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# Effects of nutrient restriction on the metabolic profile of *Bos indicus*-influenced and *B. taurus* suckled beef cows

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ABSTRACT

Recent research from our group demonstrated that *Bos indicus*-influenced suckled beef cows had greater resilience to withstand nutrient restriction and establish pregnancy compared with *B. taurus* cows exposed to the same conditions. To further understand these findings, differences in metabolic profile between these same *B. indicus*-influenced and *B. taurus* females were explored. Suckled beef cows ($n = 134$) were enrolled in a completely randomized design with a $2 \times 2$ factorial arrangement of treatments. On day −21, Angus (**AN**; *Bos taurus*) and Brangus (**BN**; *B. indicus*-influenced) cows were randomly assigned to 1) a diet that met daily energy maintenance requirements (**MAINT**), or 2) a diet that restricted intake to 70% of the daily energy maintenance requirements (**RESTR**). Cows were exposed to an estrus synchronization protocol and received an embryo 7 d after ovulation was pharmacologically induced on day 0. Blood samples were collected on days −21 and 19 to determine circulating concentrations of non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHB**), insulin, glucose, and IGF-1. Pregnancy status after embryo transfer was determined on day 28. As a consequence of the proposed diets, cows in the RESTR diet had less body condition score (**BCS**) on day 19 ($P = 0.008$) across breed types. Moreover, BCS change from day −21 to 19 was included as independent covariate into subsequent analyses, allowing for the comparison of breed types under an equivalent level of body reserve mobilization. A breed × diet interaction was observed for plasma insulin ($P = 0.03$) and IGF-1 ($P = 0.04$) on day 19, where AN–RESTR cows had less plasma concentrations on day 19 compared with AN–MAINT cows. Diets did not impact ($P > 0.10$) plasma insulin and IGF-1 concentrations in BN cows. No diet or breed effects were observed in circulating concentrations of NEFA, BHB, and glucose ($P > 0.10$). Across breed types and nutritional treatment, there was positive linear effect ($P \leq 0.04$) of plasma concentrations of insulin and IGF-1 on the probability of pregnancy to fixed-time embryo transfer. In summary, the negative impacts of nutrient restriction on the somatotropic axis, independently of body tissue mobilization, were heightened in *Bos taurus* females compared with *B. indicus*-influenced cohorts, which corroborate with the differences observed in fertility between these subspecies.


## Implications

The present study indicates that the impacts of energy restriction are greater in *Bos* (*B.*) *taurus* vs *B. indicus*-influenced beef cows when exposed to same restriction conditions. Plasma insulin and IGF-1 reduction as a consequence of energy restriction were more evident in *B. taurus* than within *B. indicus*-influenced postpartum cows. These results corroborate with the decreased pregnancy rates observed in *B. taurus* cows compared to *B. indicus* cows when both were exposed to energy restriction (Fontes et al., 2019), providing evidence of greater resilience of *B. indicus*-influenced cattle to disruptions in the somatotropic axis under scenarios of limited energy availability.

## Introduction

Evaluation of mitochondrial DNA sequencing and microsatellite loci indicate *Bos indicus* and *B. taurus* cattle diverged, from an evolutionary standpoint, between 110000 and 850000 years ago (Bradley et al.,

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1996; Machugh et al., 1997). During these years of separate evolution, *B. indicus* cattle have undergone natural selection in the hot and humid climates of Southern Asia, leading to the acquisition of genetic adaptation that convey greater heat tolerance compared to *B. taurus* that underwent natural and artificial selection in Europe (Hansen, 2004). Consequently, *B. indicus* cattle are capable of withstanding greater temperatures, experiencing a lesser reduction in feed intake (Allen et al., 1963; Seif et al., 1979), growth (Cartwright, 1955), and reproductive function (Rocha et al., 1998) compared to *B. taurus* cattle exposed to these conditions.

It is reasonable to expect that after years of separate evolution, the physiological differences between these subspecies go beyond their ability to withstand greater temperatures. A plethora of differences in reproductive biology have been reported between *B. indicus* and *B. taurus*, ranging from differences in ovarian function and hormonal metabolism (Sartori et al., 2016) to differences in gestational parameters and fetal development (Ferrell, 1991; O'Rourke et al., 1991; Mercadante et al., 2013). Previous reports also indicate that *B. indicus* cattle are better able to utilize low quality forages than *B. taurus* breeds (Turner, 1974; Moore et al., 1975; Warnick and Cobb, 1976). However, little is known about differences in physiological adaptation to energy restriction between these subspecies in postpartum beef cows, particularly its impacts on reproductive performance. Our research group recently reported that purebred Angus (**AN**; *B. taurus*) cows exposed to energy restriction had decreased pregnancy rates to fixed-time embryo transfer compared with energy-restricted Brangus (**BN**; *B. indicus*-influenced) cows, suggesting that *B. indicus*-influenced cows might have greater resilience to withstand energy restriction compared with *B. taurus* counterparts (Fontes et al., 2019). To further explore these findings, the present study compared plasma concentrations of circulating markers of metabolic adaptation to negative energy balance between AN and BN cows used in Fontes et al. (2019). We hypothesized that *B. taurus* cows are less able to cope with energy restriction conditions, and consequently have improved metabolic profile compared with *B. indicus*-influenced cows that are exposed to the same conditions.

