Function of the corpus luteum in beef heifers is affected by acute submaintenance feeding but is not correlated with residual feed intake\textsuperscript{1,2}

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\textbf{ABSTRACT:} Seventy-four Angus and Angus × Hereford heifers were used in 2 successive years (yr 1, \(n = 43\); yr 2, \(n = 31\)) to determine if luteal function of heifers during acute submaintenance feeding is related to variation in utilization of feed as determined by residual feed intake (RFI). Residual feed intake was determined for heifers beginning at 12.3 ± 0.1 mo of age in yr 1 and at 9.1 ± 0.1 mo of age in yr 2. Heifers were assigned to dry-lot pens (\(n = 6\) to 9 heifers/pen) with electronic gates to measure individual feed intake of a total mixed ration for 70 and 72 d in yr 1 and 2, respectively. Residual feed intake was calculated as the difference between actual DMI and expected DMI from linear regression of DMI on mid-test BW\textsuperscript{0.75} and ADG. At 14.4 ± 0.1 mo of age, all heifers were provided a restricted amount of feed to supply 40\% of their maintenance energy requirements for 21 d. Estrous cycles of heifers were synchronized with PGF\textsubscript{2α} on d −10, 0, and 11 relative to start of restriction. Concentrations of progesterone in plasma on d 14 to 21 of restriction were used to determine if heifers ovulated. Overall ADG and ADFI were 0.83 ± 0.02 and 7.37 ± 0.67 kg/d, respectively, for yr 1; and 0.50 ± 0.02 and 5.66 ± 0.09 kg/d, respectively, for yr 2. There was no correlation between RFI and BW, ADG, ADFI, or ultrasound measure of backfat, nor was RFI related to concentrations of IGF-I in plasma. All heifers lost BW and had reduced backfat (\(P < 0.001\)) at the end of restricted feeding. All heifers had reproductive cycles before dietary restriction started. During acute nutritional restriction, 4 heifers became anovulatory. Sixteen heifers had concentrations of progesterone in plasma during restricted feeding that were atypical of normal luteal function. There was no relationship between luteal function during nutrient restriction and RFI of heifers. Circulating IGF-I was greater at weaning and after restricted feeding in heifers with a smaller RFI (>0.5 SD below the mean) than heifers with a greater RFI (>0.5 SD above the mean). It is concluded that RFI is not related to luteal function during acute submaintenance feeding, but that short-term restriction of nutrient intake can alter luteal function that may compromise fertility, even in heifers that exhibit estrus and ovulate.

\textbf{Key words:} cattle, corpus luteum, insulin-like growth factor-I, nutrition, reproduction, residual feed intake

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regularly occur (e.g., early lactation or during inclement weather). However, reproductive cycles in mature cattle cease only after prolonged nutrient restriction (Richards et al., 1989; Rhodes et al., 1995; Bossis et al., 1999). Young growing females seem to be more sensitive to the effects of reduced nutrition (Foster et al., 1989; Barb et al., 1997; Amstalden et al., 2000). Mackey et al. (1999, 2000) found that programmatic feeding resulting in acute nutritional restriction (40% of maintenance) for 14 d suppressed follicular growth and induced ovulatory failure in 60% of heifers tested. We hypothesized that those heifers failing to maintain normal estrous cycles had different maintenance requirements than those that maintained normal estrous cycles.

Residual feed intake (RFI) is a measure of feed efficiency that reflects differences among animals in variation of requirements for maintenance and growth (Crews, 2005; Herd and Arthur, 2009). On this basis, we sought to apply RFI as a scientific tool to investigate our hypothesis that the ovarian function of beef heifers during acute submaintenance feeding is related to their ability to efficiently meet their requirements for maintenance. Thus, we combined RFI with a strategy for reducing nutrient intake of heifers to create a unique 2-step feeding protocol with which to test our hypothesis. Our goal was not to study RFI per se, but to use it as a tool to determine whether feed efficiency is associated with luteal function during acute reduction in intake of nutrients.

**MATERIALS AND METHODS**

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of Georgia and were conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.

