Dietary protein during gestation affects maternal insulin-like growth factor, insulin-like growth factor binding protein, leptin concentrations, and fetal growth in heifers\textsuperscript{1}

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INTRODUCTION

Fetal growth is a complex, dynamic process controlled by various maternal and fetal factors. Insulin-like growth factors are polypeptide hormones produced by the placenta and by maternal and fetal tissues (Gicquel and Le Bouc, 2006). The maternal IGF system can modulate placental growth and functional development, the delivery of substrates to the fetus, the partitioning of nutrients between maternal tissues and the conceptus in rodents, guinea pigs, and sheep, and size of fetal guinea pigs at birth (Sferruzzi-Perrini et al., 2007). The IGFBP control the biological activities of IGF by modulating IGF receptor interaction and by increasing the half-life of circulating IGF (Baxter, 1993). The IGF system is regulated by many factors, such as nutritional protein (Perry et al., 2002) and endocrine signals (Fowden, 2003) including bovine placental lactogen.
(bPL), which has stimulatory effects on the maternal IGF system in cattle (Weber et al., 2007). Another peptide hormone, leptin, is secreted by white adipose cells and is an important link between metabolic status and the neuroendocrine system, with nutrient intake causing major alterations in plasma leptin concentrations in cattle (Amstalden et al., 2000). Plasma leptin also varies with BCS in cattle (Lents et al., 2005), estrogen abundance in humans (Chardonnens et al., 1999), and among cattle genotypes (Ren et al., 2002). One role proposed for leptin during pregnancy is the partitioning of nutrients during the development of the feto-placental unit, explaining why associations between maternal leptin and birth weight were detected in sheep (Thomas et al., 2001).

There have been few studies on the impact of maternal nutrition on fetal growth in cattle, particularly the level of dietary protein. Despite Australian pastures typically containing sufficient energy, they are deficient in protein (Norman, 1963) with 76% of northern Australian beef operations requiring protein supplementation of their replacement beef heifers (Bortolussi et al., 2005). We tested the hypothesis that, in heifers, circulating concentrations of the IGF hormones would be correlated with growth of the calf in utero and affected by dietary protein, as well as by genotype and bPL. We also hypothesized that leptin would be correlated with growth of the calf in utero and affected by dietary protein, as well as by estrone sulfate (ES) and BCS.

MATERIALS AND METHODS

The project was approved by The University of Queensland Animal Ethics Committee.

Project Animals, Management, and Treatments

On feedlot premises located on an extensive grazing property in southwest Queensland, 120 composite beef breed, 23.2 ± 0.14 mo old (range 21.6 to 24.6 mo) heifers were acclimatized for a period of 45 d to their environment, a maintenance diet, and individual stall feeding and management. The heifers were then synchronized for AI using a 10-d progesterone-based estrous synchronization program. Progesterone-releasing devices (Eazi-breed CIDR Cattle Device, Pfizer Animal Health, West Ryde, New South Wales, Australia, 1.9 g intravaginally) and estradiol benzoate (Ciderol, Ge- netics Australia, Bacchus Marsh, Victoria, Australia, 1 mg intramuscularly) were administered on d −12 followed by PG (Lutalyse, Pfizer Animal Health, 25 mg intramuscularly) on d −5. Intravaginal devices were removed on d −2, and heifers were AI with semen from 1 Senepol bull on d 0 and again on d 1 for any heifers still showing signs of estrus (n = 6). The frozen semen straws used were thawed for 30 s in 37°C water before AI. With respect to genotype composition, BeefX (n = 18) were 1/2 Senepol, 1/4 Brahman, 1/8 Charolais, and 1/8 Red Angus; and CBX (n = 53) were 1/2 Senepol, 1/4 Brahman, and 1/4 Charolais.

The study utilized a 2 × 2 factorial arrangement of treatments. Heifers were divided into treatment groups at AI according to stratification by BW and genotype, and individual stall feeding was continued until parturition. The 4 treatment groups determined the level of CP fed to each heifer for first and second trimesters of gestation [i.e., high high (HH = high level of CP for first and second trimesters), high low (HL = high level of CP for first trimester and low level of CP for second trimester), low high (lowH = low level of CP for first trimester and high level of CP for second trimester), and low low (LL = low level of CP for first and second trimesters)]. First trimester was defined as d 1 to 92 and second trimester from d 93 to 179. For third trimester (d 180 to parturition) all heifers were fed at the same level of CP. Details on composition of rations and timing of ration changes are presented in Table 1. Of the 120 animals, 2 were removed for temperament, 41 because they were found to be not pregnant at 39 d postinsemination, and 6 because they aborted. This left a total of 71 animals distributed across treatment groups at calving as follows: HH = 16, HL = 19, lowH = 17, and LL = 19.

Concentrate feed rations were individually fed every morning while the heifers were in 1 × 3 m stanchions and consisted of cottonseed meal (Gossypium spp.), cracked sorghum seed (Sorghum spp.), ground limestone, and a vitamin and mineral premix. The concentrate components of the diet were consumed within 20 min, and the heifers were then returned to their respective treatment group pens (50 × 28 m). Long stem Bambatsi (Panicum coloratum) hay or barley (Hordeum spp.) straw rations were fed every evening on a treatment group basis for welfare reasons. Allocation of the roughage and concentrate components of the rations was calculated on an average intake per heifer per day basis. The nutritional content of the cottonseed meal was analyzed using wet chemistry, and all other feeds were analyzed by near infrared spectrophotometer, both by CASCO Agritech, Toowoomba, Queensland, Australia (Table 1). The rations were compared with NRC (1996) dietary recommendations for nutrient requirements of pregnant Brangus replacement heifers that were 23 mo at breeding with a mature weight of 475 kg and a calf birth weight of 32 kg (Table 1).

