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# Changes in acyl and total ghrelin concentrations and their association with dry matter intake, average daily gain, and feed efficiency of finishing beef steers and heifers

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ABSTRACT

Ghrelin is a peptide hormone produced in the gut that is implicated in signaling appetite and regulating dry matter intake (DMI). The objective of this experiment was to determine the change in acyl ghrelin, total ghrelin, and the ghrelin ratio (acyl ghrelin/total ghrelin) over an 84-d DMI and average daily BW gain (ADG) measurement period and to determine the association of those ghrelin measurements with DMI, ADG, ADG:DMI ratio (G:F), and residual feed intake in finishing beef steers and heifers. Blood samples were collected on day 0 and day 83 before feeding and between 0730 h and 1130 h. Samples were analyzed for acyl and total ghrelin using commercially available RIA. DMI in steers was greater during the last 35-d period of the experiment compared with the first 35 d ($P < 0.01$) and was greater than heifers regardless of period ($P < 0.01$). Steers had greater acyl ghrelin concentrations on day 0 than heifers, but concentrations decreased by day 83 to equal concentrations in heifers ($P < 0.01$). Total ghrelin concentrations were lower on day 0 in heifers but increased by day 83 and did not differ from steers on day 83 ($P < 0.01$). A mixed model analysis was used to determine the association of ghrelin concentrations and ratio with production traits, independent of breed and sire effects. There was an interaction of day 0 acyl ghrelin concentrations with time of sample collection for 84-d DMI ($P < 0.01$), ADG ($P < 0.01$), and G:F ($P = 0.09$), indicating a general positive association of acyl ghrelin with production traits, but the association weakened as time of sample collection increased. The mean ghrelin ratio tended ($P = 0.08$) to be positively associated with DMI in the last 35-d period. The ghrelin ratio on day 0 interacted with time of sample collection for ADG and G:F ($P < 0.05$), indicating an overall positive association of the ghrelin ratio with ADG and G:F. Results indicate that ghrelin is associated with DMI, ADG, and feed efficiency of finishing beef cattle, and data lend more evidence that ghrelin is involved in appetite regulation of ad libitum fed cattle.

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## 1. Introduction

The gut peptide ghrelin is thought to be a stimulator of appetite in many species [1–3], including cattle [4]. Ghrelin was first discovered because of its ability to bind the growth hormone secretagogue receptor when it is acylated on the serine-3 residue, which leads to the release of growth hormone [5]. In addition to the growth-hormone releasing activity of acyl ghrelin, it is also thought to increase appetite by stimulating the release of neuropeptide Y and agouti-related protein in the hypothalamus [6,7]. Foote et al [8] reported that a single measurement of acyl ghrelin was positively associated with dry matter intake (DMI) and average daily body weight gain (ADG) in ad libitum fed finishing steers and heifers, indicating that differences in circulating ghrelin concentrations could be

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partially responsible for the observed differences in DMI of ad libitum fed cattle. It was also shown that the ratio of acyl:total ghrelin (total ghrelin includes both acyl and desacyl ghrelin) was more associated with DMI and ADG than acyl ghrelin alone [8]. The single measurement of acyl and total ghrelin used by Foote et al [8] was collected after the completion of DMI and ADG collection period, which indicates that ghrelin concentrations could be more representative of previous DMI and not predictive of future DMI. Given the known biological function of ghrelin in other species [1–3], it is likely that greater ghrelin concentrations would be indicative of a greater appetite, and therefore greater DMI in ad libitum fed animals. Given the potential actions of ghrelin on growth and DMI regulation, it is possible that ghrelin concentrations could be used as a biomarker for feed efficiency evaluation; however, it is not clear if the association of ghrelin concentrations with production traits will remain significant in other groups of cattle. If ghrelin is shown to have a resilient association with production traits, the data will provide evidence that the ghrelin biological system should be evaluated further for having direct effects on observed variation in DMI of ad libitum fed cattle.

The objective of this experiment was to determine the association of acyl and total ghrelin measured at the beginning and the end of an 84-d feeding period with production traits. In addition, the changes in acyl and total ghrelin concentrations were determined. It was hypothesized that ghrelin concentrations will be associated with feed intake.

## 2. Materials and methods

This experiment was reviewed and approved by the US Meat Animal Research Center Animal Care and Use Committee (IACUC approval number 5438–31,000-092–03) under IACUC standards meeting the requirements outlined by the USDA.

