Impact of Fetal Versus Maternal Contributions of Bos indicus and Bos taurus Genetics on Fetal Embryonic Development

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Synopsis

Bos taurus recipients and embryos experienced greater pregnancy failure when exposed to nutrient restriction. In addition, Bos indicus fetuses were smaller than Bos taurus fetuses, and resulted in greater recipient circulating concentrations of pregnancy associated glycoproteins.

Summary

The aim of this study was to evaluate the impact of energy restriction on embryonic survival in Bos taurus and Bos indicus genotypes, as well as the influence of maternal and fetal systems on early fetal development. The experiment used a reciprocal embryo transfer approach in a completely randomized design with a 2 × 2 × 2 factorial arrangement of treatments. Embryo recipient cows were assigned to 1) a diet to meet daily maintenance requirements (MAINT), or 2) a diet that restricted intake of nutrients to 70% of energy maintenance requirements (RESTR). Angus (AN) and Brangus (BN) female embryos were produced and randomly transferred to either AN or BN recipients fed the respective diets for 28 d to create the following treatment combinations (AN × AN × RESTR, n = 14; AN × AN × MAINT, n = 19; AN × BN × RESTR, n = 16; AN × BN × MAINT, n = 17; BN × BN × RESTR, n = 15; BN × BN × MAINT, n = 19; BN × AN × RESTR, n = 14; BN × AN × MAINT, n = 19). Recipients remained on the dietary scheme until d 91. Pregnancy diagnosis was assessed at d 28 of gestation and fetal morphometries from d 42 to 91. Plasma pregnancy specific protein B (PSPB) was assessed weekly from d 28 to 91. Recipient body weight (BW) and BCS were significantly impacted by the dietary treatments (P<0.05). In addition, AN × RESTR recipients had greater (P<0.05) pregnancy failure at d 28 than AN × MAINT and BN in both diets. Furthermore, RESTR recipients that received an AN embryo experienced greater (P<0.05) pregnancy failure than AN embryos transferred to MAINT recipients. Angus embryos resulted in lesser (P<0.05) plasma PSPB concentration, but greater (P<0.05) fetal length when compared to BN embryos. These results indicate that Bos taurus cattle may be more susceptible to embryonic loss than Bos indicus when submitted to a feed restriction scheme during the first trimester of gestation, and that differences in growth rate and PSPB production exists between the two subspecies.

Introduction

Breeds generated from crossing Bos indicus × Bos taurus cattle breeds contain greater tolerance to elevated ambient temperatures and humidity than most Bos taurus beef breeds (Hansen, 2004). It is estimated that approximately 30% of cattle in the U.S. contain some Bos indicus genetics. In addition, 40% of beef cows and 50% of the country’s cow-calf producers are located in the southern U.S. where Bos indicus cattle and their crosses are located. It, therefore, is paramount that we learn more about the biology of Bos indicus cattle so that this new knowledge can be used to modify management systems for optimal production efficiency.

Both historical literature and recent findings from our laboratory demonstrate that Bos indicus fetuses grow at a slower rate in utero during early and mid-gestation (Mercadante et al., 2013, Ferrell, 1991; O'Rourke et al., 1991). Moreover, there are indications that Bos indicus fetuses undergo compensatory growth in late gestation to generate fetuses that can be larger than their Bos taurus counterparts at birth (Mercadante et al., 2013). Subspecies genotype also affects plasma pregnancy-associated glycoproteins (PAG) concentrations in early pregnancy, where Brangus cows have higher PAG concentrations than

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Angus (Mercadante et al., 2013). Nutrient utilization and feed efficiency are also thought to be different between *Bos indicus* and *Bos taurus* animals (Elzo et al., 2009).

Therefore, the objective of this study was to determine how maternal and fetal systems influence early fetal development in *Bos indicus* and *Bos taurus* cattle. We hypothesize that early fetal and placental development is modified by inclusion of *Bos indicus* genetics into the maternal genotype. In addition, we hypothesize that dietary restriction in early pregnancy will limit fetal development in both *Bos indicus* and *Bos taurus* cows, but the magnitude of the comprised development will be less evident in *Bos indicus* cattle.