Heifers were weighed monthly during gestation, and heifer BCS was assessed monthly after d 124 of gestation by a single technician on a 1 to 5 scale as described by Graham (2006). Heifer age at AI and gestation length were recorded. Calf birth weight and crown rump length (CRL) at calving were measured, as previously reported in preliminary communications (Micke et al., 2009). Crown rump length was measured from the tip of the calf nose to the base of the tail (Rakha and Igboeli, 1971).
Maternal Blood Sampling and Hormone Assays

Blood was collected 14 d before AI (d −14) and monthly during gestation by tail venipuncture of the vena caudalis mediana directly into 10-mL lithium heparin vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) using 1.2 mm × 38 mm needles. Vacutainer tubes were gently rotated by hand for 5 to 10 s, labeled, and stored on ice for 1 to 2 h before centrifugation (Hettich Universal Zentrifugen, Tuttlingen, Germany) at 3,000 × g for 10 min at room temperature, and plasma was harvested. Samples were stored frozen at −22°C until analyzed.

IGF and Total IGFBP. Concentrations of IGF-I and -II and total IGFBP were measured in heifer plasma at d −14, 28, 82, 179, and 271 of gestation by RIA after separation of IGF and IGFBP by size-exclusion HPLC under acidic conditions, as detailed by Owens et al. (1990). Four fractions of eluate (fraction 1, containing IGFBP; fraction 2, interpeak; fraction 3, containing IGF; and fraction 4, postpeak) were routinely collected for each acidified plasma sample, using collection times based on elution times of 125I-labeled IGF-I and IGF immunoreactivity. Recovery of 125I-IGF-I was 93.3 ± 0.93% for 10 HPLC runs of heifer plasma. Samples were assayed in triplicate in each assay, and all samples from the same animal were extracted in a single HPLC run and run together in the same assay. Plasma IGF-I concentrations were measured by analysis of neutralized HPLC fraction 3, in an RIA specific for IGF-I (Francis et al., 1989), using a rabbit polyclonal antibody to human IGF-I (GroPep, Adelaide, Australia). Total IGFBP concentrations were measured by analysis of neutralized fraction 1 in the same assay. Because IGFBP bind to and sequester 125I-IGF-I in this assay, they can be measured due to their effect of reducing the amount of 125I-IGF-I in the immunoprecipitated pellet, giving an apparent IGF concentration that reflects the total amount and binding affinity of IGFBP present in plasma. Interassay CV for an HPLC eluate fraction 3 pool containing 101.2 ng/mL of IGF-I was 7.1% (n = 15 assays). Covariance for extraction and assay of a pregnant bovine quality control (QC) plasma pool containing 59.8 ng/mL of IGF-I was 14.5%. Plasma IGF-II concentrations were measured by analysis of HPLC fraction 3 in a RIA specific for IGF-II (Carr et al., 1995), using a rabbit polyclonal antibody against human IGF-II (GroPep). The antibody used for human IGF-I and IGF-II have 100 and 85% cross-reactivity with bovine IGF-I and IGF-II, respectively (GroPep Novozymes, product information). Interassay CV for an HPLC eluate fraction 3 pool containing 339 ng/mL of IGF-II was 5.5% (n = 9 assays). Covariance for extraction and assay of a pregnant bovine QC plasma pool containing 296.6 ng/mL of IGF-II was 25.2%. The ratios of IGF-I:total IGFBP and IGF-II:total IGFBP were calculated at each of d −14, 28, 82, 179, and 271.

ES. Plasma ES concentrations at d 28, d 271, and at calving were assayed in duplicate by RIA using kits obtained from Diagnostic Serum Laboratories (Webster, TX). The intraassay CV for all samples was <10%. The interassay CV for low QC values was 4.35% (mean 0.51 ng/mL) and for high QC values was 10.48% (mean 14.38 ng/mL). The sensitivity of the assays was 0.05 ng/mL.

Leptin. Leptin concentrations at d 28, 82, 179, 271, and at calving were measured by RIA as developed by Blache et al. (2000) and subsequently used for bovine samples by Kadokawa et al. (2000). Intraassay CV

<table>
<thead>
<tr>
<th>Item</th>
<th>Trimester 1 (d 1 to 93)</th>
<th>Trimester 2 (d 94 to 180)</th>
<th>Trimester 3 (d 181 to term)</th>
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<tbody>
<tr>
<td></td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Sorghum, kg</td>
<td>0.65</td>
<td>1.56</td>
<td>1.00</td>
</tr>
<tr>
<td>Cottonseed meal, kg</td>
<td>2.45</td>
<td>0.00</td>
<td>2.50</td>
</tr>
<tr>
<td>Bambatsi hay, kg</td>
<td>7.88</td>
<td>2.73</td>
<td>5.79</td>
</tr>
<tr>
<td>Barley straw, kg</td>
<td>0.00</td>
<td>5.14</td>
<td>2.21</td>
</tr>
<tr>
<td>Limestone, kg</td>
<td>0.07</td>
<td>0.02</td>
<td>0.12</td>
</tr>
<tr>
<td>Premix kg</td>
<td>0.07</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>DML kg</td>
<td>9.95</td>
<td>8.64</td>
<td>10.51</td>
</tr>
<tr>
<td>DML kg/kg of BW</td>
<td>27.81</td>
<td>24.67</td>
<td>25.91</td>
</tr>
<tr>
<td>Energy, MJ of ME</td>
<td>76.29</td>
<td>62.54</td>
<td>82.43</td>
</tr>
<tr>
<td>Energy, % NRC</td>
<td>243</td>
<td>199</td>
<td>229</td>
</tr>
<tr>
<td>CP, kg</td>
<td>1.37</td>
<td>0.41</td>
<td>1.40</td>
</tr>
<tr>
<td>CP, % NRC</td>
<td>250</td>
<td>75</td>
<td>228</td>
</tr>
</tbody>
</table>

