.

Following the adaptation period, the experiment was organized as a crossover design having two 48-h treatment/sampling periods that were separated by a 5-d washout period. During the 5-d washout period, all four steers were allowed to consume ad libitum. Treatments were nutritional status (fed or fasted). The fed treatment group (FED steers) consisted of two steers that remained on the ad libitum feeding regimen that was established during the adaptation period. The fasting treatment group (FAST steers) consisted of two steers that were not feed for a total of 48 h.

Indwelling jugular catheters were inserted 1 d prior to the initiation of the sampling period. Catheter integrity for blood sample collection was maintained using potassium-EDTA as an anticoagulant. Serial blood samples were collected from all four steers over a 26-h period beginning when the FAST steers had been withheld from feed for 22 h and continuing through the point at which the FAST steers had been withheld from feed for 48 h. Blood samples were collected at 10-min intervals from 18:00 to 20:00 on day 1 of sampling. On the second day of sampling, blood samples were collected at 10-min intervals from 06:00 to 08:00 and at 15-min intervals from 08:15 to 20:00.

At each sampling time, 15 mL of blood was collected. One aliquot was collected into a glass tube containing 15% potassium-EDTA for plasma separation, and a second aliquot was placed in a glass tube containing no anticoagulant for serum separation. Samples were placed on ice, and the plasma or serum was separated by centrifugation. A 1.5-mL aliquot of plasma was acidified to preserve the integrity of the ghrelin. A 1.0-mL aliquot of serum was harvested for quantification of glucose and nonesterified fatty acids (NEFA). Plasma and serum samples were stored at  $-20^{\circ}$  C for subsequent analyses.

Plasma ghrelin concentration was quantified by using a competitive inhibition rat ghrelin radioimmunoassay (Linco

Research, St. Charles, MO). We have validated in our laboratory that this assay can be used to quantify ghrelin in bovine plasma. Glucose concentration was determined by a colorimetric assay (Sigma, St. Louis, MO), and NEFA concentrations also were determined by colorimetric assay (WAKO Chemicals USA Inc., Richmond, VA).

Plasma glucose, NEFA, and ghrelin concentrations were analyzed statistically as repeated measures in time. Steer nested in period by treatment and period were used as random variables with the MIXED procedure of SAS. Differences in treatment means at each sampling time were separated by using least squared means.

## **Results and Discussion**

### *Experiment 1.*

The pattern of ghrelin mRNA expression in digestive tract tissues was similar in all cattle regardless of gender, age, or nutritional management. An example of a northern blot from one animal is shown in Figure 1. The observed pattern of expression is predictable, based on data from rats where expression is greatest in gastric tissue with minimal but detectable message expressed in the small intestine.

### *Experiment 2.*

Serum glucose and NEFA concentrations were quantified to verify nutritional status of the steers during the sampling period. Glucose, which is absorbed directly from the digestive tract in rats, has been reported to diminish plasma ghrelin concentrations. In contrast, glucose in the ruminant results largely from *de novo* synthesis from propionate in the liver. As a result, serum glucose concentration fluctuates minimally relative feed intake in ruminants. Our data support that serum glucose concentration is relatively constant at 75 – 95 mg/dL (Figure 2). Although serum glucose concentration fluctuates over the course of the sampling period, serum glucose concentration was similar for FED steers and FAST steers.

Fasting ruminants mobilize body tissue as a means of supplying energy to support vital bodily functions during periods of inadequate nutrient intake. Mobilization of body tissue results in elevated serum NEFA concentrations, and in contrast to serum glucose concentrations, serum NEFA concentrations are a better predictor of energy status in ruminants. Serum NEFA concentrations in this experiment were elevated ( $P \leq 0.003$ ) for FAST steers (Figure 3.). These data suggest that FAST steers were mobilizing body tissue as a result of inadequate energy intake, whereas FED steers had lesser NEFA concentrations because they were receiving adequate energy from their diet and did not need to mobilize body tissue to support bodily functions.

Figure 4 illustrates mean plasma ghrelin concentrations for FED steers and for FAST steers from 22 through 48 h fasting. Mean plasma ghrelin concentration for FAST steers (649 pg/mL) was 5-fold higher than that of FED steers (115 pg/mL). For each sampling time point, plasma ghrelin concentrations were higher ( $P < 0.0001$ ) for FAST steers

compared with those assigned to FED steers. Additionally, plasma ghrelin concentrations for FAST steers remained elevated throughout the fasting period. For steers assigned to the FED steers, plasma ghrelin concentrations are elevated just prior to daily feeding time and diminish after meal consumption. This pattern in plasma ghrelin concentration was observed for each of the four steers when assigned to the FED treatment (Figure 5.). A pattern in plasma ghrelin concentration was not observed for FAST steers. Elevated plasma ghrelin concentrations just prior to feeding time is consistent with data reported for sheep, which suggests that plasma ghrelin concentration peaks just prior to feeding time in meal-fed sheep and dissipates subsequent to feeding.