## Material and methods

### *Experimental design*

As previously described (Fontes et al., 2019), a total of 197 (year 1: $n = 96$, and year 2: $n = 101$) suckled beef cows (BW: 522 ± 66.4 kg; body condition score [BCS]: 5.3 ± 0.61, 1 to 9 scale according to Wagner et al., 1988) were enrolled as candidate embryo recipients in a completely randomized design with 2 × 2 factorial arrangement of treatment over 2 consecutive years (Fig. 1). Fourteen days prior to the beginning of the experiment, all cows were commingled and fed a common diet in order to adapt cows, which were previously grazing, to a total mixed ration. At day −21, AN and BN cows were then stratified by days postpartum, BW, and BCS and were randomly assigned to one of the two diets: 1) diet formulated to meet the total daily energy requirements of a 550 kg lactating beef cow (**MAINT**; NASEM, 2016), and 2) diet formulated to meet 70% of total daily energy requirements (**RESTR**). Both diets were formulated to include the same ingredients in an attempt to minimize any dietary effects not related to total energy intake and were fed ad libitum. The proportion of the fibrous and concentrate ingredients within each diet (mainly peanut hulls and soybean hulls) was varied in order to generate the required differences in energy intake between treatments. The diets were formulated to achieve the desired restriction levels based on the most recent NASEM (2016) publication. The MAINT diet was composed of 60% of a pellet made from cotton and peanut fiber by-products (Fiber Pellet; AFG Feed LLC, Donalsonville, GA, USA), 30% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls. The RESTR diet was composed of 70% of a pellet made from cotton and peanut fiber by-products (Fiber Pellet; AFG Feed LLC), 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls. During the experimental period, all cows were housed at the University of Florida Feed Efficiency Facility at the North Florida Research and Education Center (30°46′35″N, 85°14′17″W), equipped with a GrowSafe System to measure individual feed intake (GrowSafe System Ltd., Airdrie, AB, Canada). The RESTR diet was formulated based on previous data from our research group on ad libitum feed intake of cows, so that overcompensation of energy intake by increased feed intake in this diet was minimized and restricted by gut fill due to the increased fiber content of the RESTR diet. In addition, individual feed intake combined with the dietary nutrient analysis results allowed for the estimation of the actual percentage of total daily maintenance energy requirement met (NASEM, 2016) by the diet, which allowed for the evaluation of how effective the superimposed RESTR diet was in restricting energy intake. Nutrient composition of the diets was determined on feed samples that were collected once every 2 weeks throughout the feeding period for both years. Feed samples were analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA) and results are reported in Table 1. Additionally, BW and BCS (Wagner et al., 1988) were collected on the mornings (from 0700 to 1000) of days −21 and 19 in order to evaluate the effectiveness of the diets in modulating body nutritional reserves. Body weight and BCS on day −21 represent the average of two assessments with a 24-h interval between each assessment, whereas values on day 19 represent a single measurement.

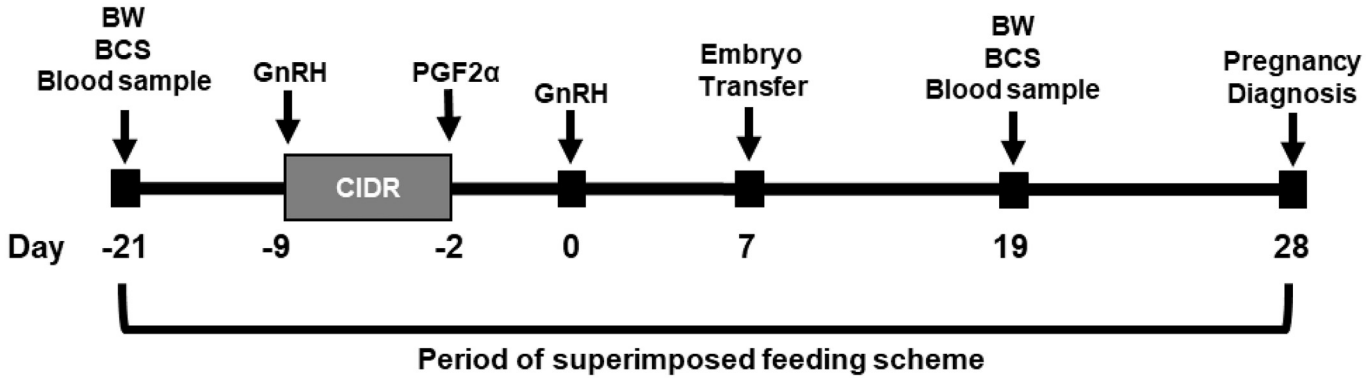


**Fig. 1.** Outline of the experimental design. Postpartum Angus (AN; *B. taurus*) and Brangus (BN; *B. indicus*-influenced) embryo recipients were randomly assigned to 1) a diet that met daily energy maintenance requirements (MAINT), or 2) a diet that restricted intake to 70% of the daily energy maintenance requirements (RESTR) in a completely randomized design with a 2 × 2 factorial arrangement of treatments. BCS: body condition score (Wagner et al., 1988). GnRH: gonadotropin-releasing hormone. $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$; CIDR: controlled internal drug release device.