1Data are presented on as-fed basis per heifer per day.
2Premix containing 17 g of calcium, 9 g of phosphorous, 2.91 g of magnesium, 5 g of sulfur, 27,200 IU of vitamin A, 60 mg of vitamin E, 70 mg of iron, 150 mg of zinc, 100 mg of manganese, 55 mg of copper, 0.5 mg of selenium, 3.4 mg of cobalt, and 4.2 mg of iodine per 100 g.
3Average BW at start of trimester.
4Comparison of ration to NRC (1996) recommended nutrient requirements for pregnant Brangus replacement heifers bred at 23 mo with a mature weight of 475 kg and a calf birth weight of 32 kg.
Table 2. Logarithmic means ± SEM, df, and P-values for significantly different plasma IGF measures by heifer treatment groups during gestation

<table>
<thead>
<tr>
<th>Item</th>
<th>Day of gestation</th>
<th>Treatment group</th>
<th>SEM</th>
<th>df²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HH</td>
<td>HL</td>
<td>HH + HL</td>
<td>LowH</td>
</tr>
<tr>
<td>IGF-I</td>
<td>28</td>
<td>4.735</td>
<td>4.383</td>
<td>4.578</td>
<td>0.307</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>179</td>
<td>4.817</td>
<td>4.082</td>
<td>4.843</td>
<td>3.991</td>
</tr>
<tr>
<td></td>
<td>271</td>
<td>4.317</td>
<td>4.248</td>
<td>4.326</td>
<td>4.322</td>
</tr>
<tr>
<td>IGF-II</td>
<td>82</td>
<td>6.023</td>
<td>5.964</td>
<td>6.014</td>
<td>6.032</td>
</tr>
<tr>
<td>Total IGFBP</td>
<td>82</td>
<td>5.463</td>
<td>5.382</td>
<td>5.631</td>
<td>5.203</td>
</tr>
<tr>
<td></td>
<td>271</td>
<td>5.051</td>
<td>5.116</td>
<td>5.215</td>
<td>4.820</td>
</tr>
<tr>
<td>IGF-I:total IGFBP</td>
<td>28</td>
<td>-0.444</td>
<td></td>
<td>-0.674</td>
<td></td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>-0.55</td>
<td></td>
<td>-0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>179</td>
<td>-0.65</td>
<td>-1.40</td>
<td>-0.79</td>
<td>-1.22</td>
</tr>
<tr>
<td></td>
<td>271</td>
<td>0.59</td>
<td>0.51</td>
<td>0.43</td>
<td>0.86</td>
</tr>
<tr>
<td>IGF-II:total IGFBP</td>
<td>179</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>271</td>
<td>0.99</td>
<td>0.86</td>
<td>0.79</td>
<td>1.22</td>
</tr>
</tbody>
</table>

1Plasma was collected from heifers that were fed high or low CP diets during first and second trimesters of pregnancy in a crossover design (low-low, LL; low-high, lowH; high-low, HL; high-high, HH).
2The number of parameters used as variables in the linear regression model, the number of independent scores in the linear regression model.

were estimated using 3 QC standards containing leptin at concentrations of 0.54 ng/mL (4.2%), 0.86 ng/mL (5.2%), and 1.85 ng/mL (4.8%) at a 0 binding of 30%. The sensitivity of the assay was 0.05 ng/mL.

**bPL.** Plasma bPL concentrations at d 179 and 271 were determined by RIA of duplicate samples according to procedures established by Wallace (1993). The sensitivity of the assay was 0.05 ng/mL. The intraassay CV was 7.7%, and interassay CV was 3.3%.

**Statistical Analyses**

A series of GLM was performed to evaluate these data using individual heifer as the experimental unit. Heifer BCS and BW were analyzed using GLM to assess for the effect of treatment group, age, and breed. Initial models were then run on d −14 values for IGF-I, IGF-II, IGFBP, IGF-I:IGFBP, and IGF-II:IGFBP to assess the effect of treatment group assignment and to assess for effect of age and breed. Normality of residuals was assessed using visual assessment of histograms and Q-Q plots (Dohoo et al., 2003). All of the values at d −14 involved logarithm transformation of outcome data to normalize the distributions. Because P-values for comparisons of treatment groups or breeds of all IGF d −14 values were P < 0.1, d −14 values were then considered as variables in all subsequent IGF GLM. Day −14 values were then only retained as variables in each GLM if P < 0.1.

Separate GLM were performed using outcomes based on the concentration of IGF-I on d 28, 82, 179, and 271, and similarly for IGF-II, IGFBP, and the ratios of IGF-I and IGF-II to IGFBP. Separate analyses at each measurement point were applied instead of a repeated measures approach because the treatments were different over time (i.e., d 28 and 82 had 2 treatment levels and the rest had 4). All IGF axis measures were right skewed and were transformed using the natural logarithm. Results are presented as transformed predicted means (Table 2) and back transformed predicted means ± SEM (Table 3). Each model included fixed effects for treatment, heifer age at AI (expressed as deviations from the mean), and breed. Bovine placental lactogen concentrations measured at d 179 and 271 were incorporated into models for those days.