**RFI**

Forty-three Angus and Angus × Hereford heifers (beginning at 12.3 ± 0.1 mo of age; 316 ± 5 kg of BW) were used in yr 1. Heifers were blocked by BW and assigned to pens (n = 5 pens with 8 to 9 heifers/pen) equipped with electronic gates (American Calan Inc., Northwood, NH). After a 2-wk adaptation period, heifers were individually fed a total mixed ration (12.6% CP, 1.86 Mcal/kg of NE\textsubscript{m}, 1.3 Mcal/kg of NE\textsubscript{g}) for 70 d at 2.2% of BW (RFI feeding period). Feed was offered twice daily in 2 equal parts at 0800 and 1600 h daily and was adjusted at mid-test to maintain a targeted ADG of 0.68 kg of BW/d. In yr 2, 31 heifers (beginning at 9.1 ± 0.1 mo of age; 263 ± 4 kg of BW) were blocked by BW and assigned to pens (n = 4 pens with 6 to 8 heifers per pen) with electronic gates as before. After a 2-wk adaptation period, heifers were individually fed a total mixed ration (12% CP, 1.5 Mcal/kg of NE\textsubscript{m}, 0.8 Mcal/kg of NE\textsubscript{g}) for 72 d at 2.0% of BW. Feed was offered in 2 equal parts at 0800 and 1600 h daily and was adjusted at mid-test to maintain a targeted ADG of 0.5 kg of BW/d. The ADG of heifers was reduced in yr 2 so that they would not be as fat at the start of restricted feeding. Body weights were determined as in yr 1. Animals were maintained in these dry-lot pens for the duration of the experiment in each year.

**Restricted Feeding**

At 14.4 ± 0.1 mo of age, all heifers were fed a restricted amount of feed for 21 d (restricted feeding period). For each heifer, BW\textsuperscript{0.75} and the calculated NE\textsubscript{m} of the diet were used to determine the amount of feed necessary to provide the maintenance energy requirement of each animal (NRC, 1996). The amount of feed was then adjusted for each individual heifer to provide 40% of her individual maintenance energy requirement for 21 d, which ranged from 0.6 to 0.8 kg/d. In yr 1, this occurred immediately after the RFI feeding period (d 71). In yr 2, the RFI feeding period occurred earlier to separate it from the start of restricted feeding. This allowed standardization of feed intake for all heifers at 120% of their individual maintenance energy requirement for a 2-wk adaptation period before the start of restricted feeding. Feed was offered twice daily during restriction in 2 equal parts, and BW was determined twice weekly. Because the objective of the experiment was to determine whether luteal function during submaintenance feeding was associated with a phenotype for RFI, no animals were fed above maintenance during this time because it was not reasonably expected that feeding above maintenance would have any negative consequence for ovarian function.

**Ultrasound Carcass Measures**

Ultrasonic measurements for each heifer were taken on the left side, and measurements included backfat thickness at 12th rib, area of LM at the 12th rib, and intramuscular fat of the LM at the beginning, middle, and end of the RFI feeding period, and again at the beginning and end of the restricted feeding period. A wave guide was used to ensure proper fit for images collected of fat thickness and LM area. Images were collected using an Aloka 500V imaging system equipped with a 3.5-MHz, 17.2-cm linear array transducer (Corometrics Medical Systems Inc., Wallingford, CT). Ultrasound images were evaluated using Beef Information Manager software (Critical Vision Inc., Atlanta, GA).

**Synchronization of Estrous Cycles**

All heifers received PGF\textsubscript{2α} (25 mg intramuscularly, Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI)
10 d before the start of restricted feeding. On the day that restricted feeding began, and then again 11 d later, heifers received an injection of PGF$_{2\alpha}$ to induce regression of the corpus luteum (CL) and provide the appropriate endocrine environment for ovulation of the first dominant follicle that developed during restriction. Observations for estrus were not recorded for these animals, so the patterns of luteal activity, as defined by plasma concentrations of progesterone, are reported.

**Blood Sampling and Assays**

A jugular blood sample was collected from each heifer at weaning. Blood samples were also collected each week during the residual feeding period, every other day from d 0 to 11 of restricted feeding, and daily thereafter until the end of the experiment, via coccygeal venipuncture. In yr 2, weekly blood samples were collected between the RFI and restricted feeding period to access the cyclic status of heifers. Blood samples (10 mL) were collected into tubes containing EDTA and placed on ice. Plasma was obtained by centrifugation (2,500 × g for 20 min at 4°C) and stored at −20°C until analyzed for hormones and metabolites.

Concentrations of progesterone in plasma were quantified with a solid-phase RIA (Siemens Healthcare Diagnostics, Tarrytown, NY), which has been validated for use with bovine plasma (Vizcarra et al., 1997). The addition of 5 ng of progesterone to 1 mL of bovine plasma resulted in 108% recovery (n = 10). When different volumes of bovine plasma were assayed, concentrations were parallel to the standard curve. Sensitivity of the assay was 0.1 ng/mL. Inter- and intraassay CV were 11.3 and 5.6%, respectively.