**Table 1**
Composition and nutrient analysis[1] of dietary treatments[2] fed to cows.

| Item | Diets | |
|---|---|---|
| | RESTR | MAINT |
| Ingredient, % (as-fed basis) | | |
| Fiber pellets[3] | 70.0 | 60.0 |
| Soybean hulls | 10.0 | 30.0 |
| Bermuda grass hay[4] | 5.0 | 5.0 |
| Peanut hulls | 15.0 | 5.0 |
| Nutrient profile | | |
| DM, % | 92.3 | 94.8 |
| CP, % of DM | 11.2 | 12.5 |
| NDF, % of DM | 69.2 | 67.9 |
| ADF, % of DM | 63.0 | 56.2 |
| Total digestible nutrients, % of DM | 37 | 46 |
| $NE_l$, Mcal/kg[5] | 0.73 | 0.97 |
| $NE_m$, Mcal/kg[6] | 0.68 | 0.98 |
| $NE_g$, Mcal/kg[7] | 0.15 | 0.43 |

[1] Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY, USA).
[2] MAINT: Diet formulated to meet the daily requirements of a 550 kg suckled beef cow (NASEM, 2016); REST: Diet formulated to meet 70% of daily requirements.
[3] Commercial pellets made of cotton and peanut by-products (AFG Feed LLC, Donalsonville, GA, USA).
[4] Cynodon spp.
[5] Net energy for lactation.
[6] Net energy for maintenance.
[7] Net energy for growth.

*Estrus synchronization, embryo transfer, and pregnancy diagnosis*

All cows were submitted to the 7 d CO-Synch + CIDR estrus synchronization protocol (Larson et al., 2006) starting 12 d (day −9) after the feeding scheme was initiated. Briefly, the protocol consisted of an injection of 100 μg of gonadotropin-releasing hormone (2 ml Factrel; Zoetis Animal Health, Parsippany-Troy Hills, NJ, USA) concurrent with the insertion of a new controlled intravaginal drug release insert (EAZI-BREED CIDR, Zoetis Animal Health) containing 1.38 g of progesterone. Seven days later (day −2), the insert was removed and recipients received a 25-mg injection of prostaglandin $F_{2\alpha}$ (5 ml Lutalyse, Zoetis Animal Health). A second injection of 100 μg of gonadotropin-releasing hormone was administered 48 h after insert removal (day 0). Day 0 was considered the first day of gestation, as embryo donors were artificially inseminated on the same day. Transrectal ultrasonography was performed 7 d after the second gonadotropin-releasing hormone injection to verify the presence of a corpus luteum using an Ibex ultrasound equipped with a linear 5 MHz multifrequency transducer (E. I. Medical Imaging, Loveland, CO, USA). Recipients were selected based on the presence of a corpus luteum and a subjective assessment of the corpus luteum function by an experienced technician. A total of 134 recipients were eligible for embryo transfer based on the presence of a corpus luteum on day 7 (AN × RESTR, $n = 30$; AN × MAINT, $n = 36$; BN × RESTR, $n = 30$; BN × MAINT, $n = 38$) and the proportion of recipients considered eligible for transfer was not different between dietary treatments, breed, or their respective interaction ($P \geq 0.26$). On the same day, donors ($n = 46$) were flushed, and a single fresh embryo was transferred into the lumen of the recipient's uterine horn ipsilateral to the corpus luteum. Embryos were produced through donor superovulation and artificial insemination using sex-sorted semen for females. Embryos were assigned to recipients in order to equally distribute the effects of sire, donor cow, and embryo grade score (Bó and Mapletoft, 2013). More detailed description of the in vivo production of embryos for the present experiment was previously described (Fontes et al., 2019). Pregnancy status after embryo transfer was determined via transrectal ultrasonography on day 28 (21 d after embryo transfer). A cow was considered pregnant if uterine fluid and an embryo were visualized in the ultrasound image. All cows diagnosed as non-pregnant were removed from the experiment on the same day as the pregnancy diagnosis was performed.

*Blood collection and analyses*

Blood samples were collected via venipuncture of the jugular vein into heparinized blood collection tubes (Vacutainer, 10 ml; Becton Dickinson and Company, Franklin Lakes, NJ, USA) between 0700 and 1000 on days −21 and 19 in order to determine circulating concentrations of non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHB**), IGF-1, insulin, and glucose. The days in which blood samples were collected for the analysis were determined based on the experimental objectives by Fontes et al. (2019), which were to characterize interferon-stimulated genes expression in peripheral blood leukocytes and circulating concentrations of pregnancy-associated glycoproteins in *B. indicus*-influenced vs *B. taurus* cattle. Also, characterizing the metabolic profile on day 19 was selected based on the abundant literature reporting the importance of this period of gestation for pregnancy establishment in domestic ruminants (Hansen et al., 2017; Bazer et al., 2018). Blood samples were cooled on ice after collection followed by centrifugation at 1 500 ×*g* at 4 °C for 15 min. The plasma was transferred into polypropylene tubes and stored at −20 °C until further analysis. A 96-well microplate reader spectrophotometer with commercial kits was used to determine circulation concentrations of NEFA (Wako Chemicals USA, Inc., Richmond, VA, USA) and glucose (Thermo Electron Corp., Waltham, MA, USA). Plasma concentrations of BHB were estimated through the use of DL-β-hydroxybutyric acid sodium salt, β-nicotinamide adenine dinucleotide hydrate, and 3-hydroxybutyrate dehydrogenase (Sigma-Aldrich, St. Louis, MO, USA) as previously described (McCarthy et al., 2015). Plasma concentrations of insulin were estimated through radioimmunoassay (EMD Millipore's Porcine Insulin RIA; EMD Millipore Corporation, Billerica, MA, USA) using a Wizard Gamma Counter (PerkinElmer, Inc., Waltham, MA, USA) as previously reported by Hobbs et al. (2017). To determine circulating concentration of IGF-1, a commercial ELISA kit (Quantikine ELISA Human IGF1 Immunoassay; R&D Systems, Inc., Minneapolis, MN, USA) designed for human IGF-1 but with 100% cross-reactivity with bovine IGF-1 (Moriel et al., 2012; Mercadante et al., 2016) was utilized. For all the abovementioned analyses, intra-assay CV was calculated using within assay replicates of each sample. Inter-assay coefficients were calculated based on control samples that were present in all assays. Control samples were selected to represent the physiological range of each response variable analyzed (IGF-1, insulin, BHB, NEFA, and glucose). Intra- and inter-assay CV were 7.36 and 8.74% for NEFA, 6.38 and 13.22% for BHB, 5.42 and 9.02% for glucose, 4.9 and 11.5% for insulin, and 7.64 and 3.58% for IGF-1, respectively.

