FIMPACT OF FETAL VERSUS MATERNAL CONTRIBUTIONS OF *BOS INDICUS* AND *BOS TAURUS* GENETICS ON FETAL DEVELOPMENT AND POSTNATAL PERFORMANCE

A Dissertation

by

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ABSTRACT

The present research focused on examining how energy restriction, fetal and maternal subspecies (*Bos taurus indicus* and *Bos taurus taurus*) interact to impact fetal development events, which can potentially alter nutrient utilization and efficiency of offspring in cattle. A reciprocal embryo transfer approach (n=134) was used in a completely randomized design with a 2×2×2 factorial arrangement of treatments in order to generate 55 pregnancies over 2 consecutive years. Recipient 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 requirements (**RESTR**). Angus (**AN**; *Bos taurus*) and Brangus (**BN**; *B. indicus*) embryo donors were superovulated and artificially inseminated with female sexed-sorted semen from the same breed. Embryos were then transferred to either AN or BN recipients fed their respective diets for 28 d. Recipients remained on the dietary scheme until d 91 of gestation. In utero female offspring development, postnatal growth, feed efficiency and puberty attainment were assessed. The main findings included greater pregnancy failure by d 28 of gestation in AN recipient (recipient breed×diet, $P<0.01$) and embryos (embryo breed × diet; $P=0.03$) when cows were exposed to the RESTR diet. Brangus cows had smaller ($P<0.05$) fetuses than AN, regardless of the breed of the embryo. Moreover, profile of circulating pregnancy associated glycoproteins differed during early gestation depending on the breed of the embryo ($P<0.05$), but not the breed or diet of the recipient ($P>0.10$). Analysis of the metabolic profile of the recipients during early gestation energy restriction corroborates with the differences in pregnancy failure. Angus×RESTR cows had greater body weight loss ($P<0.05$), and lower circulating concentrations of IGF-1 ($P<0.05$) and insulin ($P<0.05$) than AN×MAINT, whereas BN×MAINT and BN×RESTR did not differ

(P>0.10). Maternal diet or breed did not influence any postnatal response variables (P>0.10). Instead, AN heifers were more feed efficient (P<0.05), and achieved puberty earlier than BN heifers (P<0.05). In conclusion, pregnancy establishment and early fetal development was influenced by diet, maternal and fetal subspecies. However, maternal subspecies and diet did not alter postnatal growth and performance in the female offspring.

DEDICATION

To cattle producers and passionate scientists across the globe.

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1. INTRODUCTION AND LITERATURE REVIEW

Assessments performed by the United Nations estimated that the world population will increase by over a third between 2009 and 2050, likely reaching overall numbers close to 9 billion people (FAO, 2009). During the same period of time, individual average incomes are also expected to increase as shown by recent trends of growing economies in developing countries (Mensbrugghe et al., 2009). A positive correlation exists between per capita income and demand for animal food products (milk, meat, and eggs; Sans and Combris, 2015). These projections indicate that overall food production will have to increase by 70% and meat production by 42% in order to meet the demands for this growing population. Although considerable increases in food produced are needed, land availability for agriculture is estimated to increase only by 5-12% (FAO, 2009). Taken together, these projections indicate that our current food production systems will have to undergo considerable changes in order to optimize production efficiency and meet the growing demand for food, while at the same time while maintaining ecological stewardship and proper use of limited natural resources.

In a scenario where the amount of land for agriculture will not increase at the rate that food demand increases, ruminant species will play a key role on sustainable intensification of food production due to its ability of converting human-inedible sources of energy and protein to human-edible food (Tedeschi et al., 2015). At least 70% of the increase in beef production required to meet this growing demand is expected to come from tropical/subtropical regions of the planet (FAO, 2009), including Southern US, Mexico, Central/South America, Africa, Asia, and Oceania. Furthermore, approximately 70% of the world's cattle population is located in these regions (Robinson et al., 2014),

being exposed to environments characterized by high temperatures and humidity, as well as diets based on forages and agricultural by-products. Hence, the development of sustainable food production strategies that optimize human-edible protein production under these conditions are required.

Rising environmental temperatures is currently impacting and will continue to impact production efficiency of animal production. A recent Council for Agriculture and Science Technology (CAST) report estimates an increase in U.S. temperatures by 2 to 4°C within the next 25 years that will continue to provoke erratic weather patterns and intensify the severity of climate disruptions. Beef production schemes that utilize *Bos indicus* genetics might have the potential to limit the severity of the consequences that climate change might induce in future beef production. Therefore, continued efforts in understanding differences biological differences between *B. indicus* and *B. taurus* cattle, and how these differences impact beef production is paramount to sustaining beef production systems in tropical, subtropical, and other climates that may be adversely influenced by global climate changes over the next several decades and centuries.

A comparison of beef production data between 2007 and 2017 with data from 1967 and 1977 indicates that beef production in the United States has considerably changed over the last 50 years (adapted from FAOSTAT, 2017). The 10-year average number of animals slaughtered yearly decreased 20.5%, whereas the average beef production per year increase 11.9% in the same period of time. This occurred as a consequence of a substantial increase in carcass weight at slaughter. Average carcass weights went from 251.3 kg between 1967 and 1977, to 353.4 kg in the last decade (40.6% increase; FAOSTAT, 2016). These substantial gains in production efficiency were realized through the development and

adoption of a variety of technologies, including the optimization of grain feeding production systems, pharmaceutical-based technologies, and the application of crossbreeding and selection programs focused on performance traits.

Further gains in beef production efficiency will require new insights into the physiological, endocrine, and molecular mechanisms controlling various aspects of animal growth and development. The newfound insights into the developmental origins of health and disease provide new opportunities to develop strategies that will improve production efficiency of food-producing livestock. The specific interest of this dissertation is to examine how nutrition-based modifications in early fetal development impacts pre- and postnatal development, feed efficiency and reproduction. The present research efforts focused specifically on examining how nutritional restriction, fetal and maternal genetics interact to impact fetal programming events in ways that can potentially alter nutrient utilization and nutrient efficiency of offspring. Consequently generating changes in the quality and efficiency of producing meat and generating replacement heifers in beef production systems.

1.1. Benefits and Limitations for Using *Bos indicus* Genetics in the United States Beef Industry

Domestic cattle are classified phylogenetically in the *Bovidae* family. This classification contains both humped and non-humped cattle in the same genus and species designation as two subspecies (*Bos taurus taurus* and *Bos taurus indicus*, respectively). For the purposes of this dissertation, the species designation will be replaced by the subspecies designation throughout this dissertation. Although these two subspecies originated from the same ancestor, mitochondrial DNA and microsatellite sequencing

indicates they diverged from one another 110,000 to 850,000 years ago (Bradley et al., 1996; MacHugh et al., 1997). Exposure to the hot and humid climate of the Indian subcontinent induced selection for heightened tolerance of *B. indicus*, or Zebu cattle, to heat and parasites. Conversely, *B. taurus* cattle underwent environmental and artificial selection in the arid climates of Europe and Africa. It appears that minimal selection pressure for production traits (e.g. meat, milk production) has been placed on *B. indicus* cattle. The current challenge is to maximize the benefits of using *B. indicus* genetics while minimizing their limitations in production efficiency in the intensive, feedlot-based U.S. beef production system.

Approximately 30% of cattle in the U.S. contain some *B. indicus* genetics, and approximately 40% of beef cows and 50% of the country's cow-calf producers are located in the southern U.S. where *B. indicus* cattle and their crosses are located (Morrison, 2005). These cattle and the various breeds generated from crossing *B. indicus* cattle with European breeds contain a greater tolerance to elevated ambient temperatures and humidity than most European beef and dairy breeds (Hansen, 2004). They also contain a greater resistance to internal and external parasites (Turner, 1980). When Zebu cattle are exposed to warmer conditions, they experience less reduction of feed intake (Allen et al., 1963; Seif et al., 1979), growth rate (Cartwright, 1955) and reproductive function (Rocha et al., 1998) compared with *B. taurus* cattle exposed to the same conditions. However, *B. indicus* cattle also are characterized as slow-growing in the feedlot, and their carcasses usually grade lower and produce less tender beef than *B. taurus* breeds (Turner, 1980). The utility for these cattle in the U.S. beef industry lies in their use in generating either F1 crosses or new

breeds of cattle that contain the heightened thermal and parasite tolerance of Zebu cattle while retaining reproductive, growth, and carcass characteristics of European breeds.

Several differences exist between *B. indicus* and *B. taurus* cattle in their ability to utilize feeds. Literature from the 70's and 80's indicates that *B. indicus* cattle utilize low quality forages more efficiently than *B. taurus* breeds (Turner, 1980). *Bos indicus* breeds also have greater dry matter intake and digestibility than European breeds when provided low quality forages (Ikhatua et al., 1985; Moore et al., 1975; Warnick and Cobb, 1976). They also have greater apparent nitrogen digestibility and reduced protein clearance rates than *B. taurus* cattle (Ashton, 1962; Karue et al., 1972). In addition, *B. indicus* cattle are thought to have lower maintenance requirements than *B. taurus*, which may benefit them during times of nutrient restriction. In one study, Brahman (*B. indicus*) cattle exhibited a greater growth potential during feed restriction than Shorthorn and Hereford cattle (Frisch and Vercoe, 1977). Zebu cattle also excel in stressful environments. Growth rates of Brahman calves were greater than those of Hereford × Shorthorn crosses when calves received no heat abatement and no control for internal and external parasites (Frisch, 1978).

The majority of the studies evaluating subspecies differences and interaction between subspecies and different environments were performed 20 to 30 years ago. Because cattle genetics change substantially over time, it is paramount that these subspecies differences are reassessed. Recent reports indicate that these cattle are more efficient at utilizing low quality feeds and preserving body composition during periods of feed restriction and environmental stress (Elzo et al., 2009). Brahman cattle were more feed efficient than Angus (**AN**; *B. taurus*) based on residual feed intake, suggesting that

even today's *B. indicus*-based cattle appear to be more efficient at utilizing feed than their *B. taurus* counterparts.

1.2. Alterations in Early Fetal and Placental Development in *Bos indicus* cattle

A series of experiments have been conducted by our group comparing differences in early fetal development between *B. indicus* and *B. taurus* cattle (Mercadante, et al., 2013). The first indication that early developmental changes in fetal growth rates exists in *B. indicus* cattle came from studying the multi-breed Angus-Brahman herd at the University of Florida (Gainesville, FL). A single in vivo measurement through ultrasonography from crown rump length (CRL) of the fetuses at d 53 of pregnancy determined that cows containing primarily AN genetics (>80% AN) had larger fetuses than cows with at least 20% Brahman genetics (>20% Brahman; Figure A1-Panel A). A second study (Marianna, FL) examined fetus size at d 35 and 62 of gestation (Figure A1- B). Fetus size did not differ in AN and Brangus (**BN**; *Bos indicus*-influenced: 5/8 AN and 3/8 Brahman) cows at d 35, but fetuses in AN cows were larger than fetuses in BN cows at d 62. In a third study (Figure A1-C; Alachua, FL), fetus size was assessed weekly from between d 33 and 55 of gestation. There were no differences between AN and BN cows on d 33-34, 40-41, and 47-48 of pregnancy, but fetus size was greater in AN cows at d 54-55 of pregnancy. Collectively, these studies indicate *B. taurus* fetuses were larger than *B. indicus* fetuses at d 53-62 of pregnancy but were similar at earlier time points in gestation.