### 2.1. Animals

Details of the animals and experimental procedures have been previously described [9]. Calves ($n = 236$ total; $n = 127$ steers and $n = 109$ heifers) used in this experiment were the progeny from composite breed cows that were mated to Angus, Charolais, Gelbvieh, Limousin, Red Angus, and Simmental bulls that were in current use on industry ranches. Both steers (Angus- [$n = 35$], Charolais- [$n = 8$], Gelbvieh- [$n = 21$], Limousin- [$n = 21$], Red Angus- [$n = 17$], and Simmental-sired [$n = 25$]), and heifers (Angus- [$n = 25$], Charolais- [$n = 6$], Gelbvieh- [$n = 30$], Limousin- [$n = 18$], Red Angus- [$n = 14$], and Simmental-sired [$n = 16$]) were used in the study. At the beginning of the study, heifers weighed 395 ± 3.5 kg and steers weighed 416 ± 3.5 kg. Mean age of cattle at the beginning of the study was 298 ± 0.4 d. Calves were housed in a facility with Calan-Broadbent electronic headgates (American Calan, Inc, Northwood, NH, USA) to measure individual feed intake. The calves were sorted and penned by BW before beginning the study to minimize aggressive behavior of larger cattle toward smaller cattle. Steers and heifers were penned separately. Thirteen pens were used in this experiment and housed either 19 or 20 animals each. The cattle were trained to use individual Calan headgates during the adaptation period (approximately 45 d). The steers were implanted (200-mg trenbolone acetate and 40-mg estradiol 17β; Revalor XS, Merck Animal Health, Kenilworth, NJ, USA) 86 d before the start of the experiment. Heifers received a Revalor H implant (140-mg trenbolone acetate and 14-mg estradiol; Merck Animal Health) 86 d before the beginning of the study and a Revalor 200 implant (200-mg trenbolone acetate and 20-mg estradiol; Merck Animal Health) 57 d after the study began.

Cattle were placed on a diet that on a dry matter (DM) basis consisted of 67.75% dry-rolled corn, 20% wet distillers grains with solubles, 8% chopped alfalfa hay, and 4.25% commercial vitamin and mineral supplement 7 d before the feed intake and growth trial began. The supplement contained monensin to supply 300 mg/head daily. Feed bunks were evaluated visually each day of the experiment at approximately 0730 h to determine the quantity of feed to offer each animal. The bunk management approach was designed to allow for 0.25 to 0.50 kg of feed remaining in the feed bunk at the time of evaluation. After the quantity of feed to be provided to each bunk was determined, a portion of the ration sufficient to supply the feed for all pens was mixed in the feed truck (Roto-Mix IV 274–12B, scale readability ± 0.09 kg) for approximately 5 min. Cattle were fed once daily throughout the experiment, starting at approximately 0800 h. Feed was subsampled daily, and a weekly composite sample was made to determine feed DM. Orts were determined once per week. Total DMI was the sum of total DM fed minus total orts. Values reported and used in the models are average daily DMI (referred to as DMI for simplicity). The feeding study lasted 84 d and cattle were weighed on days 0, 1, 21, 42, 63, 83, and 84. A quadratic equation was used to regress BW on day of study and total BW gain was determined by solving the equation for 84 d on study. Average daily gain was calculated as total BW gain divided by days on study. The ratio of ADG:DMI (G:F) was calculated as the quotient of ADG divided by DMI. Residual feed intake (RFI) was calculated by regressing observed DMI against ADG and metabolic BW at the midpoint of the experiment, and the residual was RFI. Steers and heifers were treated as separate cohorts for the RFI calculation.

### 2.2. Sample collection, processing, and analyses

Blood (9 mL) was collected on day 0 and day 83 of the DMI and ADG measurement period before feeding, via jugular venipuncture into tubes containing EDTA (1.7 μg/mL of blood) and placed on ice immediately. Samples were then centrifuged at 3,000 × *g* for 25 min at 4°C within 1.5 h of collection to obtain plasma. Two 1-mL aliquots for acyl and total ghrelin analysis were treated with 50 μL of 1-*M* HCl and 10 μL of phenylmethylsulfonyl fluoride (10 mg/mL in 100% methanol; Sigma Aldrich, St. Louis, MO, USA). All plasma samples were stored at −20°C until analysis. Cattle were processed before feeding and samples were collected between 0730 h and 1130 h on day 0 and day 83. Pens were processed in the same order each day. An electronic time stamp was recorded for each animal when they were