Leptin concentrations were analyzed using an identical approach to that for IGF, except without transformation. An initial analysis was performed on d −14 levels to assess whether there was an effect of treatment assignment, age, or breed. Then separate statistical models were performed at each of d 28, 82, 179, and 271 and at calving, each using the concentration of leptin as an outcome. Plasma bPL was not used as a variable because there is no evidence to support bPL as influencing leptin concentrations. Plasma ES at d 82, d 271, and at calving and BCS at d 179 and d 271 were incorporated into models for those days.

Associations among calf birth weight and CRL and treatment group, IGF-I, IGF-II, IGFBP, and leptin were explored using GLM. Univariate screenings were used to identify explanatory endocrine variables that could be added to multivariable models (screening P < 0.25) using calf birth weight and CRL as the outcomes. Other explanatory variables were calf sex, breed, treatment, gestation length, and heifer age at AI.

Models were developed using a backward, stepwise approach to develop a main effects model containing only significant (P < 0.05) terms. Interactions between treatment and other terms were considered for each model and only retained if they were significant (P < 0.05). Model checking included visual inspection of a scatter plot of standardized residuals against predicted values, assessment of linearity of the relationship between the outcome and any continuous predictors, and
inspection of leverage, cooks, and DFTS statistics as outlined in Dohoo et al. (2003). All analyses were performed in STATA (StataCorp, College Station, TX), and significance was set at $P < 0.05$ and tendencies at $P < 0.10$. Model checking and inspection of residuals and unusual observations did not show any significant unusual patterns in any of the IGF or leptin models.

**RESULTS**

**Heifer Age, BW, and BCS**

The mean age of the heifers at AI by treatment group HH, HL, lowH, and LL were 23.2 ± 0.14 mo, 23.0 ± 0.17 mo, 23.0 ± 0.17 mo, and 23.0 ± 0.20 mo, respectively. There was no difference in heifer age among treatment groups ($P = 0.90$). At AI, Beef X heifers were younger than CBX heifers (22.6 ± 0.14 mo vs. 23.2 ± 0.09 mo, respectively; $P < 0.001$), but BeefX and CBX did not differ in BW (348 ± 3.4 kg vs. 356 ± 3.5 kg, respectively; $P = 0.29$).

The mean BW of the heifers during gestation by treatment group are presented in Figure 1 (Sullivan et al., 2009). There was no difference between mean BW of treatment groups at d 1 ($P = 0.36$). During first trimester, heifers on high protein diets (HH + HL) were heavier than heifers on low protein (lowH + LL) at d 28 ($P = 0.007$), 70 ($P < 0.001$), and 93 ($P < 0.001$; Sullivan et al., 2009). During second (d 124, 153, and 179) and third trimesters (d 208, 236, and 271), heifers assigned to high protein diets (HH + lowH) were heavier ($P < 0.001$) than heifers assigned to low protein diets (HL + LL) during the same period, whereas first trimester treatment also still affected BW with HH + HL heifers being heavier than lowH + LL heifers ($P < 0.001$; Sullivan et al., 2009).

Nutritional treatment affected heifer BCS measures. At d 124, heifers receiving high CP during first trimester had increased BCS ($P < 0.001$) compared with heifers receiving low CP during the same period (Figure 2). At d 153 and 179, high CP during first and second trimesters increased BCS ($P < 0.001$) compared with heifers receiving low dietary CP during those periods (Figure 2). The positive effect of high CP in first ($P < 0.01$) and second ($P < 0.01$) trimester on BCS continued at d 208, 236, and 271 (Figure 2).

**IGF-I**

Plasma IGF-I at d −14 tended to differ between animals subsequently assigned to different treatment groups ($P = 0.09$) and hence was used as a covariate for subsequent analysis. High CP increased IGF-I compared with low CP (Table 2 and Figure 3) at d 28 ($P = 0.001$) and d 82 ($P < 0.001$) of gestation. High CP in second trimester also increased IGF-I compared with low CP at d 179 ($P < 0.001$). At d 271, IGF-I was increased in HH compared with HL ($P = 0.02$). Heifer age at AI, breed, and bPL concentrations did not affect IGF-I at any stage.

**IGF-II**

Plasma IGF-II at d −14 did not differ between animals subsequently assigned to different treatment groups ($P = 0.9$) and hence was used as a covariate for subsequent analysis. High CP increased IGF-II compared with low CP (Table 2 and Figure 3) at d 28 ($P = 0.001$) and d 82 ($P < 0.001$) of gestation. High CP in second trimester also increased IGF-I compared with low CP at d 179 ($P < 0.001$). At d 271, IGF-I was increased in HH compared with HL ($P = 0.02$). Heifer age at AI, breed, and bPL concentrations did not affect IGF-II at any stage.

<table>
<thead>
<tr>
<th>Table 3. Composite breed group means for maternal plasma IGF concentrations$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
</tr>
<tr>
<td>IGF-II, ng/mL</td>
</tr>
<tr>
<td>Total IGFBP, ng/mL</td>
</tr>
<tr>
<td>IGF-I:IGFBP</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>IGF-II:IGFBP</td>
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</tr>
</tbody>
</table>

$^1$Data presented are means ± SEM.

$^2$Day of gestation relative to AI.

$^3$1/2 Senepol, 1/4 Brahman, 1/8 Charolais, and 1/8 Red Angus.

$^4$1/2 Senepol, 1/4 Brahman, and 1/4 Charolais.
Figure 1. Heifer BW of treatment groups during gestation. Body weights are from heifers that were fed high or low CP diets during first and second trimesters of pregnancy in a crossover design (low-low, LL; low-high, lowH; high-low, HL; high-high, HH). Data presented are means and SEM. Dotted vertical lines indicate trimesters of gestation. $P < 0.05$ for (HH + HL) vs. (lowH + LL) at d 28, 70, 93, 124, 153, 179, 208, 236, and 271; $P < 0.05$ for (HH + lowH) vs. (HL + LL) at d 124, 153, 179, 208, 236, and 271.