Subspecies genotype also affects plasma pregnancy-associated glycoprotein (PAGs) concentrations in early pregnancy. These are abundantly expressed products of the placenta within even-toed ungulate animals, where temporal and spatial expression in the placenta have been, to some extent, characterized in domestic ruminants, as well as in

swine (reviewed by Wallace et al., 2015). Early embryonic development and placentation in domestic large animals have unique characteristics, particularly in ruminants. In cattle, as the blastocyst hatches from the zona pellucida, the free floating conceptus undergoes dramatic morphological and functional changes prior to implantation. After hatching, the trophoblast of the spherical blastocyst elongates along the uterine lumen, increasing its surface area for apposition prior to adhesion to the uterine luminal epithelium. As the conceptus elongates from an ovoid (1-4 mm) and tubular (5-19 mm), to a filamentous (20-60 mm) form, it undergoes remarkable changes in the transcriptome which is associated with changes in the composition of the uterine histotroph and endometrial biology (Betteridge and Flechon, 1988; Bazer et al., 2018; Ribeiro et al., 2016). These series of events precede attachment and represent an orchestrated paracrine cross-talk between the preimplantation conceptus and the endometrium that is required for successful pregnancy establishment and has been comprehensively reviewed by others (Spencer et al., 2015; Bazer et al., 2018). During these period, mononuclear trophoblast cells constitute the majority of the trophoblasts in ruminants. As the ruminant conceptus develops, a second distinct populations of syncytial trophoblast cells begin to differentiate from the mononuclear trophoblast cells. These are referred to as binucleated giant cells (BNCs) in cattle, and are thought to arise through mitotic polyploidy (Klisch et al., 1999). These cells express PAGs and are first detected on the third week of pregnancy in cattle (Wallace et al., 2015). As the attachment (d 21-30 in cattle; King et al., 1981) of the conceptus trophoblast to the uterine luminal epithelium progresses, BNCs migrate to the luminal epithelium for syncytialization with epithelial cells. Pregnancy associated glycoproteins located within secretory granules of BNCs are released into the uterine stroma and some of

these proteins make their way into maternal peripheral blood circulation (Green et al., 2005). Hence, although the roles of these proteins are not completely understood (Wallace et al., 2015), they are successfully utilized as biomarkers of pregnancy in ruminants (Sasser et al., 1986; Wood et al., 1986, Ruder et al., 1986).

The preimplantation period of conceptus elongation and apposition, together with subsequent progressive attachment of the trophoblast to the apical luminal epithelium of the uterus encompasses a pivotal period of pregnancy loss in cattle. Fertilization rates are considered relatively high in cattle. Santos et al., (2004) summarized data from studies that evaluated fertilization in beef and dairy cows using different embryo retrieval techniques, including oviduct and uterine flushing in live and slaughtered females. Fertilization rates in beef cattle ranged from 75 to 98% on average in beef cows (lactating and non-lactating cows, respectively) and averaged 88% in heifers. In dairy cows, average fertilization rates reported were 76% for lactating cows and 78% for non-lactating cows (Santos et al., 2004). Although fertilization rates are relatively high and the majority of beef cows have an embryo or embryo-like structure within the first weeks post breeding, pregnancy rates in beef females that are exposed to fixed-time artificial insemination generally range between 40-60% at 30 d after breeding (Lamb et al., 2010), indicating that pregnancy loss between fertilization and the first pregnancy diagnosis after breeding affect a considerable proportion of cows and heifers. Therefore, the first 30 d of gestation represent a pivotal period for pregnancy establishment in cattle (Wiltbank et al., 2016) and have considerable implications to fertility and beef production efficiency.

Pregnancy associated glycoproteins were also proposed to be indicators of pregnancy failure in cattle (Gabor et al., 2007; Lopez-Gatius et al., 2007b; Thompson et

al., 2010). Physiological factors such as fetus number, fetus gender, parity, and lactation status and manipulations such as in vitro embryo development and nuclear cloning influence PAGs concentrations in peripheral blood circulation of cows (Chavatte-Palmer et al., 2006; Constant et al., 2011; Lopez-Gatius et al., 2007a; Patel et al., 1995). Circulating concentrations of PAGs also differ between genotypes. For example, serum concentrations of PAGs differ between Ethiopian Boran and Boran × Holstein-Friesian crossbreds (Lobago et al., 2009). Differences in PAG concentrations also exist between Texel and Suffolk ewes (Vandaele et al., 2005). A more recent report from our group determined that PAG concentrations are greater in BN and Brahman cows than AN cows in early gestation (Figure A2; Mercadante, et al., 2013). Plasma PAGs concentrations were greater in BN than AN cattle at each of the four weeks of sampling between days 33-34 and 54-55 of pregnancy. It is interesting that *B. indicus*-influenced genetics produce smaller fetuses and greater plasma concentrations of PAGs. Elevated plasma PAGs concentrations have been reported in physiological scenarios of placental insufficiencies, including pregnancies generated by somatic cell nuclear transfer technology (Chavatte-Palmer et al., 2006; Constant et al., 2011). On the contrary, lower circulating concentrations of PAGs were observed at d 24 (Reese et al., 2018) and 30 (Gatea et al., 2018; Franco et al., 2018) of gestation in cows that were diagnosed as pregnant at d 30 but lost their pregnancy between d 30 and d 100. The current understanding of the influence of PAGs on pregnancy establishment and maintenance is limited and does not provide insights into whether a placental insufficiency phenomenon exists in *B. indicus* cattle. A more reasonable presumption is that differences in PAGs profile reflect yet another genotype-dependent alteration in the normal progression of pregnancy between *B. taurus* and *B. indicus*.

1.3. Alterations in *Bos indicus* Fetal and Placental Development in Mid- and Late-gestation

The blunted growth of BN and Brahman fetuses detected during early gestation (Mercadante et al., 2013) continues into mid-gestation (Ferrell, 1991; O'Rourke et al., 1991). By examining fetal development at slaughter, O'Rourke et al (1991) identified delays in fetal growth throughout much of pregnancy by measuring CRL, heart girth, and foreleg size at slaughter in *B. indicus* breeds (Sahiwal, Africander, Brahman) and *B. taurus* breeds (Hereford, Simmental). Ferrell (1991) reported that Charolais fetuses were 70% greater in weight than Brahman fetuses at d 232 of gestation. Weights of placental membranes also were greater in Charolais cattle, and both breeds contained similar fetal to placental membrane ratios, indicating that placental efficiency might be similar between breeds. *Bos indicus* cattle may compensate for delays in fetal development by extending their gestation length. *Bos indicus*-based breeds traditionally have extended gestation lengths and yield calves with similar or greater birth weights than *B. taurus* breeds (Reynolds et al., 1980; Riley et al., 2007). In our recent work, gestation lengths were 4 to 6 days longer in BN and Brahman cattle than AN cattle (Mercadante et al., 2013).

In order to examine the relative contributions of Brahman and Charolais genotype to fetal development, Ferrel, (1991) evaluated pregnancies in Brahman and Charolais cows gestating Brahman or Charolais fetuses. Fetal genotype is a primary determinant of fetal growth rate during mid-gestation. At week 33, a direct effect of fetal breed was evident, where the weight of Charolais fetuses was greater than Brahman fetuses regardless of the cow they were gestating in. There was no effect of recipient breed and no fetal by recipient interactions at week 33. However, a recipient genotype effect was noted at week 38.5. The

rate of fetal growth between weeks 33 and 38.5 was profoundly greater in Charolais cows regardless of whether they contained Charolais or Brahman fetuses. These findings are consistent with the notion that fetal growth is delayed in *B. indicus* cattle for most of gestation and indicates that fetal genotype controls growth differences between subspecies at mid-gestation, whereas the maternal genotype controls at least some of the fetal growth potential in the last 6-7 weeks of gestation.

The maternal system also appears to play a role in establishing body composition in bovine fetuses. The reciprocal embryo transfer studies by Ferrell (1991) examined weights of various fetal tissues and organs, including the semitendinosus muscle, at weeks 33 and 38.5 of gestation. Two interesting differences were noted. First, there appears to be late compensatory muscle development in Brahman fetuses, and this is controlled to a large extent by the maternal environment. Semitendinosus weights were less in Brahman fetuses gestating in Brahman cows than Brahman fetuses gestating in Charolais cows at week 33, but weights were similar at week 38.5. Secondly, there is a marked difference in muscle growth characteristics in Charolais fetuses in late gestation. Semitendinosus muscle weights were roughly equivalent in Charolais fetuses gestating in either Charolais or Brahman cows at week 33, but the increase in muscle weight between weeks 33 and 38.5 was much greater for Charolais fetuses gestating in Charolais cows than those gestating in Brahman cows (2.5 vs. 1.3 fold increase, respectively). In an accompanying study, Ferrel, (1991b) reported lesser uterine artery blood flow in Brahman cows and suggested that placental vascularization differences between subsepecies might be controlling differences in fetal development.

A more recent report evaluating uterine artery hemodynamics and placentome vascular density also observed differences between subspecies (Lemley et al., 2018). Brahman and AN heifers were artificially inseminated with a single Hereford (*B. taurus*) sire. Brahman heifers had increased cotyledonary blood vessel density at d 175 of gestation, and increase transcript abundance of angiogenic factor compared to *B. taurus* heifers. Moreover, using a macroscopic approach to evaluate arterial perfusion of a fluorescent substrate through the cotyledonary artery, there was an increase in substrate perfusion in the placentomes of *B. indicus* vs. *B. taurus* heifers. Similar outcomes were observed in heifers that were nutrient restricted from d 30 to 175 of gestation (Lemley et al., 2018), which has been shown to blunt fetal development in cattle (Long et al., 2009). Therefore, this increase in capillary density may reflect a compensatory response of the cotyledon in an attempt to compensate for suboptimal fetal development and sparks the questions of whether differences in placental efficiency in *B. indicus* cattle might restrict fetal development.

1.4. Using Diet to Manipulate the Fetal Development and Postnatal Growth in Ruminants

Seasonal variations in temperature, length of day, and precipitation affect plant metabolism, and consequently dictates forage productivity. With the demand to select for animals that have increased growth and production, with higher energy and protein requirements (Bir et al., 2018), matching forage quality and yield with nutrient requirements becomes more challenging. In the 1980's Dr. Barker and colleagues began publishing epidemiological findings of associations between birth weight and lifetime risk of coronary heart disease in humans (Barker, 1997; Barker and Osmond, 1986; Barker et

al., 1989). Additional associations have since been made between birth weight and risk for Type II diabetes, hypertension and stroke (Barker et al., 2002; Barker et al., 2010). It is now well-accepted that birth weight is not a prerequisite for these problems but rather represents one of several scenarios of slowed fetal development that predisposes people for various physiological disorders after birth and throughout life. Interestingly, Robinson et al., (2013) explored the relationship between birth weight and different productive outcomes in cattle. These authors reported that prenatal chronic nutrient restriction from d 80 of gestation until parturition reduced birth weights by 3.7 kg. After intrauterine growth retardation, there were little or no evidence of compensatory growth after weaning. In fact, although there was considerable variation in birth weights in both prenatal treatments, a consistent positive relationship was observed between birth weight and retail beef yield at 30 months. Sheep is a well-established and recognized biomedical model for studying human fetal programming events. One well-studied approach to understand fetal programming in sheep is to induce intrauterine growth restriction (**IUGR**). Fetal growth restriction in late gestation can be induced during early gestation in ewes by heat stress exposure, surgical blood flow restriction, or nutrient restriction (Barry and Anthony, 2008; Wallace et al., 2005; Yates et al., 2012). These events impair nutrient availability to the fetus and create hypoglycemic states in utero that predispose offspring to metabolic disorders and obesity after birth. Work at the University of Wyoming extensively examined the impacts of nutrient restriction in the first half of gestation in ewes (Burt et al., 2007; Dong et al., 2008; Ford et al., 2007; Vonnahme et al., 2003). Feed restriction (50% of maintenance) in early gestation decreased fetal weight and CRL at mid-gestation. Moreover, feed restriction from d 30 to 78 of gestation was associated with an increase in

right and left ventricle growth, and decreased the fetal to caruncule and fetal to cotyledon weight ratios, suggesting potential alteration in placental efficiency (Vonnahme et al., 2003). Interestingly, fetal growth accelerated when early gestation nutrient restriction was followed by re-alimentation to 100% maintenance diet for the remainder of pregnancy, and birth weights were not different between these lambs and non-feed-restricted controls. Ford et al., (2007) explored the consequences of nutritionally altered planes of fetal on postnatal growth and glucose metabolism of the offspring. Although lambs from nutrient restricted ewes had similar birth weights, early gestation nutrient restriction altered postnatal glucose metabolism. After a glucose tolerance test, lambs from nutrient restricted ewes had greater area under the curve (AUC) for glucose and lower AUC for insulin. Moreover, lambs from restricted ewes had greater leptin concentrations, were heavier at slaughter and had greater visceral adiposity. The authors did not measure dietary intake of the offspring. Hence, it is not known whether these differences occur in response to compensatory hyperphagia.