Ghrelin mRNA is found in digestive tract tissue of ruminants, which justifies further investigation into the

effects of dietary modification and its impact on plasma ghrelin concentrations. Plasma data for cattle in different nutritional states indicate that ghrelin concentrations are elevated markedly by a fasting period as short as 22 h. Furthermore, the rapid elevation of plasma ghrelin concentrations relative to feed intake restriction warrants greater investigation to determine the impact of elevated plasma ghrelin concentrations on the composition of gain and energy expenditure in cattle.

#### Acknowledgements

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Figure 1. Ghrelin mRNA expression in ruminant digestive tissue.

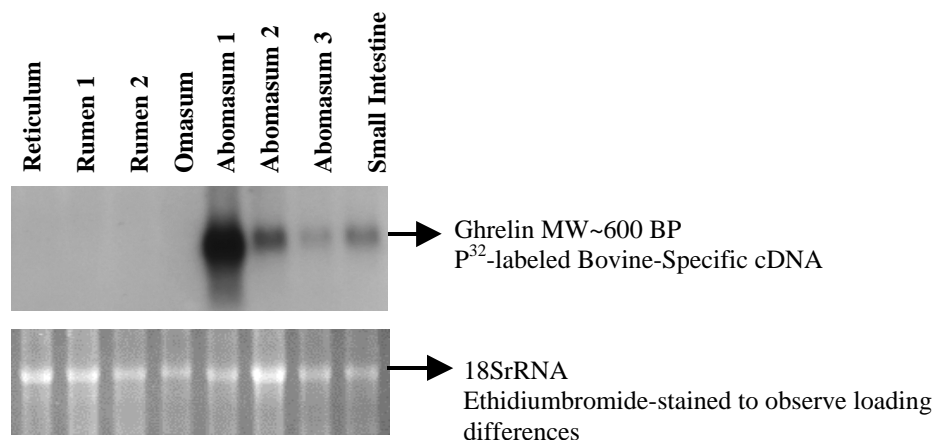


Figure 2. Comparison of serum glucose concentrations of FED steers and FAST steers. Pooled SEM 14.05. For each time point, plasma glucose concentrations did not differ for steers withheld from feed ( $P > 0.05$ ) compared with those fed ad libitum.

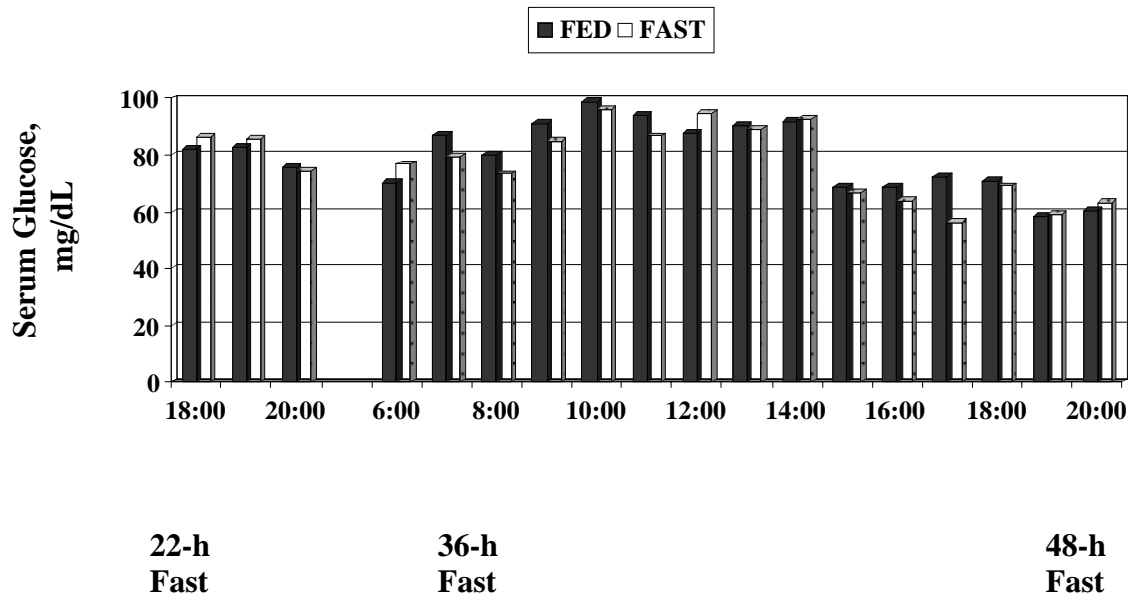


Figure 3. Comparison of serum non-esterified fatty acid (NEFA) concentrations for FED steers and FAST steers. Pooled SEM 46.51. For each time point, plasma NEFA concentrations was higher for steers withheld from feed ( $P \leq 0.003$ ) compared with those fed ad libitum. For steers assigned to FAST steers, mean NEFA concentrations were lower from 22 to 24 h of fasting than from 32 to 48 h of fasting ( $P < 0.05$ ).