Plasma concentrations of IGF-I were quantified with RIA as described and validated by Bilby et al. (1999). The final primary antibody was diluted 1:120,000, and the goat-anti-rabbit secondary antibody was diluted 1:60. The IGF-I antibody used was anti-hIGF-I (AFP4892898, A. F. Parlow, National Hormone and Peptide Program, Torrance, CA). Unknown concentrations of IGF-I were calculated using Assay Zap software (Biosoft, Cambridge, UK) utilizing counts per minute obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Inter- and intraassay CV were 5.0 and 5.7%, respectively.

Concentrations of NEFA in plasma were determined with an enzymatic colorimetric procedure (NEFA-HR2, Wako Chemicals Inc., Dallas, TX) with modification for a microplate. Briefly, reagent A and reagent B were diluted 1:2.3 and 1:1.3, respectively, with 0.05 M phosphate buffer. Standards and control serum were added (10 μL per well) in triplicate and unknown samples ran in duplicate. Reagent A was added (100 μL per well), and plates were incubated at room temperature (30 min) after shaking. Reagent B was then added (200 μL per well), mixed by shaking, and incubated for another 30 min at room temperature. Absorbance (550 nm) was measured with a microplate reader (μQuant, BioTek Instruments, Inc., Winooski, VT). Unknown concentrations were calculated using Gen5 software (BioTek Instruments) and reported as microequivalents per liter (μEq/L). When 300 μEq of oleic acid were added to a pooled bovine plasma sample containing 125 μEq/L, 91.5% was recovered (n = 10). Samples were run in a single assay. Two pools of bovine plasma with NEFA concentrations of 156 and 790 μEq/L were included in duplicate on each assay plate (n = 6); these had intraassay CV of 8.1 and 5.2%, respectively.

**Statistical Analyses**

Residual feed intake was calculated as the difference between actual DMI and expected DMI from the linear regression of DMI on mid-test BW$^{0.75}$ and ADG (Koch et al., 1963). Data from 1 animal were removed because, for unknown reasons, the RFI calculation for that heifer was not sufficiently accurate, as indicated by a poor fit of the regression model (defined as R$^2$ < 0.80). Data from an additional 6 heifers were removed because actual feed intake was unknown due to failure of the Calan gates to control animal access to feed bunks in a single pen during yr 2. Simple correlation analysis (PROC CORR, SAS Inst. Inc., Cary, NC) was used to determine linear relationships of RFI with ADG, ADFI, BW, and ultrasound carcass measures. Linear regression was used to determine the relationship of RFI with concentrations of IGF-I in plasma at weaning, during the RFI feeding period, and during submaintenance feeding. Data from each year were analyzed separately for these analyses.

Heifers were classified as having a high (>0.5 SD above the mean, n = 22) or low (>0.5 SD below the mean, n = 16) RFI. Differences in performance measures during restriction and concentrations of IGF-I of high and low RFI heifers during the RFI and restricted feeding periods were determined using a general linear model with repeated measures using the MIXED procedure of SAS. The model included year and the interaction of year with RFI classification. Because animal was the experimental unit, animal nested within RFI classification was used as the error term to test RFI effects, whereas the pooled residual was used as the error term to test the split-unit factor (day). The within-animal covariance structure was compound symmetric, and the degrees of freedom for the pooled error term were calculated using the Satterthwaite approximation. Heifers were defined to have ovulated if they had concentrations of progesterone ≥1 ng/mL in plasma on d 14 to 21 of restriction. Chi-squared distribution was used to compare the number of heifers that failed to ovulate in response to restricted feeding.

Data are reported as means ± SE. Significance was defined as P ≤ 0.05, and a tendency was defined as P ≤ 0.1 but >0.05.
RESULTS