Limited research evaluated the postnatal consequences of early gestation nutrient restriction in cattle. When cows were exposed to nutrient restriction from d 30 to 125 of gestation, IUGR was induced by d 125. Furthermore, cotyledonary weights and placentome surface area were also reduced. Interestingly, fetal weights were not different at d 245 of gestation, indicating that realimentation to the nutrient requirements induced a compensatory growth in fetuses from restricted cows (Long et al., 2009). When heifers were exposed to a diet that restricted overall intake of nutrients to 55% of the requirements from d 32 to 83 of gestation and pregnancies were carried to term, no major changes in growth postnatal growth rates we observed. However, steers tended to be heavier at feedlot entry and slaughter. Furthermore, the abundance of mRNA for genes related to glucose and

fat metabolism (*AP2, CD36, GLUT4*) were decreased in visceral adipose tissue of steers from nutrient restricted heifers: however, DMI was not measured. Hence, it is reasonable to speculate that prenatal nutrient restriction might influence postnatal nutrient utilization by the offspring and consequently alter feed efficiency. As mentioned previously, postnatal restriction during early gestation increased postnatal circulating leptin concentrations and adiposity in the sheep (Ford et al., 2007), which could be associated with hyperphagia. To our knowledge, no previous studies evaluated feed efficiency of early gestation prenatal restricted cattle.

1.5. Effects of Nutrient Restriction on Muscle Development in Cattle

Muscle fiber development can be roughly separated into prenatal and postnatal stages. In livestock species, muscle fibers are completely formed before birth and postnatal muscle growth occurs primarily as consequence of an increase in diameter and length of the existing fibers. Therefore, altering muscle fiber formation prenatally may alter postnatal muscle mass of livestock animals (Du et al., 2010). Nutrient restriction and re-alimentation of beef cows exhibits many similarities to the ovine model, including rapid in utero compensatory growth of the fetus. Fetal weight gain likely includes catch-up growth by organs and tissues but may also reflect compensatory growth of select tissues. Muscle growth in utero is asymmetric with primary fibers, large tube-like structures, forming during the embryonic and early fetal period followed by a second wave of fiber formation that establishes the final complement of muscle fibers at birth. A closer examination of the effects of nutrient restriction on primary and secondary fiber formation was previously explored in cattle (Gonzalez et al., 2013). Multiparous cows were assigned to a control diet designed to meet the nutritional requirements of a pregnant beef cow gaining 0.48 kg/d or

a nutrient restricted diet providing 70% of the net energy requirements for maintenance and crude protein recommendation. The nutrient restricted cows were re-alimented at d 85 of gestation to control dietary recommendations. Representative animals were slaughtered at d 85, 140 and 254 of gestation and fetuses harvested. Dystrophin immunohistology was performed to define the sarcolemma boundary for the measurement of muscle fiber cross sectional area (**CSA**) in the fetal infraspinatus (**INF**) muscle. Representative images are shown in Figure A3-A. The fetal INF displays distinct waves of myogenesis with primary fibers evident at d 85 of gestation followed by secondary fiber formation at d 140 and 254. Caloric restriction through d 85 is noted by the existence of large primary fibers surrounded by smaller dystrophin positive secondary fibers (Figure A3). Measurement of the fetal INF fibers at d 85 demonstrated that fibers from nutrient restricted fetuses were nearly twice as large as controls (Figure A3-B). Re-alimentation of the nutrient restricted dam allows for growth of the secondary fibers, however, the fibers remain smaller than those found in control fetuses at d 140. By contrast, nutrient restricted fetal myofiber growth exceeded that found in control fetuses resulting in a larger fiber at d 254 of gestation. The larger fiber indicates that nutrient restriction causes precocious primary myogenesis coupled with compensatory secondary myogenesis. Fetal muscle is formed from Pax7 expressing myogenic precursor cells. A subset of these cells is retained throughout gestation and comprises the adult muscle stem cell pool at birth. To further refine the mechanism underlying increased primary fiber size, the numbers Pax7 positive cells were enumerated over time in the INF of control and nutrient restricted fetuses. As shown in Figure A3-C, a severe reduction in the percentage of Pax7 positive myogenic cells is found in the nutrient restricted fetuses by comparison to control at d 85. The loss of

progenitor cells likely represents their fusion into the primary fibers and/or formation of secondary fibers. Removal of the nutritional insult does not impact Pax7 cell numbers at d 140 or d 254 suggesting no irrevocable damage to the muscle progenitor pool. Precocious differentiation and retention of a robust progenitor population in the nutrient restricted fetus is further supported by no change in myonuclear domain (MND) size (Figure A3-D). The MND is defined as the volume of a muscle fiber whose synthetic capacity is controlled by a single myonuclei. No change in MND indicates that myoblast nuclei are added to the growing fiber to support the protein synthetic demands for template mRNA. These results argue that fetal muscle is highly plastic and able to adapt to nutritional insults in utero. Moreover, the existence of compensatory growth of nutrient restricted fibers in utero without a detrimental effect on the progenitor population indicates that the muscle may more efficiently use nutrients during postnatal growth.

1.6. Considerations for Fetal Programming Experimental Design

In order to truly study the mechanisms in which different prenatal treatment alters in utero developmental programming and consequently postnatal life the offspring, it is required that the effects of the treatments during gestation are uncoupled from their postnatal consequences to the dam. These consequences include, but are not restricted to, postnatal maternal health, colostrum availability, composition, and lactation performance. Therefore, utilizing experimental designs that separate the effects of prenatal treatments from its postnatal consequences is key (Robinson et al., 2013). This is particularly true for experiments that evaluate treatments during the last trimester of gestation. Conversely, the interactions between prenatal treatments and its postnatal consequences to the dam provide

valuable information when specific experimental findings that have the potential of being directly translated to cattle producers.

Different experimental design alternatives are available in order isolate prenatal effects or to explore the interaction between the pre- and postnatal consequences of a given treatment during gestation. Artificial rearing, ideally animals are removed from dams at birth and provided with a BW-specific allowance of a standardize colostrum followed by a common pre-weaning feeding strategy (Greenwood et al., 1998). Cross-fostering and factorial designs are also valuable alternatives, which allow researchers to isolate the pre- and postnatal treatment effects, as well as explore the interaction between them (Robinson et al., 2013).

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2. IMPACT OF FETAL VERSUS MATERNAL CONTRIBUTIONS OF *BOS INDICUS* AND *BOS TAURUS* GENETICS ON EMBRYONIC AND FETAL DEVELOPMENT[*]

2.1. Introduction

As a consequence of hundreds of thousand years of separate evolution, *Bos indicus* cattle have acquired genes that bestow a greater heat tolerance in comparison to *B. taurus* cattle (Hansen, 2004). When Zebu cattle are exposed to warmer conditions, they experience less reduction of feed intake (Allen et al., 1963; Seif et al., 1979), growth rate (Cartwright, 1955) and reproductive function (Rocha et al., 1998) compared with *B. taurus* cattle exposed to the same conditions. Furthermore, zebu cattle are thought to utilize low quality feeds more efficiently than *B. taurus* breeds (Turner, 1980), having greater dry matter intake and digestibility than European breeds under these conditions (Ikhatua et al., 1985; Moore et al., 1972; Warnick and Cobb, 1976). For these reasons, cattle producers utilize *B. indicus* breeds in crossbreeding systems to optimize cattle performance in tropical/subtropical climates.

Differences between several gestational parameters are also evident *between B. indicus* and *B. taurus* cattle. Both the literature and recent findings demonstrate *B. indicus* fetuses develop at a slower rate during early (Mercadante et al., 2013) and mid-gestation (Ferrell, 1991; O'Rourke et al., 1991). During late gestation, *B. indicus* fetuses undergo compensatory growth, having similar or even greater birth weights than *B. taurus* counterparts (Mercadante et al., 2013). The plasma concentrations of pregnancy-associated

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glycoproteins (**PAGs**) during early gestation also differ between the subspecies, where *B. indicus*-influenced cows have greater PAGs concentrations than *B. taurus* (Mercadante et al., 2013).

Therefore, the objective of this study was to determine how maternal and fetal genotypes influence early fetal development in *B. indicus* and *B. taurus* cattle. We hypothesized that early fetal and placental development is modified by the inclusion of *B. indicus* genetics into the maternal genotype. In addition, since nutrient utilization and feed efficiency differ between *B. indicus* and *B. taurus* cattle (Turner, 1974; Elzo et al., 2009), we hypothesized dietary energy restriction during early pregnancy would impair conceptus development in both *B. indicus*-influenced and *B. taurus* cattle; however we hypothesized the magnitude of the compromised development would be less evident in *B. indicus*-influenced cattle.

2.2. Material and Methods

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

2.2.1. Experimental Design

A reciprocal embryo transfer approach was used in a completely randomized design with a 2 × 2 × 2 factorial arrangement of treatments in order to generate 55 pregnancies over 2 consecutive years (n = 55). Initially, recipient cows (n = 197) were randomly assigned to 1 of the 2 dietary treatments: 1) a diet to meet 100% of daily energy maintenance requirements (**MAINT**), or 2) restricted intake of nutrients to 70% of daily energy maintenance requirements (**RESTR**). Details of the diets are discussed later. Angus (**AN**) and Brangus (**BN**) embryo donors were superovulated and artificially inseminated

(**AI**) with female sex-sorted semen from their respective breed. Embryos were then collected and randomly transferred to either AN or BN recipients fed their respective diets; thereby, generating 8 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* = 15, BN × AN × MAINT, *n* = 19). Female sex-sorted semen from 4 AN and 2 BN sires were used to AI donor cows. The resulting embryos were assigned to recipients in order to randomly generate the previously described treatment combinations, and to equally distribute the effects of sire, donor cow, and embryo grade score (Bó and Mapletoft, 2013).