*Statistical analysis*

Data for the present experiment were analyzed as a completely randomized design with a 2 × 2 factorial arrangement of treatments, where the individual cow was considered the experimental unit. Continuous response variables were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary., NC, USA; Version 9.4). For the analysis of BW and BCS on day −21, the model included the fixed effects of breed, diet, and breed × diet interaction. Year and cow (breed × diet × year) were included as random effects. The same model was used to analyze the abovementioned response variables and blood indicators of cow nutritional status (NEFA, BHB, glucose, insulin, and IGF-1) on day 19; however, their respective values on day −21 and BCS change between days −21 and 19 were utilized as covariates. The use of BCS as covariate allows for metabolic comparison of breed types under an equivalent level of body reserve mobilization, alleviating the potential bias from equivocal estimation of energy requirements in *B. indicus*-influenced cows. Initial

measurements were utilized as covariates to control for individual cow variation prior to the beginning of the dietary treatments. The probability of pregnancy to embryo transfer was evaluated according to the concentration of blood markers of metabolism measured in order to explore potential relationship between these parameters and pregnancy success. The GLM procedure of SAS was used to determine if pregnancy by embryo transfer was influenced linearly, quadratically, or cubically by the different blood markers across all genotypes and diets. The LOGISTIC procedure was then utilized to generate a regression model according to the maximum likelihood estimates from each continuous order effect. The probability of pregnancy was estimated based on the following equation: Probability of pregnancy $= (e^{\text{logistic equation}})/(1 + e^{\text{logistic equation}})$. Logistic curves were constructed and reported for the variables that were significant. Significance was declared at $P \leq 0.05$ and tendencies were determined when $P > 0.05$ and $\leq 0.10$.

## Results

Average ± SEM for days postpartum was 74 ± 2.7 at the initiation of the estrus synchronization protocol, which was not different between breed, diet, and breed × diet ($P > 0.10$). Results for energy intake between days −21 and 19, BW and BCS on days −21 and 19, together with BW and BCS change are summarized in Table 2. No effect of breed ($P = 0.45$) or breed × diet interaction ($P = 0.40$) was observed for feed intake. However, an effect of diet ($P = 0.01$) was observed, where cows in the MAINT diet consumed 1.5 kg of diet DM/d more than those in the RESTR diet. As proposed by the experimental design, there was an effect of diet on the percentage of daily energy requirements met based on individual cow intake ($P < 0.001$), where cows in the MAINT diet consumed a greater percentage of their net daily energy requirements for maintenance compared with cows in the RESTR diet regardless of cow breed (breed or breed × diet, $P > 0.10$). Additionally, there were no effects of breed, diet, or breed × diet interaction ($P > 0.10$) on cow BW and BCS at the beginning of the experiment (day −21). As expected, cows in the RESTR diet had lower BCS than cows in the MAINT diet on day 19 ($P = 0.008$). Yet, no effects were observed for cow BW on day 19 ($P > 0.10$). Body weight change was also not influenced by diet or breed ($P > 0.10$), but RESTR cows experienced greater BCS loss compared with MAINT cows ($P = 0.003$), regardless of the breed (breed: $P = 0.91$; breed × diet: $P = 0.30$).

Results for plasma circulating concentrations of NEFA, BHB, insulin, and IGF-1 at day 19 are summarized in Fig. 2. There were no effects ($P > 0.10$) of cow breed, diet, or cow breed × diet on circulating concentrations of NEFA, BHB, and glucose (72.2 ± 29.03 mg/mL; mean ± SEM) on day 19. There was a breed × diet interaction on plasma concentrations of insulin ($P = 0.03$), where AN–RESTR cows tended ($P = 0.08$) to have lower plasma concentration of insulin compared with AN–MAINT cows and had lower plasma concentration of insulin than BN–RESTR cows ($P = 0.04$). No differences in plasma insulin were observed between AN–RESTR and BN–MAINT, and there were no differences between BN cows in both MAINT and RESTR diets ($P \geq 0.20$). A breed × diet interaction was also observed ($P = 0.04$) for plasma concentrations of IGF-1 on day 19. Angus cows in the RESTR diet had decreased ($P \leq 0.02$) plasma IGF-1 compared with all other breed × diet combinations. Similar to the results observed for insulin, there were no differences in plasma IGF-I observed ($P > 0.10$) between BN–MAINT and BN–RESTR.