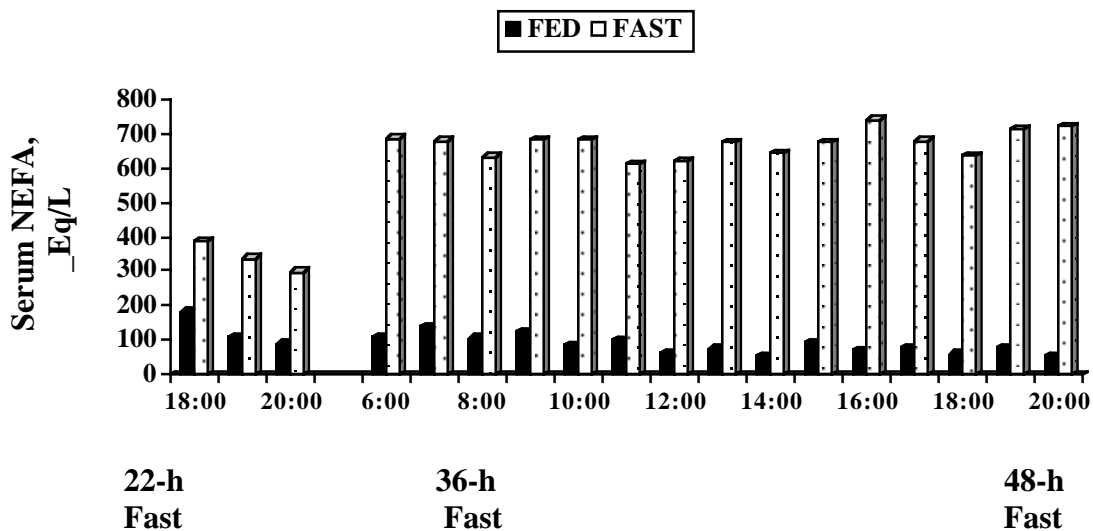


Figure 4. Plasma ghrelin concentration for FED steers and FAST steers. Pooled SEM 55.16 pg/mL. For each sampling time, mean plasma ghrelin concentrations differ ( $P < 0.0001$ ) for FED steers versus FAST steers.

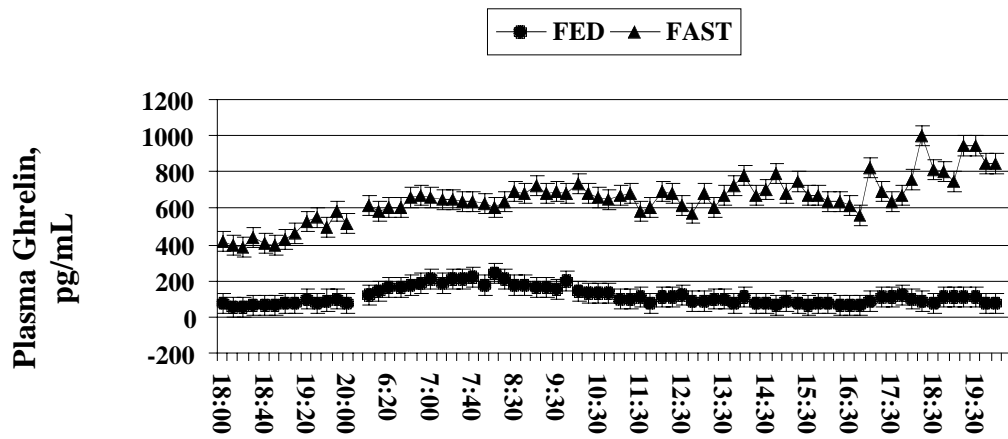


Figure 5. Individual plasma ghrelin concentrations for FED (a.) and FAST (b.) steers. Each line on a graph represents plasma ghrelin concentration for a single steer over the course of a sampling period. For FED steers, plasma ghrelin concentrations, although considerably lower than those of FAST steers, seemed to increase just prior to feeding time and diminish after feed consumption, whereas plasma ghrelin concentrations for FAST steers were elevated but did not seem to have a specific pattern of secretion.