2.2.2. Recipient Diet

A total of 197 AN and BN suckled beef cows were enrolled as recipient candidates for this study. 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 monitor individual feed intake (GrowSafe System Ltd., Airdrie, AB, Canada). 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 energy requirements of a 550 kg beef cow (MAINT; NASEM, 2016); and 2) Diet formulated to meet 70% of daily energy requirements (RESTR; Table B1). During the 2 consecutive years, feed samples were collected once every 2 weeks throughout the feeding period and analyzed for nutrient composition by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Recipient cows remained on the described feeding scheme until d 91 of gestation, when all cows were then offered a diet meeting 100% of their energy requirements for the remainder of gestation. Average recipient BW and BCS were

obtained from 2 consecutive measurements 24 h apart at the beginning (d -22 and d -21) and at the end (d 90 and d 91) of the feeding scheme. Single measurements of body weight (**BW**) and body condition score (**BCS**) were also assessed weekly from d 28 to d 91. The RESTR diet was formulated so that overcompensation by changes in feed intake between dietary treatment groups was restricted by gut fill because of the diet's bulkiness. In addition, individual feed intake combined with the nutrient analysis results allowed for calculation of the actual percentage of energy requirement met (NASEM, 2016) by the dietary treatments during the period that the feeding scheme was superimposed.

2.2.3. Donor Management and Embryo Production

Embryo donor cows (AN: n = 22 and BN = 24) were superovulated and AI with sexed-sorted semen from their respective breed. Donors were maintained in the same pasture and submitted to the same nutritional and environmental conditions during the experimental period. The superovulation protocol consisted of an injection of 100 µg of GnRH (2 mL Factrel; Zoetis Animal Health, Parsippany-Troy Hills, NJ) concurrent with the insertion of a new controlled intravaginal drug release (**CIDR**) insert (EAZI-BREED CIDR, Zoetis Animal Health) containing 1.38 g of progesterone (0700 h). Starting 4 d (1800 h) after CIDR insertion, cows were submitted to a 3-d FSH regimen (Folltropin-V; Bioniche Animal Health USA, Borgart, GA), where FSH was administered twice daily with a 12 ± 2 h interval. The CIDR insert was removed 7 d after insertion (1800h). On the same day as CIDR removal, donors received 2 injections of 25 mg of prostaglandin F2α (5 mL Lutalyse, Zoetis Animal Health) with a 12 ± 2 h (0700h and 1800h) interval between injections. Estrus detection patches (Estrotect, Rockyway Inc., Spring Valley, WI) were applied at CIDR removal, and assessed for activation twice daily for 3 d. Estrus detection

patches were considered activated when at least 50% of the patch was discolored, or when the patch was absent. Donor cows were AI with 1 straw of sex-sorted semen at onset of estrus, and were concurrently administered 100 μg of GnRH. Cows were AI a second time 12 ± 2h after the first insemination with 2 semen straws, and a third time 24 ± 2h after first insemination with 1 straw. Nonsurgical transcervical uterine flushing was performed to recover embryos 7 d after estrus. Embryos were evaluated under a stereomicroscope, and 134 transferable embryos in the morula or blastocyst stages were considered eligible for transfer.

2.2.4. Recipient Estrus Synchronization and Embryo Transfer

All recipients (n = 96 in 2015 and 101 in 2016) were submitted to the 7 d CO-Synch + CIDR estrus synchronization protocol (Larson et al., 2006) starting 12 d after the feeding scheme was initiated (d -9). Briefly, the protocol consisted of an injection of 100 μg of GnRH concurrent with the insertion of a new CIDR insert. Seven days after CIDR insertion (d -2), the insert was removed and recipients received a 25-mg injection of prostaglandin F2α. A second 100-μg GnRH injection was administered 48 h after CIDR removal (d 0). Transrectal ultrasonography was performed 7 d after the second GnRH 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). A total of 134 recipients were eligible for embryo transfer based on the presence of a corpus luteum at d 7. On the same day, donors were flushed, and a single fresh embryo was transferred into the recipient's uterine horn ipsilateral to the corpus luteum. Average ± SEM for days postpartum was 74 ± 12 at the initiation of the estrus synchronization protocol, and was not different between treatments.

2.2.5. Pregnancy Diagnosis and Embryo Morphometry

Diagnosis of pregnancy was performed by transrectal ultrasonography on d 28 of gestation (21 days after embryo transfer). A cow was considered pregnant if a visible embryo, together with a corpus luteum and uterine fluid consistent with pregnancy were present. Cows that received an embryo but were not pregnant at d 28 were considered to have experienced pregnancy failure. Additionally, transrectal ultrasonography was performed weekly from d 42 to d 91 to assess embryo morphometry. Individual videos were recorded weekly from d 42 to 91 of gestation. The ideal position and orientation of the embryo/fetus to measure crown-to-rump length (**CRL**) was selected in a frame-by-frame manner, and CRL was measured. Two individuals independently measured the images twice and the mean of each individual was used to calculate CRL estimates. Therefore, final CRL was the average of the mean obtained by each individual. On d 63, 70, 77, 84 and 91, crown-to-nose length was used to estimate CRL as previously described (Riding et al., 2008). Only recipients that remained pregnant until parturition and gave birth to a female calf were utilized in the embryo measurements analysis. Gestation length and calf birth weight were recorded at calving.

2.2.6. Expression of Interferon Stimulated Genes In Peripheral Blood Leukocytes

In order to investigate potential differences in early embryo development in the present study, blood samples were collected on d 18 and 21 of gestation during the second year of study for the isolation of peripheral blood leukocytes (**PBL**) according to Gifford et al. (2007) from cows that were diagnosed as pregnant at d 28 of gestation (n = 23). The PBL pellet was washed with ice-cold phosphate-buffered saline, centrifuged at 300 × *g* for 10 min at 4°C, and the supernatant discarded. The pellet was resuspended with 0.8 mL of

Trizol (Molecular Research Center, Inc., Cincinnati, OH), transferred into 1.5 mL microtubes, and stored at -80°C until further analysis.

Total RNA was purified and concentrated (PureLink RNA Mini Kit; Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Total RNA concentration and quality was determined by measuring absorbance at 260 and using a 280 nm NanoDrop spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA). All RNA samples contained a 260:280 nm ratio >1.8. One microgram of total cellular RNA was treated with deoxyribonuclease (DNase I Rnase-free; Invitrogen) before synthesis of cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems Inc., Foster City, CA). Complementary DNA (50 ng) was amplified using gene-specific primers (Table B2) and SYBR Green chemistry in a 20-µL reaction (Mastercycler EP Realplex System; Eppendorf AG, Hamburg, Germany) in triplicate. Amplification parameters were 40 cycles of a 2-step amplification protocol (15 s at 95°C followed by 60 s at optimized annealing temperature of 57°C and by 15 s at 95°C followed by 15 s at 60°C). Primer efficiencies ranged from 86 to 90%. Reactions were run in triplicate and exposed to a melting curve analysis to ensure amplification of a single product. Samples lacking reverse transcriptase were also included to verify that no genomic contaminants were amplified. Threshold cycle (**Ct**) values for reference gene, *Peptidylprolyl Isomerase A* (***PPIA****;* (Green et al., 2010), were used for computation of delta cycle threshold (ΔCt) values. This internal control was chosen because its transcript abundance was not altered by breed, parity, time period, or treatment for the particular tissue of interest. Relative fold change in gene expression was calculated with the formula $2^{-\Delta\Delta Ct}$. Intra-assay variation was assessed based on the mean standard variation of the triplicates according to Bustin et

al., (2009), and values were 15 % for *PPIA,* 37 % for *Myxovirus resistance 2* (*MX2*), 25 % for *2'-5'-oligoadenylate synthetase 1* (*OAS1*), and 22 % for *interferon-stimulated gene 15* (*ISG15*).

2.2.7. Circulating Pregnancy Associated Glycoproteins

Blood samples were collected from the jugular vein into 10-mL evacuated vials containing 143 IU of sodium heparin (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) at d 28, 35, 42, 49, 56, 63, 70, 77, 84, and 91 of pregnancy. Blood samples were immediately placed on ice and later centrifuged 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. Two commercial (**A1** and **A2**) and 1 in-house ELISA were used to determine circulating concentrations of PAG in plasma. The BioPRYN Quantitative ELISA (A1; BioTracking Inc., Moscow, ID) was used to quantify circulating pregnancy-associated glycoprotein 1 (PAG1; also known as pregnancy specific protein B [**PSPB**]). The assay was completed as described by the manufacturer using plasma diluted 1:10 in phosphate-buffered saline (pH 7.2) containing 0.5% (w/v) BSA. All standards, controls, and samples were ran in duplicates. Absorbance was read at 650 nm using an Infinite M1000 PRO microplate reader (Tecan, Männedorf, Switzerland). Two plasma controls containing moderate (2.0 ng/mL) and low (1.0 ng/mL) PAGs concentrations were included in each plate. Plasma concentrations of PAGs were also measured by a monoclonal-based PAGs in-house ELISA (**in-house**) as described by Green et al., (2005), and modified by Pohler et al., (2016); Reese et al., (2018), as well as a second commercial assay (A2; Idexx, Westbrook, ME). The in-house assay uses a polyclonal antibody raised against first-secreted PAGs (Ab 63; Reese et al., 2018), whereas the antigen used to create a detection

antibody for A2 consisted of a mixture of PAGs (PAG 4, 6, 9, 16, 18, 19; US Patent no. 7,604,950B2). Both assays (in-house and A2) were ran with a standard curve, pooled samples from a pregnant cow on d 60 of gestation, and pooled samples from a non-pregnant cow. Standards, controls, and samples were ran in duplicates. Absorbance was read at 405 nm using an ELx808 microplate reader (Bio-Tek, Winooski, VT). Intra and inter-assay coefficients of variations were 2.19 and 3.98% for A1, 6.32 and 7.23% for A2, 6.85 and 6.02% for the in house assay, respectively.

2.2.8. Statistical Analysis

All data were analyzed using the SAS (SAS Inst. Inc., Cary, NC; Version 9.4). The present study was a completely randomized design with a 2 × 2 × 2 factorial arrangement of treatments, where recipient breed (AN or BN), embryo breed (AN or BN) and recipient diet (MAINT and RESTR) represented the 3 different factors. Recipient cow individual intake was obtained daily during the experiment; therefore, cow was considered the experimental unit. The effects of the explanatory variables on recipient BW, BCS, plasma concentrations of PAGs, as well as fetal CRL and interferon-stimulated genes (**ISG**) expression were analyzed as repeated measures using the MIXED procedures of SAS. The covariance structure for all the analyses was selected based on the smallest Akaike information criterion for each variable analyzed. The models used for BW and BCS included the fixed effects of diet, day and diet × day interaction. When analyzing cow BW and BCS, initial BW and BCS were used as covariates for the respective analysis. The models for recipient plasma concentrations of PAGs and fetal CRL included the fixed effects of recipient breed, embryo breed, diet, day, and all the interactions that included day. When analyzing ISG expression, data was log transformed and models included the

fixed effects of recipient breed, embryo breed, diet, day and all possible interactions. Recipient BW and BCS loss during the feeding period were also analyzed by the MIXED procedure of SAS. The model included the fixed effect of diet. All models included the random effect of year.

Pregnancy failure was analyzed by ANOVA using the GLIMMIX procedure of SAS. The models included recipient breed, embryo breed, diet and the respective interactions as fixed effects, and year as a random effect. All embryo flushing, handling, and transfer was performed by the same personnel. Hence, their effects were not included in any of the analysis. In addition, embryos were assigned to recipients to equally distribute the effects of sire and donor across the different recipient breed × diet combinations; therefore, those variables were not included in the analysis. For a more conservative interpretation of the data and to reduce the chances of type I error, Tukey adjustment was used for all simultaneous pairwise comparisons. Significance was declared at $P \leq 0.05$.