The observed differences in circulating concentrations of insulin and IGF-1 led us to explore a potential relationship between metabolites on days −21 or 19 and the probability of pregnancy to embryo transfer on day 28 through the use of a logistic regression. A linear relationship was observed between plasma concentrations of insulin on day −21 ($P = 0.001$) and the probability of pregnancy, where the probability of pregnancy increased as the plasma concentrations of insulin increased (Fig. 3A). A similar relationship (linear; $P = 0.04$) was observed between the probability of pregnancy and plasma concentrations of IGF-1 on day 19, where the probability of pregnancy increased as plasma concentration of IGF-1 increased (Fig. 3B).

## Discussion

Although it has been well documented that several physiological differences exist between *B. indicus* and *B. taurus* cattle (Hansen, 2004; Sartori et al., 2016), the majority of applied strategies utilized to enhance production efficiency in commercial *B. indicus*-influenced beef herds were researched and developed using *B. taurus* cattle. It is important to acknowledge and investigate these differences between subspecies to facilitate development of strategies tailored to increase production efficiency in *B. indicus*-influenced cattle. In the present study, we further explored a previous report which indicated that *B. taurus* suckled beef cows have decreased reproductive performance compared with *B. indicus*-influenced counterparts when both subspecies are exposed to the same energy restriction environment (Fontes et al., 2019). Because the objective of the present study was to better understand if these subspecies respond differently to energy restriction, rather than characterizing intrinsic differences in metabolic profile between them, initial measurements were used as covariates to control for individual cow variation prior to the beginning of the dietary treatments. No differences were observed between breeds for BW and BCS during the experiment. Yet, BCS change was used as a

**Table 2**
Effects of cow breed and dietary treatments[1] on recipient BW and body condition score (BCS).[2]

| Item | Diet | | *P*-value | Breed | | *P*-value |
|---|---|---|---|---|---|---|
| | RESTR | MAINT | | Angus | Brangus | |
| DM intake, kg/d | 18.5 + 0.50 | 20.2 + 0.45 | 0.01 | 19.6 + 0.48 | 19.1 + 0.50 | 0.45 |
| $NE_m$ requirements met,[3] % | 77.5 + 12.94 | 93.9 + 12.90 | <0.001 | 87.3 + 12.92 | 84.1 + 12.92 | 0.29 |
| Initial BW,[4] kg | 532.5 + 8.58 | 514.4 + 8.73 | 0.12 | 522.9 + 8.22 | 524.1 + 8.11 | 0.92 |
| BW at day 19, kg | 496.2 + 8.94 | 500.3 + 8.05 | 0.30 | 498.7 + 8.52 | 497.9 + 8.42 | 0.83 |
| BW change, kg | −59.6 + 9.00 | −47.9 + 8.10 | 0.82 | −52.8 + 8.60 | −54.7 + 8.50 | 0.18 |
| Initial BCS | 5.4 + 0.18 | 5.3 + 0.17 | 0.16 | 5.3 + 0.17 | 5.4 + 0.17 | 0.23 |
| BCS at day 19 | 4.9 + 0.06 | 5.1 + 0.06 | 0.008 | 5.0 + 0.06 | 5.1 + 0.06 | 0.40 |
| BCS change | −0.4 + 0.07 | −0.1 + 0.07 | 0.003 | −0.3 + 0.07 | −0.3 + 0.07 | 0.91 |

[1] MAINT: Diet formulated to meet the daily energy requirements of a 550 kg of BW suckled beef cow (CP = 12.5%, total digestible nutrients = 46%; NASEM, 2016), comprised of 60% fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls; RESTR: Diet formulated to meet 70% of daily energy requirements (CP = 11.2%, total digestible nutrients = 37%), comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls.
[2] There was no breed × diet interaction for these response variables ($P \geq 0.23$).
[3] Percentage energy requirements for maintenance ($NE_m$) that was met by the diet. Values were calculated based on nutrient analysis and individual intake of nutrients between days −21 and 19, according to the NASEM (2016).
[4] Initial BW and BCS represent estimates assessed on day −21 of the experiment.