RFI Feeding Period

Body weight increased (P < 0.01) during the RFI feeding period as heifers gained an average of 0.83 ± 0.02 and 0.50 ± 0.02 kg of BW/d in yr 1 and 2, respectively. Fat thickness increased (P < 0.05) in a linear fashion during the RFI feeding period in yr 1, with similar results in yr 2 (data not shown). There was a similar day effect (P < 0.05) on LM area (data not shown). Overall means for ADFI and G:F were 7.37 ± 0.10 kg/d and 99.3 ± 2.2 g/kg of DM, respectively, in yr 1. In yr 2, ADFI and G:F were 5.66 ± 0.09 kg/d and 89.9 ± 3.4 g/kg of DM, respectively. Differences (P < 0.01) between years in BW, LM area, fat thickness, and intramuscular fat were expected (data not shown) because heifers were younger at the start of the RFI feeding period in yr 2. Likewise, because the targeted rate of BW gain of heifers was less in yr 2 than in yr 1, differences (P < 0.001) in ADG, ADFI, and G:F were observed as expected. Residual feed intake was not correlated with BW at the start, middle, or end of the RFI feeding period (r < 0.036, P > 0.77). Similarly, RFI was not correlated with LM area, fat thickness, or intramuscular fat at these time points (r < 0.25, P > 0.35). There was no correlation of RFI with ADG (r = −0.003, P = 0.99), ADFI (r = 0.07, P = 0.59), or G:F (r = −0.21, P = 0.10).

In each year, RFI values of low RFI heifers (yr 1, n = 9; yr 2, n = 7) were less (P < 0.001) than for high RFI heifers (yr 1, n = 14; yr 2, n = 8). However, the magnitude of this difference was greater (P < 0.05) in yr 1 (0.083 ± 0.009 vs. −0.113 ± 0.013 for high and low RFI heifers, respectively) when compared with yr 2 (0.053 ± 0.013 vs. −0.062 ± 0.012 for high and low RFI heifers, respectively). Within year, LM area, fat thickness, ADG, and ADFI variables were not different between high and low RFI heifers.

Restricted Feeding Period

There was no effect of year or year x day interaction on BW or fat thickness during restricted feeding. Body weight and fat thickness were greater (P < 0.001) at the start of restricted feeding than at the end (371.7 ± 4.0 vs. 324.6 ± 3.5 kg and 0.61 ± 0.01 vs. 0.46 ± 0.02 cm for BW and fat thickness, respectively). Overall, heifers in yr 1 had smaller LM area with less intramuscular fat than heifers in yr 2 (P < 0.001). In yr 1, heifers had greater (P < 0.05) LM area at the start of restriction than at the end (54.35 ± 0.69 vs. 52.01 ± 0.86, respectively), whereas in yr 2, LM area only tended (P = 0.07) to be less at the end of restriction (data not shown). Heifers had less (P < 0.05) intramuscular fat at the start of restricted feeding in yr 1 when compared with intramuscular fat of heifers in yr 2 (3.30 ± 0.16 vs. 3.77 ± 0.17%, respectively). At the end of the restricted feeding period, intramuscular fat was similar for all heifers and was independent of year. There were no differences in BW, fat thickness, LM area, or intramuscular fat between high and low RFI heifers either at the start or end of the restricted feeding period (Table 1).

All heifers were pubertal, having demonstrated a luteal phase of normal duration, before d −10 of restricted feeding. Plasma concentrations of progesterone (≥1 ng/mL) on d 14 to 21 of restriction were used to classify the luteal function of heifers during restricted feeding. Data from 67 animals were available for analysis (Table 2). Sixty of these animals had a CL on d 11 of restriction that was capable of responding to PGF2α, and the profile of plasma progesterone in these heifers is shown in Figure 1. Of the 67 animals, 4 heifers were classified as anovulatory, whereas 53 heifers were classified as having ovulated. Of the 53 heifers that ovulated, 6 formed a CL with a short lifespan after PGF2α on d 0 of restriction. The ovulatory response to restricted feeding in 10 of 67 heifers was unclassified because of abnormal CL function or development. In 3 of these 10 heifers, the CL regressed after PGF2α on d 0 of restriction, but formation of a new functional CL (progesterone ≥1 ng/mL) was delayed, and consequently, it was not responsive to PGF2α on d 11 of restriction. By d 12 of restriction, all 3 of these heifers had concentrations of progesterone greater than 1 ng/mL, and remained elevated for the duration of the study. Two of the 10 unclassified heifers were considered to have formed a short-lived CL after PGF2α on d 0 of restriction because progesterone concentrations fell below 1 ng/mL.