**Materials and Methods**

A reciprocal embryo transfer (ET) approach was used in a completely randomized design with a $2 \times 2 \times 2$ factorial arrangement of treatments in order to generate 55 pregnancies over two consecutive years ($n = 55$). Recipient cows ($n = 134$) were randomly assigned to 1 of the 2 dietary treatments: 1) a diet to meet 100% daily maintenance requirements (MAINT), or 2) restricted intake of nutrients to 70% of energy maintenance requirements (RESTR). Angus (AN) and Brangus (BN) embryo donors were superovulated and artificially inseminated (AI) with female sexed-sorted semen from the same breed. Embryos were then collected and randomly transferred to either AN or BN recipients fed the respective diets; therefore generating 8 treatment combinations (AN × AN × RESTR, $n = 15$; AN × AN × MAINT, $n = 19$; AN × BN × RESTR, $n = 16$; AN × BN × MAINT, $n = 17$; BN × BN × RESTR, $n = 15$; BN × BN × MAINT, $n = 19$; BN × AN × RESTR, $n = 14$; BN × AN × MAINT, $n = 19$). Female sex-sorted semen from 4 AN and 2 BN sires was utilized to AI donor cows. In addition, the generated embryos were not only assigned to recipients in order to equally generate the previously described treatment combinations, but also to equally distribute the effects of sire, donor cow, and embryo grade score (Bo and Maplesoft, 2013) between the different recipient breeds, dietary treatment, and parity (primiparous or multiparous).

Angus and BN cows were randomly assigned to 1 of 2 dietary treatments 28 d prior to embryo transfer (d-21): 1) diet formulated to meet the daily requirements of a 1213 lb of BW beef cow (NRC, 2000), comprised of 60% fiber pallets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*), and 5% peanut hulls (CP = 12.5%, TDN = 46%; MAINT); and 2) diet formulated to meet 70% of daily requirements, comprised of 70% fiber pallets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%; REST). Recipient cows remained on the described feeding scheme until d 91 (91 d of gestation), then all cows were submitted to a diet to meet 100% of the maintenance requirements for the remaining of gestation.

Diagnosis of pregnancy was assessed by transrectal ultrasonography on d 28 with an Ibex ultrasound equipped with a linear 5 MHz multifrequency transducer. A ultrasound video was recorded at d 42, 49, 56, 63, 70, 77, 84 and 91 and in a frame-by-frame fashion, the ideal position and orientation of the conceptus was chosen to measure embryo length (CRL) as previously described (Riding et al., 2008). The BioPRYN Quantitative pregnancy-specific protein B (PSPB) ELISA (BioTracking Inc., Moscow, ID) was used to quantify circulating pregnancy-associated glycoprotein (PAG) concentrations in plasma harvested from blood collected at d 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91 of pregnancy. This assay was developed to recognize PSPB (also known as PAG1), but it also detects other PAGs in circulation (Sasser et al. 1986, Xie et al. 1997). Intra-assay coefficients of variation averaged 2.78% and 1.60% for the two controls. Inter-assay coefficients of variation were 3.89% and 4.09% for the same two controls, respectively.

All data were analyzed using the SAS statistical package (SAS Inst. Inc., Cary, NC; Version 9.4). The present study was a completely randomized design with a $2 \times 2 \times 2$ factorial arrangement of treatments, where recipient breed (AN and BN), embryo breed (AN and BN) and recipient diet (MAINT and REST) represented the 3 different levels of factorials. Recipient cow’s individual intake of nutrients were obtained during the experiment, therefore cow was considered the experimental unit. Continuous variables were analyzed by the MIXED procedure and categorical variables were analyzed using the
For a more conservative interpretation of the data, the Tuckey’s method was used for all simultaneous pairwise comparisons. \( P \)-values \( \leq 0.05 \) were considered significant, and no tendencies will be discussed throughout this report.

Results
The dietary approach used in this experiment successfully induced an energy restriction scenario, as shown by the different percentage of energy requirements met by the diets (MAINT = 107.3% and REST = 81.4%), along with the significant impact of diet in recipient BW (\( P = 0.02 \)) and BCS loss from d-21 to d 91(\( P = 0.02 \); Table 1). When weekly repeated measurements of BW and BCS were evaluated, a diet \( \times \) day interaction was observed for both recipient BW (\( P < 0.01 \); Figure 1) and BCS (\( P < 0.01 \); Figure 2), where recipients in the REST diet had lower BW on d 28, 35, 56, 70, 77 and 84 (\( P < 0.05 \)), together with lower BCS at d 56, 63, 70, 77, 84 and 91 (\( P < 0.05 \)).

A recipient breed \( \times \) diet interaction was observed on pregnancy failure by d 28 of gestation (\( P < 0.01 \)), where AN cows submitted to the REST diet had increased pregnancy failure compared to AN in the MAINT diet and BN cows in both REST and MAINT diets (Figure 3). In addition, there was an embryo breed \( \times \) diet interaction (\( P = 0.01 \)) on pregnancy failure at d 28. Recipients in the REST diet that received an AN embryo experienced greater pregnancy loss than recipients in the REST diet receiving BN embryos, regardless of the recipient breed (Figure 4).