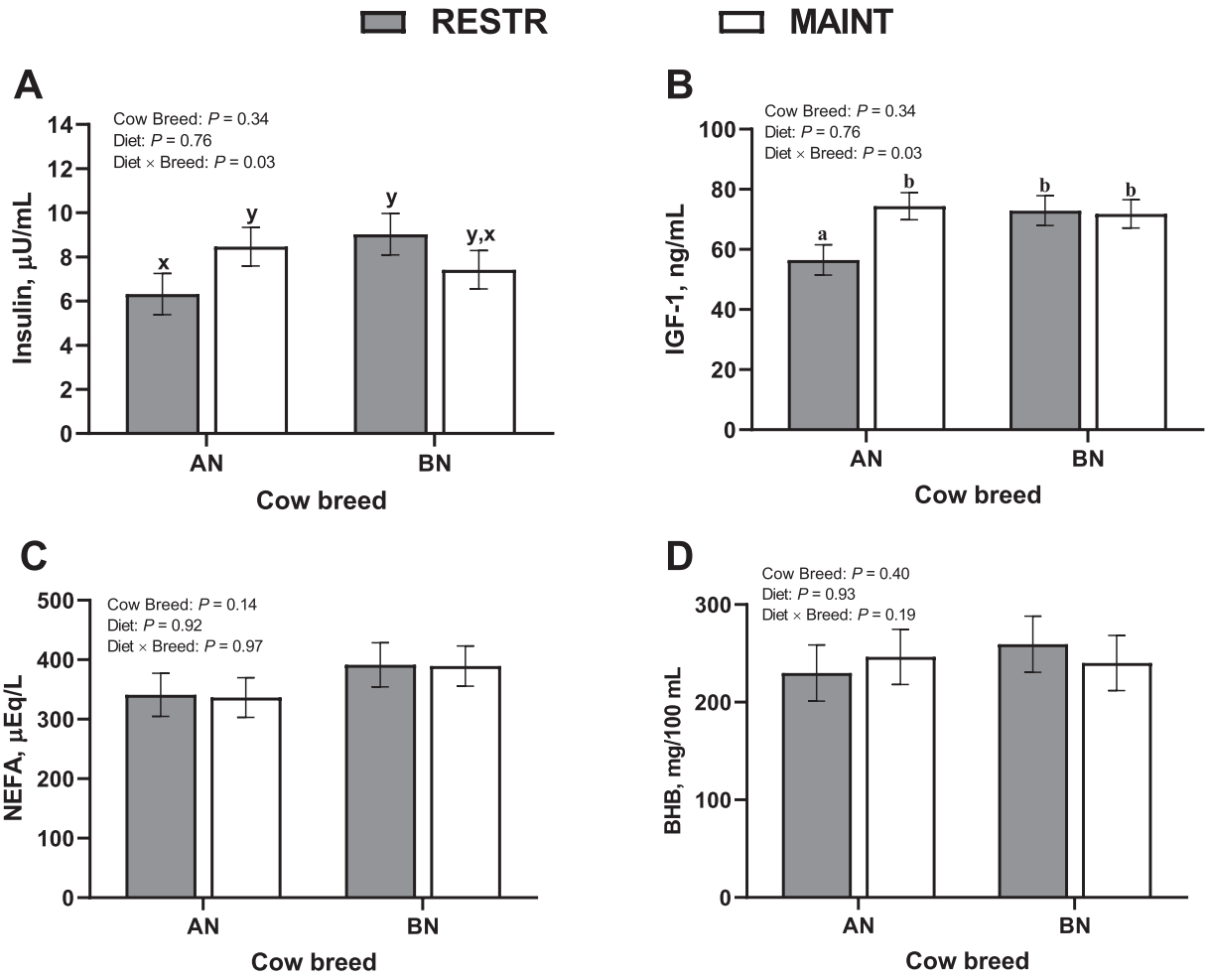


**Fig. 2.** Effects of cow breed and dietary treatments on plasma concentrations of insulin, insulin-like IGF-1, non-esterified fatty acids (NEFA), and β-hydroxybutyrate (BHB) on day 19 of the experiment (the first day of gestation was considered day 0). MAINT: Diet formulated to meet the daily energy requirements of a 550 kg suckled beef cow (NASEM, 2016). RESTR: Diet formulated to meet 70% of daily energy requirements. Dietary scheme initiated at day −21. Least square means reported were covariate-adjusted to cow BCS change from day −21 to 19 of the experiment and circulating concentrations of respective metabolic parameter at day −21. Error bars represent SE of the means. Panel A: $^{x,y}$ represent pairwise comparison differences ($P < 0.10$). Panel B: $P = 0.04$. $^{a,b}$ represent pairwise comparison differences ($P < 0.05$). AN: Angus; BN: Brangus; BCS: body condition score.

covariate in order to account for potential numerical differences in BCS change between AN and BN cows that could have driven differences in circulating concentration of markers of negative energy balance. Additionally, the use of BCS as covariate allows for metabolic comparison of breed types under an equivalent level of body reserve mobilization, alleviating the potential bias from equivocal estimation of energy requirements in *B. indicus*-influenced cows. Hence, the results reported in this study reflect intrinsic physiological response differences to a similar degree of nutritional deficit between *B. taurus* and *B. indicus*-influenced cattle.

It is reasonable to speculate that differences in energy requirements between the subspecies might be driving the differences observed in the present study. *Bos taurus* cattle are thought to have approximately 10% greater energy requirements for maintenance compared with purebred *B. indicus* breeds, and 5% greater requirements than *B. indicus* × *B. taurus* crossbred cattle (Vercoe, 1970; Patle and Mudfal, 1975; van der Merwe and van Rooyen, 1980s). However, more recent studies showed no differences in energy requirements between these subspecies (Tedeschi et al., 2002; Chizzotti et al., 2008), indicating that generalization of lower energy requirements for maintenance in *B. indicus*-influenced cattle might not be appropriate. This is particularly relevant for differences between breeds that are undergoing extensive genetic selection for greater productivity (e. g., rate of growth or milk production) and suckled beef cows, in which limited data on subspecies comparisons are available (NASEM, 2016). As proposed by the design of the present experiment, there was an effect of diet on the percentage of energy requirements met by the diets; however, the magnitude of the difference between RESTR and MAINT was less than we expected. This indicates that RESTR cows were able to partially compensate the lack of energy in their diet by increasing their intake, which might explain the lack of differences observed in glucose, BHB, and NEFA. Moreover, it might indicate that the differences reported here for insulin and IGF-1 could potentially be greater if the targeted energy restriction was achieved. These changes in intake when energy concentration of the diet is decreased have been reported previously as a potential mechanism to compensate (or even overcompensate) for daily energy intake (DiLorenzo and Galyean, 2010). Further supporting this concept, a positive linear relationship between dietary NDF and DMI was observed in a meta-analysis of literature data (Arelovich et al., 2008). Nevertheless, there were no differences between breeds on the percentage of energy requirements consumed during the experimental period. The difference in energy intake between dietary treatments led to differences in BCS by day 19. Although cows submitted to the RESTR and MAINT diets had similar BCS on day −21, cows in the RESTR diet had lower BCS than cows in the MAINT diet by day 19 across breed types. Not surprisingly, there were no effects of diet on cow BW on day 19, which was likely