2.3. Results and Discussion

The aim of the present study was to better understand how the inclusion of *B. indicus* genetics in both maternal and fetal systems alters fetal plane of development during early gestation. In addition, we were interested on the effects of feed restriction during early gestation on embryo and fetal development of both *B. indicus* and *B. taurus* cattle. Therefore, a feeding scheme was applied to recipient cows during the first trimester of gestation in order to create conditions similar to those in the field, when feed intake sometimes fails to meet the energy requirements of the postpartum beef cow. The dietary approach utilized in this experiment induced an energy restriction scenario, as shown by

the differences ($P < 0.01$) in the percentage of energy requirements consumed by cows in the different diets (Table B3), along with the impact of diet on the average recipient BW ($P = 0.02$) and BCS change ($P < 0.01$; Table B3). When weekly BW and BCS were evaluated from d 28 to d 91 of gestation, a diet × day interaction was observed for both recipient BW ($P < 0.01$; Figure A4) and BCS ($P < 0.01$; Figure A5). Recipients in the RESTR diet had lesser ($P < 0.05$) BW on d 28, 35, 70, 77 and 84, as well as lesser ($P < 0.05$) BCS at d 70, 77, 84 and 91. When looking only at BW change from the beginning of the feeding scheme (d -21) until the first pregnancy diagnosis (d 28), cows in the RESTR diet were already experiencing greater BW loss ($P = 0.03$; Table B3).

Although fertilization rates in beef females are estimated to be close to 90% (Santos et al., 2004), the average pregnancy rates to fixed-time AI at around d 30 of gestation ranges between 45 to 60% (Lamb et al., 2010). Therefore, the interval from fertilization to the first pregnancy diagnosis is a critical period for the establishment of pregnancy in cattle (Wiltbank et al., 2016). In the present study, we were able to evaluate if nutrient restriction during early gestation affects the establishment of pregnancy in *B. indicus*-influenced and *B. taurus* cattle. A recipient breed × diet interaction was observed on pregnancy failure by d 28 of gestation ($P < 0.01$), where AN-RESTR had increased pregnancy failure compared to AN-MAINT diet and BN recipients in both RESTR and MAINT diets (Figure A6). In addition, there was an embryo breed × diet interaction ($P = 0.03$) on pregnancy failure at d 28. Restricted recipients receiving an AN embryo experienced greater pregnancy failure than recipients in the MAINT diet receiving AN embryos, regardless of recipient breed (Figure A7). Undernutrition prior to breeding is known to have deleterious impacts on postpartum resumption of estrous cycles, pregnancy

success, and reproductive efficiency of beef cows (Randel, 1990; Diskin et al., 2003). Interestingly, the negative effects of undernutrition are not restricted to the modulation of the hypothalamic-pituitary-gonadal axis and the resumption of postpartum anestrus. Studies restricting intake shortly after AI had a negative impact on reproductive performance of beef heifers (Perry et al., 2015; Kruse et al., 2017), indicating nutrient restriction might also impact early embryonic development. When embryos were recovered from heifers submitted to energy restriction immediately after AI, the collected embryos had decreased quality, decreased number of blastomeres, and tended to have a lower percentage of live cells (Kruse et al., 2017).

Interferon-stimulated genes are upregulated in PBL in response to interferon-τ production by the developing conceptus (Hansen et al., 2017). Since interferon-τ is required for pregnancy establishment in cattle, and ISG mRNA expression in PBL was positively correlated to the amount of interferon- τ produced by the conceptus (Matsuyama et al., 2012), we were interested in evaluating if the pregnancy failure observed in this study was associated with impaired conceptus development and interferon-τ production. However, the only effect observed on ISG mRNA expression in PBL was an increase in the mean ISG15 mRNA expression in AN cows from d 18 to 21 ($P = 0.01$), which provides no insight on the biology controlling the differences in pregnancy failure observed in this study. Interferon-stimulated genes mRNA expression was only measured in the second year of the study might have contributed to the absence of significant differences, due to the lack of statistical power in the analysis.

Although most experiments evaluating the effects of nutrient restriction on reproductive performance in beef cows have been performed in *B. taurus* cattle (Randel,

1990; Diskin et al., 2003; Perry et al., 2015), data indicate *B. indicus* females in a poor nutritional status also experience impaired reproductive performance (Baruselli et al., 2004; Filho et al., 2010). Our results, however, indicate the magnitude of the detrimental effect of nutrient restriction differs between *B. taurus* and *B. indicus*-influenced cattle. The *B. taurus* genotype in both maternal and fetal systems decreased pregnancy when nutrient restriction was superimposed, indicating *B. taurus* cows were more susceptible to experience embryonic loss when exposed to the same negative plane of nutrition. A potential explanation for the greater impact of energy restriction on *B. taurus* cattle could be the different energy requirements between the subspecies. Crossbred cattle consisting of both *B. indicus* and *B. taurus* genetics are thought to have approximately 5% lesser energy requirements when compared with *B. taurus* animals (Frisch and Vercoe, 1977). Hence, the magnitude of restriction in the scenario proposed by this study could potentially be less for *B. indicus*-influenced cows. In contrast, more recent reports do not support the concept of reduced energy requirements for maintenance in *B. indicus* × *B. taurus* cattle (Chizzotti et al., 2007; Chizzotti et al., 2008), indicating that genetic selection towards greater productivity might have resulted in increased nutrient requirements for *B. indicus*-influenced cattle. The generalization of reduced energy requirements for a specific group of cattle (i.e., *B. indicus*) might not be appropriate since breed genetics have changed over time. Unfortunately, little is known about the differences in physiological adaptation of *B. indicus* vs. *B. taurus* females in the postpartum period under limited energy availability. Therefore, further research is required to better understand how suckled *B. indicus* and *B. taurus* cows differ with regards to homeorhetic adaptations to energy restriction, and might

provide insights as to why *B. taurus* cattle experience greater pregnancy loss under nutrient restriction.

Previous reports indicate that the pattern of fetal growth during gestation differs between *B. indicus* and *B. taurus* cattle. In a series of experiments, our research group previously showed that *B. indicus*-influenced fetuses grow at a slower rate during early gestation compared with *B. taurus* counterparts (Mercadante et al., 2013). Furthermore, fetuses harvested during mid-gestation had reduced growth rates in *B. indicus* compared with *B. taurus* cattle (C. L. Ferrell, 1991; O'Rourke et al., 1991). In the present study, differences in CRL were also detected; however, only at d 91 of gestation (recipient breed × day; $P < 0.01$). Angus recipients had larger fetuses than BN recipients, regardless of the embryo breed or diet (Figure A8). When a similar embryo transfer approach was used to compare fetal development of Charolais (*B. taurus*) and Brahman (*B. indicus*) cattle, recipient breed was also associated with fetal size during mid to late-gestation (Ferrel, 1991). Similar to the results from our studies, the growth of Charolais fetuses was reduced in Brahman cows compared with Charolais cows, indicating that recipient genotype may restrain fetal development. Intriguingly, calf birth weight can be similar or sometimes greater in *B. indicus* cattle compared with *B. taurus* counterparts (Mercadante et al. 2013), indicating that *B. indicus* cattle undergo compensatory gain during late gestation. In the present study, recipient breed, embryo breed, diet, or any of the respective interactions did not affect calf birth weight ($P > 0.10$). In addition, although there was an interaction between recipient breed and embryo breed on gestation length ($P = 0.05$), the least square means comparisons were not different ($P > 0.10$). Overall, our results corroborate the idea

B. indicus-influenced cattle grow at a slower rate during early and mid-gestation, and then undergo compensatory growth during the last trimester, resulting in similar birth weights.

The differences in fetal development between cattle subspecies could be associated with different placental function or efficiency (Ferrell, 1991). The ruminant synepitheliochorial placenta has binucleate cells that arise from mononuclear trophoblast cells through mitotic polyploidy (Wathes and Wooding, 1980; Wooding, 1984). The binuclueate cells can migrate through the uterine-placental interface and fuse with uterine luminal epithelial cells. Interestingly, these cells have secretory granules that release their contents via exocytosis into the uterine stroma. Pregnancy-associated glycoproteins are located within these secretory granules, and can make their way into the maternal circulation early in gestation (Wallace et al., 2015). Although the function of PAGs are not well understood, changes in plasma concentration of PAG shave been observed in physiological scenarios of placental insufficiency, such as somatic cell nuclear transfer cloning (Chavatte-Palmer et al., 2006; Constant et al., 2011) and increased incidence of embryonic mortality (Franco et al., 2018; Gatea et al., 2018). Previous research from our laboratory indicates *B. indicus*-influenced females (BN) have greater plasma concentrations of PAGs when compared with *B. taurus* counterparts (AN; Mercadante et al., 2013). Nevertheless, there are no previous reports of circulating PAGs differences between *B. indicus* and *B. taurus* animals using a reciprocal embryo transfer approach. The results of the present study are unique and provide a notion on whether the maternal or the fetal genotype is controlling the previously observed differences in circulating PAGs. An embryo × day interaction was observed in A1 ($P < 0.01$) with recipients receiving BN embryos having greater concentrations of PAGs at d 91 (Figure A9-A). Similarly, a main

effect of embryo breed ($P < 0.01$) was observed in A2, where cows that received a BN embryo had greater concentrations of PAGs compared with cows receiving an AN embryo throughout all measurements (Figure A9-B). There was also an embryo breed × day interaction when the in-house assay was used. However, as opposed to the other assays, cows receiving AN embryos had greater concentrations of PAGs at d 28 and 35 of gestation than cows receiving BN embryos (Figure A9-C). Recipient breed or diet did not affect any of the 3 PAG assays ($P > 0.10$).

The PAG family of trophoblast-expressed genes encode for several PAG products, and the differences observed between assays in this study are because the assays are probably detecting different PAGs. Both the A1 and A2 are commercially available pregnancy tests, where A1 is an assay that detects mostly PAG1, and A2 uses a polyclonal antibody that detects different members of the PAG family (PAG 4, 6, 9, 16, 18, 19; Gatea et al., 2018). Conversely, the in-house assay using Ab 63 is specific to early-secreted PAGs, which have been shown to differ in maternal circulation between *B. indicus* and *B. taurus* cattle (Franco et al., 2018). When multiparous Nelore (*B. indicus*) cows were AI with Nelore or AN (*B. taurus*) sires, cows inseminated to AN sires had greater circulating PAGs (Franco et al., 2018). These results corroborate our findings, where cows receiving *B. taurus* embryos had greater plasma concentrations of first-secreted PAG and support the idea the profile of PAG production during early gestation differs between *B. indicus* and *B. taurus* conceptuses.

In summary, the *B. taurus* genotype in either maternal or fetal systems decreased the probability of pregnancy when nutrient restriction was superimposed, indicating that *B. taurus* cattle are more susceptible to experiencing early pregnancy failure when exposed to

a negative plane of nutrition. This study also provides additional evidence that differences in fetal growth rate and PAGs production between *B. indicus* and *B. taurus* exist. The differences observed between subspecies on plasma concentrations of PAGs were always associated with the conceptus breed. Therefore, the previous reports in the literature where *B. indicus* and *B. taurus* females have different plasma concentrations of PAGs (Mercadante et al., 2013) are likely associated with the conceptus genotype rather than the maternal genotype. In addition, our results indicate that the maternal environment potentially controls fetal growth rate to some extent, and thus raises questions as to whether these differences in growth rate can program postnatal development.