Table 1. Mean BW and live animal ultrasound measures for fat thickness, LM area, and intramuscular fat of the LM at the start and end of the restricted feeding period for heifers with high or low residual feed intake (RFI).^1

<table>
<thead>
<tr>
<th>Item</th>
<th>High^2</th>
<th>Low^2</th>
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<tbody>
<tr>
<td>n</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>BW, kg</td>
<td>374.5 ± 7.8</td>
<td>369.1 ± 9.0</td>
</tr>
<tr>
<td></td>
<td>327.6 ± 6.8</td>
<td>320.9 ± 8.0</td>
</tr>
<tr>
<td>Fat thickness, cm</td>
<td>0.64 ± 0.04</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.48 ± 0.04</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>LM area, cm^2</td>
<td>56.6 ± 1.3</td>
<td>58.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>57.2 ± 2.0</td>
<td>56.6 ± 2.3</td>
</tr>
<tr>
<td>Intramuscular fat, %</td>
<td>3.63 ± 0.21</td>
<td>3.27 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>3.75 ± 0.20</td>
<td>3.81 ± 0.23</td>
</tr>
</tbody>
</table>

^1Animals were fed 40% of their maintenance energy requirement for 21 d.
^2High and low residual feed intake are ≥0.5 SD above and below the mean, respectively.
^3Ultrasound fat thickness at the 10th rib.
^4Ultrasound LM area at the 10th rib.
^5Ultrasound intramuscular fat of the LM at the 10th rib.
before PGF$_{2\alpha}$ on d 11. Each of these heifers formed a subsequent CL by d 16 of restriction, and plasma concentrations of progesterone remained increased for the duration of the experiment. In 2 more of the unclassified heifers, the CL failed to regress after PGF$_{2\alpha}$ on d 11 of restriction. Finally, 3 of the 10 unclassified heifers had concentrations of progesterone in plasma from d 14 to 21 of restriction that reached the predetermined cutoff level. However, in contrast to the characteristic continued increase in progesterone concentrations seen in heifers that ovulated, progesterone concentrations in these heifers remained low (1.78 ± 0.18 ng/mL) during this time (Figure 1). This was interpreted as an indication of the possible presence of a luteinized follicle, but this was not confirmed with ultrasound examination.

There was no difference in the proportion of high or low RFI heifers that ovulated ($\chi^2 = 0.259, P = 0.88$).

For high RFI heifers, 18 of 22 heifers ovulated, 1 failed to ovulate, and 3 were unclassified. For low RFI heifers, 12 of 16 ovulated, 1 failed to ovulate, and 2 were unclassified.

**Blood Metabolites**

When data from all heifers were included in the analysis, there were differences between years in concentrations of IGF-I in plasma. In yr 1, circulating concentrations of IGF-I tended ($P = 0.07$) to be less at weaning but were increased ($P < 0.05$) at the start of the RFI feeding period when compared with yr 2 (Table 3). There was no effect of year on concentrations of IGF-I in plasma at the middle or end of RFI feeding, nor at the start of restricted feeding. However, at the end of restricted feeding, concentrations of IGF-I in plasma were again less ($P < 0.001$) in yr 1 than in yr 2. When data from all heifers were evaluated with linear regression, there was no relationship of RFI with concentrations of IGF-I in plasma at weaning, during the RFI feeding period, or during submaintenance feeding (data not shown). Concentrations of IGF-I in plasma at weaning were greater ($P < 0.05$) in low-RFI than high-RFI heifers (Table 4). Concentrations of IGF-I in heifers ranked as either high or low RFI were less at the end of restricted feeding ($P < 0.001$) in yr 1 than in yr 2 (Table 4), but there was no interaction of year with RFI rank. Overall, concentrations of IGF-I at the end of restricted feeding were similar to those observed at weaning, in that low-RFI heifers tended ($P = 0.06$) to have greater circulating IGF-I than high-RFI heifers (Table 4).

Concentrations of NEFA in plasma were increased ($P < 0.001$) by d 2 of restriction and remained increased for the duration of submaintenance feeding (Table 5). In contrast, concentrations of IGF-I in plasma were reduced ($P < 0.001$) beginning on d 2 of restriction and remained suppressed throughout the remainder of restricted feeding (Table 5). There was no difference in NEFA concentrations during restriction for high-RFI compared with low-RFI heifers.

**DISCUSSION**

The main finding of this experiment is that acute submaintenance feeding did not suppress ovulation in heifers but may have compromised function of the CL, which has important consequences for fertility. This was not, however, associated with RFI of the animal. Previous reports are that RFI is not correlated with ADG and BW in growing male cattle (Herd and Bishop, 2000; Arthur et al., 2001; Nkrumah et al., 2007). The current experiment used fewer animals, but data in these limit-fed replacement beef heifers are consistent with this and confirm other reports in growing female cattle (Kelly et al., 2010a; Shaffer et al., 2011). Heifers were 3 mo younger during the RFI feeding period in yr 2, but this likely had little impact on classification of heifers. Positive phenotypic correlations have been reported for RFI of heifers between the postweaning period and later growth periods (Archer et al., 2002; Loyd et al., 2011). Kelly et al. (2010b) reported that