Figure 2. Heifer BCS (1 to 5 scale) of treatment groups from d 124 to 271 of gestation (Sullivan et al., 2009). Body condition scores are from heifers that were fed high or low CP diets during first and second trimesters of pregnancy in a crossover design (low-low, LL; low-high, lowH; high-low, HL; high-high, HH). Data presented are BCS means ± SEM; $P < 0.05$ for (HH + HL) vs. (lowH + LL) at d 124, 153, 179, 208, 236, and 271; $P < 0.05$ for (HH + lowH) vs. (HL + LL) at d 124, 153, 179, 208, 236, and 271.
There was a tendency for total IGFBP at d −14 to differ among animals subsequently assigned to different treatment groups \((P = 0.07)\), and hence total IGFBP values for d −14 were used as covariates for subsequent analysis. At d 82, heifers fed a high level of CP in first trimester had greater \((P = 0.002)\) total IGFBP concentrations than heifers fed a low level of CP \((242 \pm 18 \text{ ng/mL vs. } 232 \pm 26 \text{ ng/mL, respectively; Table 2 and Figure 3})\).

At d 179, heifers fed a high level of CP in second trimester had greater \((P = 0.005)\) total IGFBP than heifers fed a low level of CP \((257 \pm 44 \text{ ng/mL vs. } 209 \pm 27 \text{ ng/mL, respectively})\). At d 271, heifers fed a high level of CP in second trimester had greater \((P = 0.03)\) total IGFBP than heifers fed a low level of CP \((169 \pm 29 \text{ ng/mL vs. } 144 \pm 18 \text{ ng/mL, respectively})\).

Breed effects were detected for plasma total IGFBP at d −14, with CBX having increased total IGFBP compared with BeefX \((P = 0.02; \text{Table 2})\). Heifer age at AI and bPL concentrations did not affect total IGFBP at any stage of gestation.

**IGFBP**

Ratios of IGF-I:Total IGFBP and IGF-II:Total IGFBP

The maternal plasma IGF-I:total IGFBP ratio was less in CBX than in BeefX cattle at d −14 \((P = 0.008)\), d 28 \((P = 0.002)\), and d 82 \((P = 0.02; \text{Table 2})\), and hence IGF-I:total IGFBP values for d −14 were used as covariates for subsequent analysis. Plasma IGF-I:total IGFBP at d −14 did not differ among animals subsequently assigned to different treatment groups \((P = 0.21)\). At d 28, heifers fed a high level of CP in first trimester had greater \((P < 0.001)\) ratios of IGF-I:total

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**Figure 3.** Plasma IGF-I, IGF-II, and total IGFBP concentrations of heifer treatment groups during gestation. Plasma was collected from heifers that were fed high or low CP diets during first and second trimesters of pregnancy in a crossover design \((\text{low-low, LL; low-high, lowH; high-low, HL; high-high, HH})\). Data presented are geometric means and SEM \((\text{left-hand panel})\) and back-transformed predicted means and SEM \((\text{right-hand panel})\). Dotted vertical lines indicate trimesters of gestation.
IGFBP than heifers fed a low level of CP (0.642 ± 0.081 vs. 0.514 ± 0.090, respectively). At d 82, heifers fed a high level of CP in first trimester had greater (P = 0.008) ratios of IGF-I:total IGFBP than heifers fed a low level of CP (0.581 ± 0.078 vs. 0.513 ± 0.089, respectively). At d 179, heifers fed a high level of CP in second trimester had greater (P < 0.001) IGF-I:total IGFBP ratios than heifers fed a low level of CP (0.494 ± 0.081 vs. 0.272 ± 0.031, respectively). The heifers assigned to the HH diet had greater IGF-I:total IGFBP than heifers assigned to HL (P < 0.001) and LL (P < 0.001) diets (0.521 ± 0.11 vs. 0.246 ± 0.031 and 0.300 ± 0.059, respectively). Heifers assigned to lowH diets had a greater IGF-I:total IGFBP ratio (0.449 ± 0.12) than LL and HL heifers (P < 0.001). At d 271, heifers fed a high level of CP in second trimester had decreased (P = 0.04) ratios of IGF-I:total IGFBP than heifers fed a low level of CP (0.441 ± 0.070 vs. 0.507 ± 0.066, respectively). At d 271, heifers assigned to lowH diets had a decreased (P = 0.02) IGF-I:total IGFBP ratio compared with heifers assigned to LL diets (0.41 ± 0.10 vs. 0.61 ± 0.12, respectively).

Dietary treatment group assignment did not alter maternal plasma IGF-II:total IGFBP at d −14, before the commencement of treatments (P = 0.20). Dietary treatment altered the ratio of IGF-II:total IGFBP in maternal plasma during second and third trimesters of gestation. At d 179, heifers fed a high level of CP in second trimester had reduced (P = 0.01) IGF-II:total IGFBP ratios than heifers fed a low level of CP (1.66 ± 0.35 vs. 1.97 ± 0.38, respectively). Heifers assigned to lowH diets had a decreased (P = 0.006) IGF-II:total IGFBP than the heifers assigned to LL diets (1.53 ± 0.50 vs. 2.35 ± 0.67, respectively). At d 271, heifers fed a high level of CP in second trimester had reduced (P = 0.02) ratios of IGF-II:total IGFBP than heifers fed a low level of CP (2.42 ± 0.51 vs. 2.81 ± 0.54, respectively). Heifers assigned to lowH diets had reduced (P = 0.008) IGF-II:total IGFBP concentrations compared with heifers assigned to LL diets (2.21 ± 0.71 vs. 3.37 ± 0.96; respectively).