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3. EFFECTS OF NUTRIENT RESTRICTION ON THE METABOLIC PROFILE OF *BOS INDICUS*-INFLUENCED AND *B. TAURUS* SUCKLED BEEF COWS

3.1. Introduction

Mitochondrial DNA sequencing and microsatellite loci indicates *B. indicus* and *B. taurus* cattle diverged, from an evolutionary standpoint, between 110,000 to 850,000 years ago (Bradley et al., 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 South Asia, leading to the acquisition of genetic adaptation that convey greater thermotolerance compared to *B. taurus*, which have undergone natural and artificial selection in Europe (Hansen, 2004). Consequently, *B. indicus* cattle are capable to withstand higher temperatures, experiencing less reduction of 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 warmer 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 (Moore et al., 1972; Turner, 1974) (Turner, 1980; Moore et al., 1975; Warnick and Cobb, 1976). However, little is known about differences in physiological

adaptation to energy restriction between these subspecies in suckled beef cows, particularly its impacts on reproductive performance. Our research group recently reported that Angus (**AN**; *B. taurus*) cows under energy restriction had lower pregnancy rates through embryo transfer compared to 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 circulating markers of metabolic adaptation to negative energy balance between the AN and BN cows utilized by Fontes et al. (2019).

3.2. Material and Methods

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

3.2.1. Experimental Design

A total of 197 (year 1: n = 96, and year 2: n = 101) suckled beef cows (body weight [**BW**]: 522 ± 66.4; body condition score [**BCS**]: 5.3 ± 0.61) were enrolled as candidate embryo recipients in a completely randomized design with 2 × 2 factorial arrangement of treatment over 2 consecutive years as previously described (Fontes et al., 2019). Fourteen days prior to the beginning of the experiment, all cows were comingled and fed a common diet in order to adapt cows, which were previously grazing, to a total mixed ration. At d -21, Angus (**AN**; *B. taurus*) and Brangus (**BN**; *B. indicus*-influenced) cows were then stratified by days postpartum (**DPP**), BW and BCS, and were randomly assigned to 1 of the 2 diets: 1) Diet formulated to meet the daily energy requirements of a 550 kg beef cow

(**MAINT**; NASEM, 2016); and 2) Diet formulated to meet 70% of daily energy requirements (**RESTR**; Table B1). 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 monitor individual feed intake (GrowSafe System Ltd., Airdrie, AB, Canada). The RESTR diet was formulated so that overcompensation by changes in feed intake between dietary treatment groups was restricted by gut fill due to the RESTR diet's bulkiness. In addition, individual feed intake combined with the nutrient analysis results allowed for calculation of the actual percentage of energy requirement met (NASEM, 2016) by the diet, which allowed for evaluating how effective the superimposed RESTR diet was in restricting energy intake. Nutrient composition of the diets were 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) and results are reported in Table B1. Additionally, BW and BCS were collected on days -21, 19, 56 and 91 in order to evaluate the effectiveness of the diets in modulating body nutritional reserves. Body weight and BCS at d -21 and d 91, represent the average of 2 assessments with a 24-h interval between each assessment, whereas values at d 19 and 56 represent a single measurement.

3.2.2. 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 after the feeding scheme was initiated (d -9). Briefly, the protocol consisted of an injection of 100 µg of GnRH (2 mL Factrel; Zoetis Animal Health, Parsippany-Troy Hills, NJ) concurrent with the insertion of a new

controlled intravaginal drug release (**CIDR**) insert (EAZI-BREED CIDR, Zoetis Animal Health) containing 1.38 g of progesterone. Seven days after CIDR insertion (d -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 GnRH was administered 48 h after CIDR removal (d 0). Day 0 represents the first day of gestation since embryo donors were artificially inseminated on the same day. Transrectal ultrasonography was performed 7 d after the second GnRH 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). A total of 134 recipients were eligible for embryo transfer based on the presence of a corpus luteum at d 7. On the same day, donors 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 sexed-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 determine via transrectal ultrasonography at d 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. The rational for removing non-pregnant cows from the experiment was related to the objectives of Fontes et al., (2019), which was to evaluate differences in embryonic and fetal development in *B. indicus* and *B. taurus* fetuses exposed

to the uterine environment of *B. indicus* or *B. taurus* cows. This was accomplished by characterizing not only pregnancy establishment and subsequent fetal growth, but also expression of interferon-stimulated genes in peripheral blood leukocytes, and circulating pregnancy-associated glycoproteins (Fontes et al., 2019). Hence, as cows were diagnosed open, they were removed from the experiment.

3.2.3. Circulating Blood Concentrations of Non-Esterified Fatty Acids, β-Hydroxybutirate, Insulin-Like Growth Factor 1, Insulin and Glucose

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) at d -21, 19, 56 and 91 in order to estimate circulating concentrations of non-esterified fatty acids (**NEFA**), β-hydroxybutirate (**BHB**), insulin-like growth factor 1 (**IGF-1**), insulin and glucose. On d -21 and 19, blood samples were collected from all cows that received an embryo (n = 134). On d 56 and 91, samples were collect only from cows that remained pregnant until d 91 of gestation (n = 51). 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. Blood samples were cooled 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 to determine circulation concentrations of NEFA (Wako Chemicals USA, Inc., Richmond, VA) and glucose (Thermo Electron Corp., Waltham, MA). 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) as previously described (McCarthy et al., 2015). Plasma concentrations of insulin were estimated through radioimmunoassay (EMD Millipore's Porcine Insulin RIA; EMD Millipore Corporation, Billeerica, MA) using a Wizard Gamma Counter (PerkinElmer, Inc., Watham, MA). To determine circulating concentration of IGF-1, a commercial enzyme-linked immunosorbent assay kit (Quantikine ELISA Human IGF1 Immunoassay; R&D Systems, Inc., Minneapolis, MN) designed for human IGF-1 but with 100% cross-reactivity with bovine IGF-1 (Moriel et al., 2012; Mercadante et al., 2016) was utilized. Intra- and inter-assay coefficient of variation were, respectively, 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, 7.64 and 3.58% for IGF-1.

3.2.4. Statistical Analysis

Data for the present experiment were analyzed as a completely randomized design with a 2 x 2 factorial arrangement of treatments, where the individual cow was considered the experimental unit. Continuous and binary response variables were analyzed using the MIXED and GLIMMIX procedures of SAS (SAS Inst. Inc., Cary., NC; Version 9.4), respectively. For the analysis of BW, BCS and blood indicators of cow nutritional status (NEFA, BHB, glucose, insulin, and IGF-1) of all cows at d -21, the model statements included the fixed effects of breed, diet, and breed × diet interaction. The same model was used to analyze the abovementioned response variables at d 19: however, their respective values at d -21 were utilized as covariates. The analysis also included the random effects of year and cow(breed × diet). A separate analysis was performed within the subset of cows

that remained pregnant until d 91. In this case, continuous response variables were analyzed as repeated measures within the same experimental unit. The models included the fixed effects of cow breed, diet, day, and all the potential interaction between them. Additionally, year and cow(breed × diet) were included as random effects. The values at d -21 were also utilized as covariates for the repeated measures analysis and the covariance structures was selected based on the lowest Aikike information criteria. 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. 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 logistic equation)/(1 + e logistic equation). Logistic curves were constructed and reported for the variables that were significant. Throughout the analysis for the present experiment, significance was declared at $P \leq 0.05$ and tendencies were determined when $P > 0.05$ and ≤ 0.10. Least square means were reported when the *P*-value for the main effect was ≤ 0.10.

3.3. Results

Average ± SEM for days postpartum was 74 ± 12 at the initiation of the estrus synchronization protocol, which were not different between breed, diet, and breed × diet ($P > 0.10$). Results for BW, BCS and blood indicators of cow nutritional status at d -21 and d 19 for all recipient cows included in the experiment are summarized in Table B4. As

proposed by the experimental design, there was an effect of diet on the percentage of energy requirements met based on individual cow intake ($P < 0.001$), where cows in the MAINT diet consumed a greater percentage of their energy requirements compared to the 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 (d -21). As expected, cows in the RESTR diet had less BCS than cows in the MAINT diet at d 19 ($P = 0.008$). No significant effects were observed for cow BW at d 19 ($P > 0.10$). There was an effect of cow breed on circulating concentrations of NEFA and BHB, where BN cows had greater concentration of NEFA and BHB than AN cows on d -21 ($P = 0.02$ and $P = 0.01$, respectively). Covariately-adjusted circulating concentrations of NEFA and BHB were not different at d 19 ($P < 0.10$). No breed or diet effects were observed on glucose, insulin and IGF-1 ($P > 0.10$) at d -21. There was a breed × diet interaction on plasma concentrations of insulin ($P = 0.03$), where AN - RESTR cows tended ($P = 0.06$) to have less plasma concentration of insulin compared to AN - MAINT cows, and had less plasma concentration of insulin than BN - RESTR cows. No differences in plasma insulin were observed between BN cows in both MAINT and RESTR diets ($P = 0.24$). A breed × diet interaction was also noted ($P = 0.03$) for plasma concentrations of IGF-1 at d 19. Angus cows in the RESTR diet had less ($P \leq 0.02$) plasma IGF-1 compared to all other breed × diet combinations. No differences in plasma IGF-I were observed ($P > 0.10$) between BN - MAINT and BN - RESTR.

Results for BW, BCS, together with the changes in BW and BCS between d -21 and d 91 for the cows that remained pregnant until d 91 are summarized in Table B5. At the beginning of the experiment (d -21), cows assigned to the RESTR diet tended ($P =$

0.07) to have greater BW and had greater BCS (P = 0.04) compared with cows assigned to the MAINT diet. Body weight and BCS were not different between breeds or diets at d 91 ($P > 0.10$). Nevertheless, there was a tendency ($P = 0.08$) for a cow breed × diet interaction on BW change between d -21 and 91, where AN - RESTR cows experienced greater ($P = 0.01$) BW loss compared to AN - MAINT cows. Brangus cows in the RESTR diet did not experience greater BW loss compared to the other breed × diet combinations ($P > 0.10$). Cows in the RESTR diet, regardless of the breed (breed and diet × breed: $P > 0.10$), had greater ($P < 0.001$) BCS loss between d -21 and d 91.

Changes in plasma concentrations of NEFA, BHB, insulin and IGF-1 for cows that remained pregnant until d 91 are summarized in Figure A10. A diet × day interaction was observed for plasma concentrations of NEFA ($P = 0.01$). There were no differences in plasma concentrations of NEFA on d 19 ($P > 0.10$): however, BN - RESTR cows had greater circulating concentration of NEFA at d 56 compared to BN - MAINT ($P = 0.006$), AN - RESTR ($P = 0.04$), and AN-MAINT cows ($P = 0.002$). On d 91, AN - RESTR diet had greater circulating NEFA compared to AN - MAINT diet ($P = 0.05$). Additionally, BN - MAINT cows tended ($P = 0.07$) to have less plasma concentrations of NEFA compared to BN - REST cows. There was also a diet × day interaction on plasma concentrations of BHB. Brangus cows in the RESTR diet had greater concentration of BHB at d 19 compared to both AN - MAINT ($P = 0.03$) and BN - MAINT ($P = 0.05$). Similarly, AN cows in the RESTR diet tended ($P = 0.08$) to have greater concentrations of BHB than AN cows in the MAINT diet. There were no differences in plasma concentration of BHB on d 56 and 91 ($P > 0.10$). A 3-way interaction was observed for circulating insulin (breed × diet × day; P = 0.04). Although no differences in plasma concentrations of insulin were

observed between BN × RESTR cows and BN × MAINT cows throughout all measurements ($P > 0.10$), concentrations of insulin at d 19 were less in AN × RESTR compared to AN × MAINT cows ($P = 0.02$). There were no differences between BN × MAINT and BN × RESTR at d 19 ($P = 0.36$). There were also no differences in circulg concentrations of insulin at d 56 and 91 ($P > 0.10$). Similar to the results observed for insulin, circulating concentrations of IGF-1 were not altered by diet in BN × MAINT and BN × RESTR cows ($P > 0.10$): however, differed between AN cows in the RESTR compared to the MAINT diet. Angus cows in the RESTR diet had less plasma concentration of IGF-1 then AN cows in the MAINT diet at d 19 ($P = 0.04$), 56 ($P = 0.04$), and 91 ($P = 0.008$). No differences ($P > 0.10$) were observed for plasma concentrations of glucose on d 19, 56 and 91 (95.1 ± 8.44 mg/mL; mean ± SEM).