**Table 2.** Number of heifers that ovulated by d 21 of restricted feeding (RST) and luteal activity (LA) of anovulatory heifers throughout RST$^{1,2}$

<table>
<thead>
<tr>
<th>Item</th>
<th>Ovulated by d 21 of RST</th>
<th>Unclassified</th>
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</thead>
<tbody>
<tr>
<td>Animals, n</td>
<td>4</td>
<td>53</td>
</tr>
<tr>
<td>Duration of LA after PGF$_{2\alpha}$ on d 11 of RST</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal, n</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Short, n</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Delayed formation of CL$^3$ after PGF$_{2\alpha}$ on d 0 of RST, n</td>
<td>3</td>
<td></td>
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<tr>
<td>CL formed after PGF$<em>{2\alpha}$ on d 0 of RST, but regressed before PGF$</em>{2\alpha}$ on d 11, n</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Failed to regress CL after PGF$_{2\alpha}$ on d 11 of RST, n</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Possible luteinized follicle,$^4$ n</td>
<td>3</td>
<td></td>
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</table>

$^1$Heifers fed 40% of their maintenance energy requirement for 21 d.

$^2$Progesterone ≥1 ng/mL in serum indicated normal luteal function and was used (d 14 to 21 of RST) to determine if heifers ovulated in response to PGF$_{2\alpha}$, on d 11 of RST.

$^3$CL = corpus luteum.

$^4$Indicated by failure of plasma progesterone to increase above 1.78 ± 0.18 ng/mL on d 14 to 21 of RST.
RFI rank of heifers between the growth and finishing phases was repeatable.

Because these heifers were limit fed during the RFI feeding period, they could not express their genetic potential for growth or appetite. This was by design because the objective was for RFI to be more reflective of variation in maintenance than growth. As a consequence, variation in RFI was reduced. Despite this, the phenotypic relationship of RFI with ADG and BW described here is in agreement with that reported by Roberts et al. (2007). Roberts et al. (2007) found that this relationship is similar for limit-fed heifers compared with heifers fed ad libitum, which further supports the expectation that heifers are correctly classified in the current experiment. Appetite is a biological process that contributes to variation in RFI (Richardson and Herd, 2004). These data further provide evidence that limit feeding cattle may be useful in determining the amount of variation in RFI associated with other biological processes beyond appetite.

Residual feed intake is reported to be genetically and phenotypically correlated with traits for body fatness in heifers (Arthur et al., 2001; Lancaster et al., 2009). No correlation between RFI and ultrasound measures of LM area or backfat was observed, and this is in agreement with other reports for heifers (Roberts et al., 2007; Kelly et al., 2010b). Shaffer et al. (2011) reported that ultrasound measures of rib and rump fat were positively correlated to RFI in yearling heifers of the British breed type because heifers with low RFI were less fat than those with high RFI. Shaffer et al. (2011) also reported a positive association of RFI with LM area, but only when LM area was expressed as a ratio with BW. Our heifers, although of a similar breed type, were growing at a slower rate than those used by Shaffer et al. (2011), which likely accounts for the reported differences.

Acute restriction of nutrients failed to inhibit ovulation in the majority of beef heifers, and no association with RFI can therefore be drawn. This is important because it illustrates that acute submaintenance feeding of heifers that have been phenotyped for RFI is likely not a reliable approach to study how maintenance requirements of heifers are related to ovulatory response during restricted feeding. It may be that this degree of submaintenance feeding is insufficient to inhibit ovulation in 1 follicular cycle. However, Mackey et al. (1999) reported that ovulation was suppressed in 60% of what were described as yearling heifers when submitted to a similar protocol of acute nutritional restriction as used in this study. Differences in body composition of animals may explain divergent results. Although heifers lost fat during restriction, the net loss was not great enough to inhibit reproductive cycles. Using the same restricted feeding protocol, White et al. (2000) found that 40% of heifers failed to ovulate. Animals in that study were approximately 30 d younger than heifers in the current experiment. It may be that sensitivity of heifers to this level of acute restriction is dependent upon age. Alternatively, the response of heifers to acute nutritional restriction may not necessarily be dependent on age or a particular amount of body fat, but may be more a function of growth and composition of BW gain before the start of restriction. Growth rate of heifers was limited, but was in line with other studies where reproductive performance was not adversely affected (Freetly et al., 2001; Funston and Deutscher, 2004). Limiting growth rate alters the composition of