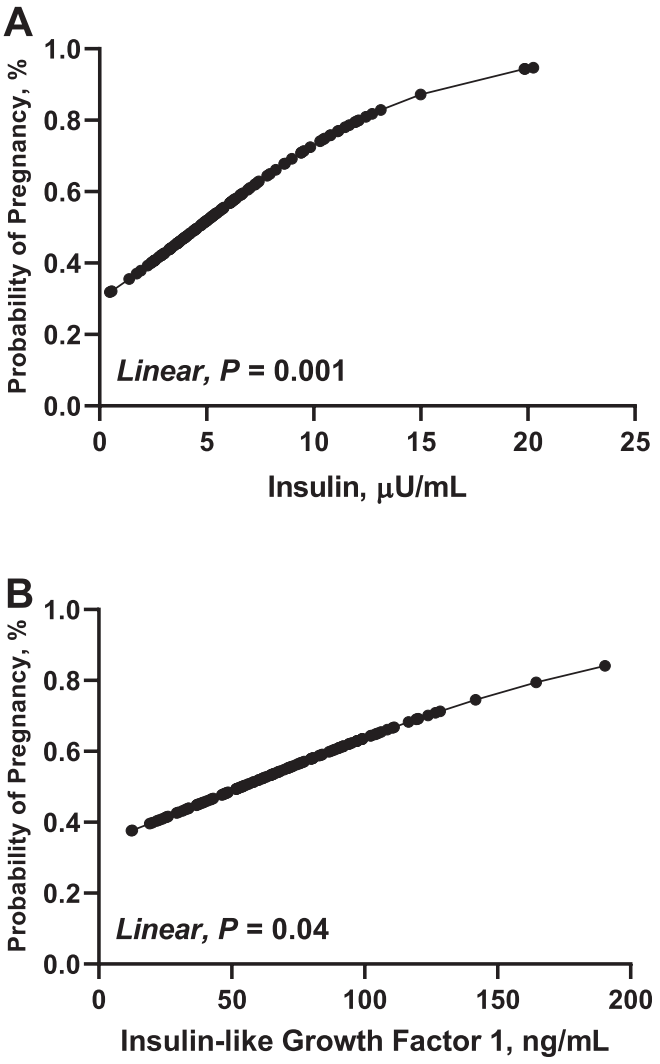


**Fig. 3.** Probability of pregnancy to fixed-time embryo transfer in suckled beef cows according to the plasma concentrations of insulin at day −21 (A) and IGF-1 at day 19 of gestation (B). Pregnancy status was assessed through transrectal ultrasonography on day 28 of gestation. Hosmer–Lemeshow goodness of fit: Panel A: $\chi^2 = 3.456$, df = 8, $P = 0.90$. Panel B: $\chi^2 = 12.913$, df = 8, $P = 0.12$.

associated with the fact that gut fill introduces considerable variation to BW data, and BW was assessed by a single measurement on day 19. Furthermore, the RESTR diet was formulated to decrease passage rate and increase gut fill due to the greater inclusion of peanut hulls, which could have decreased our ability to detect a main effect of diet on BW on day 19. These results indicate that the proposed diets were able to successfully induce a difference in energy intake between MAINT and RESTR diets. Additionally, no clear differences in energy intake, BW, and BCS were observed between subspecies.

Non-esterified fatty acids are a product of the breakdown of triglyceride ester bonds within adipocytes and are utilized to investigate the magnitude of lipolysis during negative energy balance in dairy and beef cattle (Richards et al., 1989; Staples et al., 1990; Marques et al., 2019). Beta-hydroxybutyrate is a product of the butyrate metabolism in the ruminal epithelium and hepatic metabolism of circulating NEFA, increasing in circulation when dietary energy fails to meet the requirements of the animal (Herdt, 2000). Hence, we expected to observe an increase in circulating concentrations of NEFA and BHB, indicating a negative energy balance in cows exposed to the RESTR diet compared with cows in the MAINT diet. Yet, no effects of diet or cow breed were observed on covariate-adjusted NEFA or BHB concentrations on day 19, indicating no subspecies differences in response to dietary treatments for these markers. Because BCS change was used as a covariate, it is not surprising that NEFA and BHB did not differ. Previous studies have reported that *B. indicus* females have greater circulating concentrations of NEFA compared to *B. taurus* in the postpartum when both were exposed to the same dietary conditions (Coleman et al., 2017). Similar results were observed when covariates were not utilized (estimates not reported) in the present study, where BN cows had greater NEFA and BHB compared to AN cows, regardless of the diets. Because the aim of this experiment was to evaluate how each subspecies respond to an energy restriction scenario in the postpartum period, covariates were included in the analysis to account for initial differences in NEFA and BHB. In this context, our results suggest similar lipolytic activity between subspecies when exposed to the same level of BCS loss.