When exploring a potential relationship between metabolites at d -21 and the probability of pregnancy to embryo transfer at d 28 through the use of a logistic regression, there was a linear relationship between plasma concentrations of insulin at d -21 ($P = 0.002$) and the probability of pregnancy, where the probability of pregnancy increased as the plasma concentrations of insulin increased (Figure A11-A). A similar relationship was observed between the probability of pregnancy and plasma concentrations of IGF -1 at d 19 (linear; $P = 0.04$), where the probability of pregnancy increased as plasma concentration of IGF-1 increased (Figure A11-B).

3.4. 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 on, and developed for *B. taurus* cattle. Therefore, it is important to acknowledge and further explore these differences between subspecies in order to facilitate the 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 than *B. indicus*-influenced counterparts when both subspecies are exposed to the same energy restriction environment (Fontes et al., 2019). We hypothesized that *B. taurus* cows are less resilient to energy restriction, and consequently have a different metabolic profile compared to *B. indicus*-influenced cows that are exposed to the same conditions. 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.

As proposed by the design of the experiment, when all cows were included in the analysis, cows submitted to the RESTR and MAINT diets had similar BCS at d -21. By d 19, cows in the RESTR diet had lower BCS than cows in the MAINT diet, regardless of the breed. Not surprisingly, there were no effects of diet in cow BW at d 19, which was likely associated with the fact that gut fill introduces considerable variation to BW data, and BW was assessed by a single measurement at d 19. When the effects of the proposed diets were evaluated for a longer period of time in the pregnant cows, AN cows in the RESTR diet had greater BW loss between d -21 and 91 compared to AN × MAINT cows. Supporting our hypothesis, there were no differences in BW loss between BN × RESTR and BN × MAINT. Additionally, there was only an effect of diet, but no effects of breed or

breed × diet interactions on the percentage of energy requirements met by the diets. These results indicate that the proposed diets were able to successfully induce a difference in energy intake between MAINT and RESTR diets, which resulted in greater BW and BCS loss in cows that were in the RESTR diet. Furthermore, it indicates that RESTR cows were already experiencing the impacts of energy restriction at d 19: however, breed differences were only observed after cows were exposed to the diets for a longer period of time.

Non-esterified fatty acids are a product of the breakdown of triglyceride ester bounds within adipocytes and are utilized to investigate the magnitude of lipolysis during negative energy balance in dairy and beef cattle (Richards et al., 1987; Staples et al., 1990; Marques et al., 2019). Beta-hydroxibutyrate is a product of the hepatic metabolism of circulating NEFA, and also increases in circulation when dietary energy fail to meet the requirements (Herdt, 2000). The plasma concentrations of NEFA and BHB were greater in BN cows at the beginning of the experiment (d -21) compared to AN cows, corroborating similar data of previous reports in postpartum *B. indicus* cows in Southern U.S. (Coleman et al., 2016). However, no effects of diet or cow breed were observed on covariately-adjusted NEFA or BHB concentrations at d 19, indicating no subspecies differences in response to dietary treatments for these markers when all cows were included in the analysis. Similar results were observed when plasma concentrations of NEFA and BHB were evaluated only in cows that remained pregnant throughout the experiment. Although differences were observed for both NEFA and BHB within specific time points, there was not a clear difference between subspecies within the RESTR diet.

While no clear differences between subspecies were observed for NEFA or BHB, AN cows in the RESTR diet had less concentrations of both insulin and IGF-1 compared to

AN cows in the MAINT diet, and BN cows in both MAINT and RESTR diet when all cows were included in the analysis at d 19. Additionally, there were no differences in both insulin and IGF-1 between BN × RESTR and BN × MAINT, corroborating with the proposed hypothesis. When these hormones were analyzed only in pregnant cows, a breed × diet interaction was also present. Although no differences were observed between BN × MAINT and BN × RESTR, AN cows submitted to the RESTR diet had lesser concentrations of insulin and IGF-1 than AN × MAINT cows. These differences were maintained throughout all measurements of IGF-1 in cows that remained pregnant: however, were only observed at d 19 for insulin. Insulin concentrations during the postpartum period has been previously shown to be decreased in cows that are losing BCS compared to cows that are maintaining BCS (Sales et al., 2015; Sheehy et al., 2017). In addition, dietary strategies that induce an increase in circulating concentrations of insulin can alleviate the effects of negative energy balance in the postpartum, 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., 2003).

Since the results of plasma concentrations of insulin and IGF-1 corroborated with the differences in fertility reported by Fontes et al., (2019), a logistic regression was conducted to explore the relationship between plasma concentrations of IGF-1 and insulin at d -21 and 19 with the probability of pregnancy after embryo transfer. The probability of pregnancy linearly increased as the concentrations of insulin at d-21 and IGF-1 at d 19

increased. Growth hormone (GH) and IGF-1 receptors are expressed by the bovine uterus and conceptus (Kolle, 1997; Kolle et al., 2001), and these hormones were shown to influence preimplantation conceptus development in ruminants (Spencer et al., 1999; Kolle, 2002; Moreira et al., 2002). In fact, exogenous supplementation of recombinant bovine GH post-insemination increased circulating concentrations of IGF-1, 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. Noteworthy, although literature supports the idea that the somatotropic axis plays a role in pregnancy establishment and might mediate the differences in fertility between *B. indicus*-influenced and *B. taurus* cattle under energy restriction, the present experiments provides no direct evidence of causative relationship between insulin, IGF-1 and fertility.

It is reasonable to speculate that differences in energy requirements between the subspecies might be driving the differences observed in the present study. *B. taurus* cattle are thought to have approximately 10% less energy requirements for maintenance compared to purebred *B. indicus* breeds, and 5% lesser 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 indicated 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. Particularly, 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). In conclusion, the results of the present study indicate that the magnitude of the impacts of energy restriction might be 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, differences in BCS, plasma insulin and IGF-1 between MAINT and RESTR cows were more evident in comparisons within *B. taurus* than within *B. indicus*-influenced cows. These results corroborate with the differences in fertility between subspecies reported when cows are exposed to energy restriction (Fontes et al., 2019), providing further evidence of a greater resilience of *B. indicus*-influenced cattle to perform under energy restriction conditions.

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4. IMPACTS OF *BOS INDICUS* VS. *B. TAURUS* GENETICS AND NUTRIENT ENERGY RESTRICTION DURING EARLY GESTATION ON OFFSPRING PERFORMANCE AND FEED EFFICIENCY

4.1. Introduction

Approximately 30% of cattle in the U.S. contain some *Bos indicus* genetics, and approximately 40% of beef cows and 50% of the country's cow-calf producers are located in the Southern U.S., where *B. indicus* cattle and their crosses are located (Chase et al., 2005). These cattle and the various new breeds generated from crossbreeding *B. indicus* cattle with European breeds contain a greater tolerance to elevated ambient temperatures and humidity than European breeds (reviewed by Hansen, 2000). However, a variety of physiological differences that go beyond the acquisition of genes for thermotolorance exist between *B. indicus* and *B. taurus*, including differences in gestational parameters. *B. indicus* (Ferrell, 1991) and *B. indicus*-influenced (Mercadante et al., 2013; Fontes et al., 2019) fetuses are smaller during early gestation compared to B. taurus fetuses. Interestingly, *B. indicus* fetuses undergo a relative compensatory growth compared to *B. taurus* during mid- and late-gestation, resulting in calves with similar or sometimes greater body weight at birth (Mercadante et al., 2013; Fontes et al., 2019).

Exposure of pregnant cows to nutrient restriction during early gestation impairs placental vascularization (Vonnahme et al., 2007), resulting in differences in fetal growth during the first trimester of gestation (Vonnahme et al., 2007; Long et la., 2009). When early gestation maternal undernutrition is followed by dam realimentation to recommended requirements, differences in fetal size are eliminated by the end of gestation (Long et al.,

2009; Vohname et al., 2003; Ford et al., 2007), indicating that dietary-induced intrauterine growth restricted (IUGR) fetuses can undergo compensatory growth in utero. These patterns of fetal development resembles the growth rate of *B. indicus* fetuses (Ferrell et al., 1991), and resulted in altered postnatal glucose metabolism and adiposity of offspring in the sheep (Ford et al., 2007). Hence, we hypothesized that differences in postnatal growth and development between *B. indicus* and *B. taurus* cattle are to some extent influenced by differences in intrauterine development. Therefore, the objective of the present study was to evaluate the fetal and maternal contributions of *B. indicus* genetics to female offspring growth and feed efficiency. Moreover, we investigated if these subspecies are influenced differently by energy restriction during early gestation.

4.2. Material and Methods

The present study was conducted at the University of Florida - North Florida Research and Educational Center (Marianna, FL). All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee (protocol number 201408681).

4.2.1. Experimental Design

Over the course of 2 consecutive years, A total of 197 (year 1: n = 96, and year 2: n = 101) suckled beef cows were enrolled as candidate embryo recipients in a completely randomized design with 2 × 2 × 2 factorial arrangement of treatments as previously described (Fontes et al., 2019). Angus (**AN**; *B. taurus*) and Brangus (**BN**; *B. indicus*-influenced) cows were stratified by days postpartum (**DPP**), body weight (**BW**) and body condition score (**BCS**; 1 to 9 scale; 1 being emaciated; 9 being extremely obese), and were randomly assigned to 1 of the 2 diets starting 28 days prior to embryo transfer: 1) Diet

formulated to meet the daily energy requirements of a 550 kg beef cow (**MAINT**; NASEM, 2016); and 2) Diet formulated to meet 70% of daily energy requirements (**RESTR**). During the feeding period, all cows were housed at the University of Florida Feed Efficiency Facility (**FEF**) at the North Florida Research and Education Center (30°46′35″N, 85°14′17″W), equipped with a GrowSafe System to monitor individual feed intake (GrowSafe System Ltd., Airdrie, AB, Canada). The RESTR diet was formulated so that overcompensation by changes in feed intake between dietary treatment groups was restricted by gut fill due to the RESTR diet's bulkiness. The superimposed diets were fed rom d -28 until d 91 of gestation. Cows were then comingled and fed a common diet that met their energy requirements until calving. Individual cow intake was utilized to estimate the percentage of energy requirements met by the diets according to NASEM, 2016. As a consequence of the proposed feeding scheme, cows in the RESTR diet had significantly lower energy intake and experienced greater BW and BCS loss during the experimental period compared to cows in the MAINT diet. These differences were independent of cow breed. Estimates, SEM and P-values for these variables have been previously reported (Fontes et al., 2019): therefore, are not included in the present manuscript.

All cows enrolled in the present study were exposed to an industry standard estrus synchronization protocol (7 d CO-Synch + CIDR; Larson et al., 2006) followed by fixed-time embryo transfer. Briefly, cows received a new controlled intravaginal drug release (**CIDR**) insert (EAZI-BREED CIDR, Zoetis Animal Health) containing 1.38 g of progesterone, and an injection of 100 µg of GnRH (2 mL Factrel; Zoetis Animal Health, Parsippany-Troy Hills, NJ). Seven days after CIDR insertion, 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 GnRH was administered 48 h after CIDR removal. The day of the second injection of GnRH was considered the first day of gestation since embryo donors were artificially inseminated on the same day. Transrectal ultrasonography was performed 7 d after the second GnRH 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). Fresh embryos were transferred into the lumen of the uterine horn ipsilateral to the corpus luteum. Embryos utilized in this study were produced vivo through superovulation of AN and BN donors followed by artificial insemination with female sex-sorted semen from the donors' respective breeds. Embryos were assigned to recipient cows in order to generate the previously described treatments and to equally distribute the effects sire, donor, and embryo grade.