Breed effects were detected for IGF-II:total IGFBP throughout gestation, with BeefX displaying greater IGF-II:total IGFBP values compared with CBX (P < 0.001) at d −14, 28, 82, 179, and 271 (Table 2), and hence IGF-II values for d −14 were used as covariates for all IGF-II:total IGFBP analysis. Heifer age at AI and bPL concentrations did not affect IGF-II:total IGFBP at any stage.

**DISCUSSION**

This study has demonstrated that low dietary CP and energy reduced maternal plasma IGF-I and -II and total IGFBP concentrations in Bos indicus influenced heifers during pregnancy. Genotype affected maternal plasma IGF-II and total IGFBP before pregnancy, and IGF:total IGFBP ratios before and during pregnancy, but did not alter maternal plasma IGF-I or leptin. Maternal plasma bPL did not affect IGF. Increasing heifer age at AI decreased IGF-II and increased leptin concentrations in maternal plasma. Maternal plasma leptin was also increased by dietary protein and BCS but not ES. Therefore, the influences on maternal IGF-I and IGF-II differ, with the latter affected by genotype, age, and nutrition, whereas only nutrition affected IGF-I. Reproductive hormone bPL had no obvious influence on the IGFBP or leptin. Leptin was correlated with BCS, consistent with its known biology in mammals (Thomas et al., 2001). In turn, the maternal IGFBP system and leptin were predictors of calf birth weight and CRL, which may reflect the actions of these hormones to alter maternal nutrient partitioning and hence determining nutrient supply to, and growth of, the fetus (Fowden, 2003).

**Nutrition**

Because the high and low nutritional protein groups were not isocaloric, it is feasible to suggest that the elevated energy intake in the high CP nutritional groups may also have contributed to the nutritional effects observed for maternal responses. The CP intake of the high groups, however, was greater than suggested NRC levels, whereas the CP intake of the low groups was less than the suggested levels. This resulted in a 3.5-fold difference in CP content between groups, yet the difference in energy content between the high and low rations was 1.2 to 1.3 fold, with both groups receiving above the recommended NRC energy requirements.
In this study, increases in dietary CP and energy increased maternal leptin at calving despite the fact that dietary treatments had ceased at the end of second trimester. This is similar to sustained responses of leptin to nutrition observed in sheep, where the greater maternal leptin of sheep fed ad libitum, compared with nutrient restricted sheep, persisted for 2 mo after nutritional treatment had ceased (Bispham et al., 2003).

Table 4. Final multivariable GLM\(^1\) for birth weight of calves born to dams fed different levels of CP during gestation\(^2\)

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Level</th>
<th>Coefficient</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dam treatment</td>
<td>HH Reference</td>
<td>0.8</td>
<td>1.3</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>HL</td>
<td>0.2</td>
<td>1.3</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>−1.8</td>
<td>1.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Dam composite breed</td>
<td>CBX(^3) Reference</td>
<td>5.5</td>
<td>2.1</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>BeefX(^4)</td>
<td>−8.5</td>
<td>2.9</td>
<td>0.004</td>
</tr>
<tr>
<td>Dam treatment × breed</td>
<td>HH × BeefX(^4) Reference</td>
<td>−0.1</td>
<td>2.8</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>HL × BeefX(^4)</td>
<td>−3.3</td>
<td>2.8</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>LowH × BeefX(^4)</td>
<td>−0.1</td>
<td>2.8</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>LL × BeefX(^4)</td>
<td>−1.8</td>
<td>1.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Calf sex</td>
<td>Female Reference</td>
<td>2.4</td>
<td>0.8</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>1.5</td>
<td>0.6</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>2.3</td>
<td>1.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Dam age at AI</td>
<td></td>
<td>2.4</td>
<td>1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dam leptin at d 271(^5)</td>
<td></td>
<td>28.4</td>
<td>1.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\)Data presented are coefficients and SEM.
\(^2\)Dams were fed high or low CP diets during first and second trimesters of pregnancy in a crossover design (low-low, LL; low-high, lowH; high-low, HL; high-high, HH).
\(^3\)Dams were 1/2 Senepol, 1/4 Brahman, 1/8 Charolais, and 1/8 Red Angus.
\(^4\)Dams were 1/2 Senepol, 1/4 Brahman, and 1/4 Charolais.
\(^5\)Dam plasma leptin concentration at d 271 of gestation, ng/mL.

**Leptin**

In this study, increases in dietary CP and energy increased maternal leptin at calving despite the fact that dietary treatments had ceased at the end of second trimester. This is similar to sustained responses of leptin to nutrition observed in sheep, where the greater maternal leptin of sheep fed ad libitum, compared with nutrient restricted sheep, persisted for 2 mo after nutritional treatment had ceased (Bispham et al., 2003).
In cattle, acute total nutrient restriction in prepuber
tal heifers markedly reduced circulating leptin (Am-
stalden et al., 2000) and medium to long-term high
level of CP supplementation increased plasma leptin
in mature gestating beef cows (Lents et al., 2005). The
short-term underfeeding of energy while feeding ade-
quate protein to the nonpregnant cow results in de-
creased plasma leptin levels (Delavaud et al., 2002), as
does the medium term underfeeding of dietary protein
(1.35 fold) and energy (1.12 fold) to nonpregnant heif-
ers (Leon et al., 2004). As these aforementioned stud-
ies have demonstrated that dietary protein and energy
affect leptin independently of each other, the negative
nutritional effect on plasma leptin in the heifers in this
study is most probably attributable to joint effects of
the energy and protein differences in the high and low
diets. In this study, however, the dietary variations only
affected circulating leptin in the longer term.