While no differences between subspecies were observed for NEFA or BHB, AN cows in the RESTR diet had lower concentrations of both insulin and IGF-1 compared with AN cows in the MAINT diet, and BN cows in both MAINT and RESTR diet on day 19. Additionally, there were no differences in both insulin and IGF-1 between BN–RESTR and BN–MAINT, corroborating the proposed hypothesis that *B. indicus*-influenced cows are more resilient to reduced energy availability. Insulin concentrations during the postpartum have been previously shown to be decreased in cows that are losing compared with cows that are maintaining BCS (Sales et al., 2015; Sheehy et al., 2017). Additionally, dietary strategies that induce an increase in circulating concentrations of insulin can alleviate the effects of negative energy balance during the postpartum in cows that are not in a insulin resistant state, decrease the interval from calving until first ovulation, and increase the proportion of cows resuming postpartum cyclicity within 50 d post-calving (Gong et al., 2002). Circulating concentrations of IGF-1 were also shown to be influenced by nutrient intake (Bossis et al., 2000), energy balance (Spicer et al., 1990), and BCS (Bishop et al., 1994), where postpartum cows with greater energy intake have greater systemic concentrations of IGF-1 (Ciccioli et al., 1998). Hence, decreased circulating concentrations of insulin and IGF-1 can reflect a catabolic state associated with negative energy balance. Previous studies have reported that *B. indicus* cows have greater circulating concentrations of IGF-1 and insulin compared to *B. taurus* (Mendonça et al., 2013; Sartori et al., 2016). Insulin-like growth factor 1 is a potent stimulator of granulosa cell proliferation and steroidogenesis. Therefore, it has been proposed that these higher circulating concentrations of IGF-1 modulate differences in ovarian physiology that lead to the greater circulating concentrations of estradiol and progesterone observed in *B. indicus* females (Sartori et al., 2016). Nevertheless, the number of studies comparing these subspecies within the same environmental conditions is limited and often performed utilizing nonlactating dairy females (Sartori et al., 2016). Therefore, further research is required to better understand these underlying physiological differences between subspecies, more specifically their interaction with the nutritional status of beef females in the postpartum period.

The results for plasma concentrations of insulin and IGF-1 corroborated with the differences in fertility reported by Fontes et al. (2019), where AN cows had lower fertility compared with BN cows when both breeds were exposed to the RESTR diet. Therefore, a logistic regression was conducted to explore the relationship between plasma concentrations of IGF-1 and insulin on days −21 and 19 with the probability of pregnancy after fixed-time embryo transfer. The probability of pregnancy linearly increased as the concentrations of insulin on day 21 and IGF-1 on day 19 increased. As opposed to what is observed during early pregnancy in rodents and humans, bovine conceptuses have an increased period of preimplantation development in which they undergo considerable morphological changes before attaching to the uterine luminal epithelium (Betteridge and Flechon, 1988; Guillomot, 1995). These changes occur simultaneously with remarkable changes in the transcriptome of the endometrium and conceptus

(Ribeiro et al., 2014). Intriguingly, although fertilization rates are estimated to be greater than 80% in beef cattle (Santos et al., 2004; Reese et al., 2020), pregnancy rates at day 28 of gestation are usually around 40–60%, suggesting that pregnancy loss during the pre- and peri-implantation occurs in approximately 40% of beef females. Recent reports also indicate that these losses might be greater in *B. indicus* cattle managed under tropical and sub-tropical environments (Reese et al., 2020).

Growth hormone and IGF-1 receptors are expressed by the bovine uterus and conceptus during the same period (Kolle et al., 1997; Kolle et al., 2001), and these hormones influence conceptus development in ruminants (Spencer et al., 1999; Kolle et al., 2002; Moreira et al., 2002). Moreover, intrauterine infusion of GH increases the expression of histotroph proteins, and exogenous supplementation of recombinant bovine somatotropin post-insemination increased systemic concentrations of IGF-1, thereby increasing peri-implantation conceptus development, and fertility in lactating dairy cows (Ribeiro et al., 2014). Improved reproductive performance observed in cows in a better nutritional status throughout the literatures might, to some extent, be mediated by the role of the somatotropic axis on the female reproductive tract and conceptus. Additionally, the ability of *B. indicus*-influenced cows to maintain systemic concentrations of insulin and IGF-1 during energy restriction might be one of the mechanisms controlling the differences in pregnancy establishment between *B. indicus* and *B. taurus* cows under conditions of limited energy availability.

In conclusion, the results of the present study indicate that the magnitude of the impacts of energy restriction is greater in *B. taurus* vs *B. indicus*-influenced beef cows when cows are exposed to same conditions. Although there were no differences in energy intake, plasma insulin and IGF-1 differences between MAINT and RESTR cows were more evident by comparisons within *B. taurus* than within *B. indicus*-influenced cows. These results corroborate with the decreased pregnancy rates observed in AN cows compared to BN cows when both were exposed to energy restriction (Fontes et al., 2019), providing evidence of a greater resilience of *B. indicus*-influenced cattle to disruption in the somatotropic axis under scenarios of limited energy availability. Hence, the utilization of *B. indicus* or *B. indicus*-influenced females might be beneficial in order to partially remedy the lack of energy availability in specific beef production systems.

## Ethics approval

All procedures involving animals comply with ethical standards and were approved by the University of Florida Institutional Animal Care and Use Committee (protocol number 201408681).

## Data and model availability statement

Data and models have not been deposited in an official repository.

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## Declaration of interest

The authors declare no conflict of interest.

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