### Table 3.

Concentrations of IGF-I (least squares means, ng/mL) in plasma of all heifers in each year at weaning, during the residual feed intake (RFI) feeding period, and during restricted feeding (RST)\(^1\)

<table>
<thead>
<tr>
<th>Measured at</th>
<th>Yr 1</th>
<th>SE</th>
<th>Yr 2</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning</td>
<td>76.8</td>
<td>3.6</td>
<td>88.0</td>
<td>4.8</td>
<td>0.07</td>
</tr>
<tr>
<td>RFI, d 0</td>
<td>108.0</td>
<td>3.1</td>
<td>96.5</td>
<td>4.0</td>
<td>0.03</td>
</tr>
<tr>
<td>RFI, d 35</td>
<td>111.6</td>
<td>5.2</td>
<td>104.2</td>
<td>7.7</td>
<td>0.43</td>
</tr>
<tr>
<td>RFI, d 70</td>
<td>116.6</td>
<td>4.0</td>
<td>125.8</td>
<td>5.6</td>
<td>0.19</td>
</tr>
<tr>
<td>RST, d 0</td>
<td>116.6</td>
<td>3.6</td>
<td>124.1</td>
<td>4.7</td>
<td>0.21</td>
</tr>
<tr>
<td>RST, d 20</td>
<td>81.9</td>
<td>2.7</td>
<td>113.0</td>
<td>3.5</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

\(^1\)Heifers fed 40% of their maintenance energy requirement for 21 d.

### Table 4.

Concentrations of IGF-I (least squares means, ng/mL) in plasma at weaning, at the start, and at the end of the restricted (RST) feeding period for heifers that were ranked as having high or low residual feed intake (RFI) in each year\(^1,2\)

<table>
<thead>
<tr>
<th>Measured at</th>
<th>Yr 1 High</th>
<th>Yr 1 Low</th>
<th>SEM</th>
<th>Yr 2 High</th>
<th>Yr 2 Low</th>
<th>SEM</th>
<th>Year</th>
<th>Rank</th>
<th>Year × rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weaning</td>
<td>69.4</td>
<td>86.3</td>
<td>6.2</td>
<td>79.8</td>
<td>95.9</td>
<td>8.1</td>
<td>0.18</td>
<td>0.03</td>
<td>0.96</td>
</tr>
<tr>
<td>RST, d 0</td>
<td>114.2</td>
<td>119.9</td>
<td>8.1</td>
<td>133.6</td>
<td>114.7</td>
<td>9.2</td>
<td>0.41</td>
<td>0.44</td>
<td>0.15</td>
</tr>
<tr>
<td>RST, d 20</td>
<td>78.6</td>
<td>90.9</td>
<td>5.4</td>
<td>109.2</td>
<td>121.2</td>
<td>6.6</td>
<td>0.001</td>
<td>0.06</td>
<td>0.99</td>
</tr>
</tbody>
</table>

\(^1\)High and low RFI heifers had an RFI value \(\geq 0.5\) SD above and below the mean, respectively.

\(^2\)Heifers fed 40% of their maintenance energy requirement for 21 d.
BW gain in favor of lean tissue growth and reduces organ mass (Burrin et al., 1990). The overall effect is a reduction in maintenance requirements and an increase in efficiency, which is reflected in reduced variation of RFI among growth-restricted heifers (Roberts et al., 2007). Consequently, heifers in the current study, regardless of their RFI rank, may have been better able to cope with acute nutrient restriction than heifers in the experiments of White et al. (2000) or Mackey et al. (1999) because they were more efficient as a result of limit feeding beforehand.

In addition to the 4 heifers that demonstrated ovulatory failure during restriction, plasma concentrations of progesterone that were atypical of normal luteal function were observed in 16 of the 67 heifers. This altered luteal function was not related to the RFI of the animal. Appropriate caution should be exercised when interpreting this result because animals that were not on a restricted diet were not included in this study. However, feeding heifers above their maintenance requirement has no apparent detrimental effect on luteal function (Mackey et al., 1999, 2000; White et al., 2000).