Embryos that were transferred to AN recipients had greater CRL at d 91 of gestation when compared to embryos carried by BN recipients (recipient breed \( \times \) day: \( P < 0.01 \); Figure 5), regardless of the embryo breed. Furthermore, recipients that received BN embryos had greater plasma concentrations of PSPB at d 91 when compared to recipients that received AN embryos (embryo breed \( \times \) day: \( P < 0.01 \); Figure 6), regardless of the recipient breed.

Acknowledgements
The authors would like to thank Zoetis Animal Health (Parsippany, NJ) for their donation of PGF\(_{2\alpha}\) (Lutalyse), GnRH (Factrel), and CIDR inserts (EAZI-BREED CIDR). Sincere appreciation is also expressed to P. Folsom, M. Foran, O. Helms, D. Jones, C. Nowell, T. Schulmeister, and D. Thomas for their assistance with data collection and laboratory analysis. Furthermore, the authors would like to acknowledge the USDA for providing funding for this experiment.

Literature Cited
Mercadante et al., 2013. 91:3693–3701
Table 1. Effects of dietary treatments\(^1\) on recipient BW and BCS.

<table>
<thead>
<tr>
<th>Item</th>
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<td>-60</td>
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<td>0.02</td>
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<tr>
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<td>5.2</td>
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<tr>
<td>BCS change d-21 to 90</td>
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<td>0.4</td>
<td>0.11</td>
<td>&lt;0.01</td>
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</tbody>
</table>

\(^1\)MAINT: diet formulated to meet the daily requirements of a 1213 lb of BW suckled beef cow (NRC, 2000), comprised of 60% fiber pallets, 30%, soybean hulls, 5% bermudagrass (Cynodon dactylon) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%) and REST: diet formulated to meet 70% of daily requirements, comprised of 70% fiber pallets, 10% soybean hulls, 5% bermudagrass (Cynodon dactylon) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%). Diet effect: \(P<0.01\); Day effect: \(P<0.01\); Diet \(\times\) Day: \(P<0.01\).

Figure 1. Effects of dietary scheme on recipient BW; MAINT: Diet formulated to meet the daily requirements of a 1213 lb of BW suckled beef cow (NRC, 2000), comprised of 60% fiber pallets, 30%, soybean hulls, 5% bermudagrass (Cynodon dactylon) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%) and REST: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pallets, 10% soybean hulls, 5% bermudagrass (Cynodon dactylon) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%). Diet effect: \(P<0.01\); Day effect: \(P<0.01\); Diet \(\times\) Day: \(P<0.01\).
Figure 2. Effects of dietary scheme on recipient BCS; MAINT: Diet formulated to meet the daily requirements of a 1213 lb of BW suckled beef cow (NRC, 2000), comprised of 60% fiber pallets, 30%, soybean hulls, 5% bermudagrass (Cynodon dactylon) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%) and REST: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pallets, 10% soybean hulls, 5% bermudagrass (Cynodon dactylon) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%). Diet effect: $P<0.01$; Day effect: $P<0.01$; Diet × Day: $P<0.01$. 

![Figure 2](image-url)
Figure 3. Effects of recipient embryo breed on pregnancy failure at d 28 of gestation. MAINT: Diet formulated to meet the daily requirements of a 550 kg of BW suckled beef cow (NRC, 2000), comprised of 60% fiber pallets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%) and REST: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pallets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%). Recipient breed × diet interaction: $P<0.01$. *a,b* Means without a common letter within bar differ ($P<0.05$).
Figure 4. Effects of embryo breed and diet on pregnancy failure at d 28 of gestation. MAINT: Diet formulated to meet the daily requirements of a 550 kg of BW suckled beef cow (NRC, 2000), comprised of 60% fiber pallets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%) and REST: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pallets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%). Recipient breed × diet interaction: $P<0.01$. a,b Means without a common letter within bar differ ($P<0.05$).
Figure 5. Effects of recipient breed on fetal crown to rump length (CRL). Transrectal ultrasonography was performed weekly from d 42 to d 91, and fetal CRL was assessed. At d 63, 70, 77, 84 and 91, crown to nose length (CNL) was used to estimate CRL as previously described (Riding et al., 2008). AN: Angus recipient and BN: Brangus recipient. *Recipient breed × Day interaction: $P<0.01$. 
Figure 6. Effects of embryo breed on recipient’s plasma concentration of PSPB. Blood samples were collected weekly from d 28 to 91. AN: recipients that received an Angus embryo. BN: Recipients that received a Brangus embryo. Embryo breed × day interaction: * $P<0.01$. 