Nutritional effects on leptin appear to be mediated,
at least in part, through effects on body energy stores.
Consistent with previous studies (Lents et al., 2005),
we found that increased nutrient intake was associated
with increased BCS in heifers. In turn, heifer BCS in
this study was positively associated with the concen-
tration of leptin in bovine plasma, as also reported by
Leon et al. (2004), Meikle et al. (2004), Chilliard et al.
(2005), and Lents et al. (2005).

In adolescent sheep fed to promote rapid growth, a
negative association exists between maternal circulat-
ing leptin and fetal birth weight (Thomas et al., 2001).
This differs from our model where maternal leptin con-
centrations at d 271 had a positive relationship with
calf birth weight, probably because the heifers were not
overfed to such an extent. Similarly, in the human in-
fant, a positive correlation exists between cord blood
concentrations of leptin at delivery and birth weight
(McMillen et al., 2006). Although cord samples were
not collected in this study, maternal and fetal plasma
leptin concentrations are correlated during late gesta-
tion in the sheep (Yuen et al., 2004). Maximal growth
of the bovine fetus occurs during the final 100 d of
a gestation averaging approximately 286 d (Prior and
Laster, 1979). Together, this suggests that the nu-
tritional effect on calf birth weight as discussed by Micke
et al. (2009) may be mediated by nutrient partitioning
associated with maternal leptin concentrations in third
trimester. Interestingly, we observed that older heif-
ers had greater leptin concentrations and heavier birth

weight calves. Dam age has been reported to account
for significant variation in calf birth weight in Bos tau-
rus (Brown and Galvez, 1969; Bellows et al., 1982) and
Bos indicus (Vernon et al., 1964) when comparing cows
born in different breeding seasons. The heifers in our
study only had a 3-mo range in age and demonstrated
that dam age remains an important variable of calf
birth weight in heifers even when the age variation is
considerably smaller. A close positive correlation be-
tween maternal age and BW at AI is consistent with
reduced nutritional requirements for maternal growth
in older heifers, which would increase maternal BW,
plasma leptin, and the proportion of nutrients available
for calf growth.

Maternal plasma ES was not associated with leptin in
this study. Although estradiol enhances leptin secretion
by cultured human placental cells (Chardonnens et al.,
1999), human maternal serum leptin is not directly cor-
related with peripheral estrogens (Sivan et al., 1998).
Because the bovine placenta produces negligible leptin
(Liefers et al., 2005) in comparison with the human
placenta, it is less likely that circulating bovine mater-
nal ES would directly influence maternal leptin. The
association between ES and leptin, however, was still
explored in this study because the loss of the placenta
at parturition coincided with the fall in leptin concen-
trations as previously reported in the cow (Kadokawa
et al., 2000), and mRNA encoding for leptin has been
detected in uterine caruncular tissue of pregnant cows
(Takahashi et al., 1999).

Although genotype has been previously reported to
affect plasma leptin in cows (Ren et al., 2002), no geno-
typic differences were observed in this study, consistent
with another recent study of 3 beef breeds by Daix
et al. (2008). This may be due to the relatively small
(12%) genetic variation within beef breeds in the cattle
used in this study compared with 100% variation in
the dairy vs. beef cows used in the Ren et al. (2002)
study.

IGF System

Nutritional restriction reduced maternal plasma IGF-
I in the present study, similar to previous reports in cat-
tle by Houseknecht et al. (1988), Elsasser et al. (1989),
Rutter et al. (1989), Perry et al. (2002), and Lents et
al. (2005). These studies involved dietary manipulation
by decreasing protein (Lents et al., 2005), decreasing

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**Table 5. Final multivariable GLM for calf crown rump length**

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Level</th>
<th>Coefficient</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf sex</td>
<td>Male</td>
<td>2.31</td>
<td>1.20</td>
<td>0.06</td>
</tr>
<tr>
<td>Gestation length</td>
<td></td>
<td>0.67</td>
<td>0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dam IGF-I at d 271</td>
<td></td>
<td>−0.53</td>
<td>0.17</td>
<td>0.003</td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td>82.03</td>
<td>0.81</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

---

1 Data presented are coefficients and SEM.
2 Dam plasma IGF-I concentration at d 271 of gestation, ng/mL.
both energy and protein (Rutter et al., 1989; Perry et al., 2002), increasing protein and decreasing energy (Houseknecht et al., 1988), and decreasing protein and energy independently (Elsasser et al., 1989). Some results suggest that in cattle, protein may be the primary nutritional determinant of IGF-I but that the IGF-I response to protein may be increased by the availability of increased ME (Elsasser et al., 1989). Alternatively, reduced dietary energy has resulted in reduced plasma IGF-I concentrations despite concurrent increases in dietary protein (Houseknecht et al., 1988). The consistent positive IGF-I response to the nutritional treatment, in this study, may then indicate an increased response to protein given that these heifers also had increased availability of energy throughout gestation due to the small differences in energy between the dietary treatments. Furthermore, our study has demonstrated that increasing the protein and energy content of the diet increases IGF-I during gestation with the effect being detectable within 28 d. Our observation that maternal plasma IGF-I concentrations in the third trimester are affected by treatment in second trimester suggests a carryover effect of diet.