Mackey et al. (1999, 2000) reported that growth rate and size of the dominant follicle that developed during acute restriction was reduced compared with that of animals eating above their maintenance energy requirements. Compromises in luteal function observed in this study may have been a consequence of altered follicular development during nutrient restriction. Circulating concentrations of progesterone are related to size of the ovulatory follicle and the CL that subsequently forms after ovulation (Vasconcelos et al., 2001; Echternkamp et al., 2009). Echternkamp et al. (2010) reported that reducing dietary intake of energy did not affect size of the ovulatory follicle or the resulting CL of replacement beef heifers, but observed that serum concentrations of progesterone in those heifers with limited energy intake were reduced. This indicates that reduced energy intake could affect some aspect of the function of the CL. Consequently, it cannot be ruled out that compromises in luteal function of heifers observed in this study may have been a direct result of the nutritional restriction imposed. The implication of this is that acute limitations in nutrient intake that can occur in production cycles (e.g., negative energy balance associated with lactation or inclement weather) can alter reproductive function.

**Table 5.** Concentrations of NEFA (μEq/L) and IGF-I (ng/mL) in plasma of heifer during restricted feeding

<table>
<thead>
<tr>
<th>Day</th>
<th>NEFA</th>
<th>SE</th>
<th>IGF-I</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>145.4a</td>
<td>8.9</td>
<td>119.4a</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>531.7b</td>
<td>31.2</td>
<td>94.5 b</td>
<td>2.6</td>
</tr>
<tr>
<td>4</td>
<td>783.8b</td>
<td>37.6</td>
<td>97.3 b</td>
<td>4.1</td>
</tr>
<tr>
<td>8</td>
<td>804.4b</td>
<td>44.9</td>
<td>101.4b</td>
<td>4.4</td>
</tr>
<tr>
<td>12</td>
<td>1,020.6b</td>
<td>51.2</td>
<td>100.2b</td>
<td>5.1</td>
</tr>
<tr>
<td>20</td>
<td>800.2b</td>
<td>44.2</td>
<td>93.5b</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*a,b*Means within a column without a common superscript differ \((P < 0.01)\).

1Heifers fed 40% of their maintenance energy requirement for 21 d.

**Figure 1.** Concentrations of progesterone in plasma of heifers that had a corpus luteum and that responded to injection of PGF2α on d 11 of restriction. Heifers were restricted to 40% of their maintenance energy requirements for 21 d. Heifers were defined ovulatory if they had concentrations of progesterone ≥1 ng/mL in plasma on d 14 to 21 of restriction. If progesterone decreased to <1 ng/mL before d 21, then heifers were considered to have short-cycled. Concentrations of progesterone in 3 heifers remained suppressed (1.78 ± 0.18 ng/mL) during this time, which was interpreted as indicating the possible presence of a luteinized follicle.
function and may compromise fertility, even in heifers that display estrus and ovulate.

In this study there was no phenotypic relationship between concentrations of IGF-I and RFI when all animals were included in the analysis, but there were observed greater plasma concentrations of IGF-I at weaning in low-RFI heifers. Interestingly, at the end of the restricted feeding period, low-RFI heifers again had greater concentrations of IGF-I in plasma than high-RFI heifers. Circulating concentrations of IGF-I are positively associated with RFI in pigs (Bunter et al., 2010) and cattle (Moore et al., 2005). Others report that the relationship between RFI and IGF-I in heifers becomes more negative with advancing age (Lancaster et al., 2008). Furthermore, heifers with low RFI reach puberty at a later age (Shaffer et al., 2011) and consequently give birth later in the calving season (Arthur et al., 2005). Given that circulating concentrations of IGF-I are positively correlated with initiation or resumption of estrous cycles (Roberts et al., 1997; Stagg et al., 1998; Lents et al., 2008), it seems reasonable to speculate that RFI might have impacts on reproductive function via the IGF-I pathway. Unraveling the complex relationships between RFI, reproduction, and IGF-I is made difficult because IGF-I binding proteins play a critical role in mediating the action of IGF-I at the tissue level (Simmen et al., 1998; Velazquez et al., 2008).

The observation that the function of the CL during acute nutritional restriction in heifers is compromised implies that acute periods of negative energy balance, which regularly occur in production scenarios, can alter ovarian function. This may compromise fertility, even in heifers that exhibit estrus and ovulate. Further research is needed to determine how acute reduction in nutrient availability alters the function of the CL of heifers and what consequence this may have for fertility. Given the importance of IGF-I in the control of reproductive function, the finding that blood concentrations of IGF-I were associated with RFI, depending when measurements were made, indicates that selection for RFI could possibly affect reproductive performance of cattle through this hormonal pathway. It will be important going forward to determine if and how this might occur. Finally, it can be concluded that differences in RFI are not related to the function of the CL of heifers during acute nutrient restriction. The relationship between RFI and follicle development during submaintenance feeding, however, remains to be elucidated.

**LITERATURE CITED**