In this study, no correlations were observed between bPL and the IGF system at any stage of gestation, despite the stimulatory effect of bPL on IGF-I and -II revealed in rodents by Byatt et al. (1992), the negative correlation between IGF-I and bPL observed in the bovine by Hossner et al. (1997), and the positive relationship demonstrated between bPL and IGF-I in the bovine by Weber et al. (2007). An increased level of gestational nutrition was correlated with calf birth weight, despite not affecting calf CRL, as previously reported by Micke et al. (2009). Although maternal IGF-I was affected by diet, it was not correlated with offspring birth weight, similar to results reported for guinea pigs (Sohlstrom et al., 2001) and humans (Davidson et al., 2006). Maternal plasma IGF-I near term in fact had a negative correlation with CRL at birth. This is despite IGF-I being a known anabolic agent for the proliferation, differentiation, and mineralization of bovine osteoblasts in vitro (Li et al., 2009) and the promoter of skeletal maturation in fetal lamb infused with IGF-I (Lok et al., 1996). The negative association of maternal IGF-I with CRL is contrary to previous reports of a positive correlation of fetal CRL to maternal IGF-I concentrations in sheep (Osgerby et al., 2003) and an unchanged effect on fetal length when fetal lambs were infused with IGF-I (Lok et al., 1996). Yet, the results of our study complement the previous study by Hossner et al. (1997) where cows pregnant to sires with high expected progeny birth weights had less serum IGF-I than dams carrying light expected birth weight calves, and the fetal weight in these calves was negatively correlated to maternal IGF-I (Hossner et al., 1997).

We also observed a positive nutritional effect on maternal plasma IGF-II concentrations, contradictory to previous reports in the guinea pig (Sohlstrom et al., 2001). In the Bos taurus heifers, dam plasma IGF-II in second trimester was reduced by low nutritional protein levels (Perry et al., 2002), whereas in pregnant guinea pigs, plasma IGF-II was increased by food restriction (Sohlstrom et al., 2001). Yet, IGF-II has also been observed to be nutritionally insensitive in cattle (McGuire et al., 1992) and in sheep (McMullen and Wathes, 2003) or less responsive to undernutrition than IGF-I in general (Perry et al., 2002; Fowden, 2003). In fact, in this study, a carryover effect of previous nutritional treatment on IGF-II concentrations in third trimester, similar to IGF-I, was observed. In guinea pigs, increased circulating maternal IGF-II increased the number of viable fetuses and fetal weight (Sferruzzi-Perri et al., 2006). Yet, in the present study there was no effect of circulating IGF-II at any stage of pregnancy on fetal weight or CRL, possibly due to species differences.

Similar to the effects on IGF-I and -II, increased gestational nutrition in the present study increased total IGFBP concentrations. The major carrier of IGF-I in adult sheep plasma is IGFBP-3 (Hodgkinson et al., 1989; Gallaher et al., 1992), but pregnancy alters the abundance and stability of the IGFBP (Sohlstrom et al., 2001). Circulating IGFBP-3 concentrations are reduced, whereas IGFBP-1 and -2 are increased in maternal plasma during food restriction in guinea pigs (Roberts et al., 2001; Sohlstrom et al., 2001; Roberts et al., 2002), sheep (Gallaher et al., 1995), and cattle (Vicini et al., 1991), and a 31-kDa IGFBP (similar to sheep IGFBP-1) tended to be elevated in postpartum cows on low levels of feed intake (Roberts et al., 1997). Yet, cattle subject to diets differing in levels of energy and protein did not demonstrate a change in IGFBP-2 (McGuire et al., 1992), and sheep on high level of protein did not demonstrate a change in IGFBP-3 (Clarke et al., 1993) despite changes in circulating IGF-I in both species.

In the guinea pig, the ratio of IGF-I:IGFBP-1 in maternal plasma was reduced, and the ratio of IGF-I:IGFBP-3 was increased (Roberts et al., 2002) by nutrient restriction in late pregnancy. Changes in these ratios of IGF to IGFBP may inhibit or potentiate the bioavailability of IGF at the cellular level depending on the IGFBP and conditions used for evaluation (Jones and Clemmons, 1995). Because of their strong binding, IGFBP generally inhibit the biological effects of IGF through sequestration, although they also can have facilitating actions. The ratio of IGF-I:total IGFBP in early and mid pregnancy was increased, and in late pregnancy was decreased in our study by high protein. The results from late pregnancy are in agreement with those of Roberts et al. (2002) in the guinea pig. Conversely, in our study, high protein and energy levels in mid and late pregnancy decreased the ratio of IGF-II:total IGFBP, also in agreement with nutritional changes in the ratio of IGF-II:IGFBP-2 in the guinea pig (Roberts et al., 2001). Total IGFBP in fetal circulation are said to be negatively correlated with birth weight because they decrease the availability of IGF
for fetal growth (Gicquel and Le Bouc, 2006), but we did not find any correlation among maternal plasma IGFBP and calf parameters at birth.

This study expands on the current knowledge of the complex interactions among nutrition, genetics, and the influence of metabolic hormones on fetal growth in beef cattle. Although maternal IGF-I, IGF-II, and total IGFBP concentrations were responsive to gestation feed level, dam genotype also had a strong influence on IGF-II and total IGFBP. Leptin may have mediated an increase in fetal birth weight associated with greater IGF-II and total IGFBP. Leptin may have mediated an increase in fetal birth weight associated with greater IGFBP-2 and IGFBP-4 in the ovine foetus. J. Endocrinol. 122:681–687. Gaither, B., B. Brier, J. Harding, and P. Gluckman. 1995. Perceptual undernutrition resets plasma IGFBP levels and alters the response of IGFBP-1, IGFBP-3 and IGF-I to subsequent maternal undernutrition in the sheep. Prog. Growth Factor Res. 6:189–195.