4.2.2. Offspring Performance, Feed Efficiency and Age at Puberty

Within 12 h after calving, birth weight was collected in all calves, and male calves were identified and removed from the experiment. After removal of male calves, a total of 43 heifers remained in study until their first breeding season. From calving until weaning, all cow-calf pairs that remained in the experiment were kept in the same pasture with ad libitum access to water, minerals and hay (Cynodon dactylon). Heifers (n = 43; AN × AN × RESTR, n = 1; AN × AN × MAINT, n = 8; AN × BN × RESTR, n = 5; AN × BN × MAINT, n = 6; BN × BN × RESTR, n = 8; BN × BN × MAINT, n = 5; BN × AN × RESTR, n = 4, BN × AN × MAINT, n = 6) were then weaned at 219 ± 4.5 days of age and kept together during the remaining of the study. Three months after weaning, heifers entered the FEF and were enrolled in a 14-d acclimation period, which preceded a 70-d feeding test (Archer et al., 1997). Pens were equipped with a Grow Safe system and

individual intake of nutrients were monitored. During the adaptation period and feed efficiency test, heifers had ad libitum access to water and a roughage-based diet composed of 22.5% corn gluten, 22.5% soyhulls, 5% supplement pellets and 50% fiber pellets as fed, which was formulated to support growth rates of 1 kg/d (NASEM, 2016; Table B6). Feed samples were collected every 2 weeks and dried at 55ºC for 72 h in a forced air oven. Samples were then ground in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA, USA) using a 2.0 mm screen. After grinding, samples were composited for analysis on an equal weight basis and were analyzed in duplicate for nutritive values by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Heifer BW and BCS were collected in 2 consecutive days at the beginning and at the end of the feed efficiency test. The average of these values were utilized as the initial and final BW and BCS.

Heifers were kept in the FEF after the completion of the feed efficiency evaluation. When heifers were 427 ± 5.2 (mean ± SD) of age, 2 injections of 25 mg of prostaglandin F2α were administered with an interval of 12 d between them. After the second injection, estrus detection patches (EstrotectTM, Rockway Inc, Spring Valley, WI) were placed on the tail head of each heifer to assist with detection of estrus. Heifers were observed twice daily (0600 and 1800 h) for 29 days. Estrus detection patches were considered activated when at least 50% of the patch was discolored, or when patch was absent. Heifers detected in estrus in the morning were artificially inseminated in the afternoon, and heifers in estrus in the afternoon were artificially inseminated the following morning. Heifers were placed with cleanup bulls after the 29-d heat detection and artificial insemination (AI) period, and stayed with the heifers for 60 days. Pregnancy rates to AI were determined via transrectal ultrasonography with an Ibex portable ultrasound equipped with a 5.0 MHz linear

transducer (Ibex, E.I. Medical Imaging, Loveland, CO) 28 days after the last heifers was artificially inseminated. Final pregnancy rates were also assessed via ultrasonography 37 d after bulls were removed.

From weaning until the end of the first breeding season, blood samples were collected weekly to determine puberty achievement in each individual heifer. Samples were collected via venipuncture of the jugular vein into heparinized blood collection tubes (Vacutainer, 10 mL, Becton Dickinson, Franklin Lakes, NJ). After collection, blood samples were placed on ice, and were then centrifuged at 2000 g for 15 min to induce plasma separation. Aliquots were transferred to polypropylene tubes and stored at -20 °C. Plasma were analyzed for concentrations of progesterone using a chemiluminescent enzyme immunoassay (Immulite 2000 XPi platform; Siemens Medical Solutions Diagnostics, Los Angeles, CA) as previously validated for bovine samples (Reis et al., 2014). Heifers were considered to have reached puberty when estimated plasma concentration progesterone was ≥ 1 ng/mL for two consecutive weeks (Perry et al., 1991), and puberty attainment was declared at the first week of elevated progesterone. Heifers were only considered pubertal if these observations were followed by a cyclic pattern of high (> 1 ng/mL) concentrations of progesterone (Schubach et al., 2017).

4.2.3. Statistical Analysis

Residual feed intake (**RFI**) was computed for each heifer as the residual of the regression of dry matter intake (**DMI**) on average daily gain (**ADG**) and metabolic body weight (**MBW**) using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) as described by Black et al., (2013). The model was as follows:

$$Yj = \beta 0 + \beta 1\ ADGj + \beta 2\ MBWj + ej$$

Where Yj is the expected DMI of the jth heifer, β0 is the regression intercept, β1 is the partial regression coefficient of DMI on ADG, β2 is the partial regression coefficient of DMI on MBW, and ej is the residual error of the jth heifer. All data were analyzed using heifer as experimental unit and year as a random variable. Continuous response variables were analyzed using the MIXED procedure of SAS. Although the objectives of the present study did not include the evaluation of potential differences in binary response variables, these were analyzed by the GLIMMIX procedure and reported in the manuscript. Models for both continuous and binary response variables included the fixed effect of cow breed, heifer breed, diet, and all the 2 and 3-way interactions between them. A backward elimination approach was utilized and explanatory variables were removed if $P > 0.20$. For a more conservative interpretation of the data and to reduce the chances of type I error, Tukey adjustment was used for all simultaneous pairwise comparisons. Significance was declared at $P \leq 0.05$.

4.3. Results

The estimates and SEM for gestation length, birth weight, weaning weight, BW in the beginning and end of the feed efficiency test, as well as heifer age at puberty are reported in Table B7. There were no effects of cow breed, embryo breed, diet or their respective interactions on gestation length ($P > 0.10$; Table B7). Similarly, heifer BW at birth was not affected by the prenatal treatments ($P > 0.10$). There were also no effects of breed and diet of dam, or embryo breed on postnatal weaning weights ($P < 0.10$), and 205-d adjusted weaning weights ($P < 0.10$). Furthermore, BW and BCS at the beginning and at the end of the 70-d feed efficiency test were not different between prenatal treatments ($P > 0.10$). Although no clear differences were observed in BW and BCS at the end of the test,

there was a main effect of embryo breed on daily average DMI during the feeding efficiency test. Brangus heifers had greater daily DMI than AN heifers ($P = 0.04$), regardless of breed or diet of the dam (Figure A12). While there were differences between heifer breed on DMI, ADG was not influenced by maternal diet, cow breed, or heifer breed ($P > 0.10$). Consequently, AN heifers were more feed efficient than BN heifers, having lower RFI ($P = 0.02$) and lower feed to gain ratio ($P = 0.04$; Figure A12).

There was a significant difference in age at puberty between AN and BN heifers. As expected, AN heifers reached puberty at a younger age compared to BN heifers ($P = 0.01$; Table B7). There were no effects of cow breed or diet on heifer age at puberty ($P > 0.10$). The proportion of heifers exhibiting estrus during the heat detection period was 67.4% and was also not altered ($P > 0.10$). Additionally, average pregnancy rates during to AI after a 29-d estrus detection period was 25.6% and was not different between prenatal treatments ($P > 0.10$). Final pregnancy rates were 74.4% and also did not differ ($P > 0.10$) between prenatal treatments.

4.4. Discussion

The present experiment evaluated the hypothesis that differences in postnatal growth between *Bos indicus* and *B. taurus* are, to some extent, associated with the prenatal differences in fetal development between these subspecies of cattle. Similar to previous reports evaluating fetal size in early gestation nutrient-restricted cows (Long et al., 2009; Vohname et al., 2003; Ford et al., 2007), *B. indicus* fetuses are smaller during early gestation compared to *B. taurus* counterparts (Mercadante et al., 2013). Moreover, *B. indicus* fetuses have been reported to undergo a relative compensatory growth in utero during mid and late gestation, reaching similar sizes during late gestation (Ferrel, 1991)

and birth (Mercadante et al., 2013) compared to *B. taurus*. This pattern of fetal development resembles previous reports of early gestation nutrient-restricted cows that are realimented to their maintenance requirements during mid and late gestation. Undernutrition during gestation has shown to reduce fetal growth in the sheep, pigs and horses (Vonnahme et al., 2003; Schoknecht et al., 1994; Pugh, 1993, respectively). Accordingly, when cows were nutrient-restricted (68.1% of NEm and 86.7% of MP recommendations) from d 30 to 125 of gestation, IUGR was induced by d 125 of gestation. In the same study, nutrient restricted cows carrying IUGR fetuses had reduced cotyledonary weights and placentome surface area at d 125. Interestingly, although cotyledonary weights and cotyledonary to caruncular weights ratio were still reduced by d 245, realimentation to NRC requirements eliminated differences in fetal weight (Long et al., 2009). Early gestation nutrient restriction have also been shown to reduce cotyledonary vascularity during late gestation as shown by immunohistochemistry-based techniques to quantify differences in capillary area and surface density, suggesting a potential placental programming effect through nutrient restriction (Vonnahme et al., 2007). Similar to the results observed in the bovine, early to mid-gestation nutrient restriction have also repeatedly elicited IUGR in the sheep (Vohname et al., 2003; Kwon et al., 2004) without altering birth weights. Moreover, lambs born from early gestation nutrient restricted ewes had postnatal hyperglycemia and disordered insulin secretion following a glucose tolerance test, hyperleptinemia, and greater adiposity at slaughter (Ford et al., 2007). Although the impacts of early gestation nutrient restriction in utero are similar in sheep and cattle, and the postnatal consequences observed in sheep might have considerable application to beef

production, few studies have evaluated the postnatal effects of early gestation nutrient in the bovine (Long et al., 2010).

Previous reports indicated that gestation length is greater in *B. indicus* vs. *B. taurus* cattle (Reynolds et al., 1980). Moreover, fetal development differs between these subspecies (Ferrell et al., 1991). Using a similar embryo transfer approach as the present study, Ferrell et al., (1991) showed that the genotype of the recipient can influence fetal weight during mid and late-gestation. The growth of Charolais (*B. taurus*) fetuses produced through embryo transfer was reduced in Brahman (*B. indicus*) recipients compared to Charolais recipients. Using the same experimental approach, our group showed that AN cows had greater fetuses at d 91 gestation compared to BN cows, regardless of the genotype of the fetuses (Fontes et al., 2019). In a recent study, Brahman heifers had increased macroscopic density of cotyledonary blood vessels compared AN heifers. Moreover, Brahman heifers had increased expression of ANGP1, FLT1 and KDR in the caruncle and cotyledon and increased cotyledonary capillary area (Lemley et al., 2018). Similar placental adaptation were observed in early gestation nutrient-restricted cows (Lemley et al., 2018; Vonnahme et al., 2007). Although gestation length has been previously shown to differ between subspecies (Reynolds et al., 1980; Mercadante et al., 2013), gestation length was not influenced by cow breed, embryo breed, or diet in the present study. Additionally, restriction during early gestation and subspecies differences were shown to alter birth weights (Long et al., 2007; Mercadante et al., 2013): however, there were no effects of diet or subspecies in the present study.

When heifers were exposed to nutrient restriction from d 32 to 83 of gestation, no differences were observed in offspring BW at birth, weaning (Long et al., 2009). However,

steers from nutrient restricted heifers were heavier at feedlot entry and tended to have greater BW at slaughter (Long et al., 2009). In the same study, the abundance of mRNA for genes related to glucose and fat metabolism (*AP2*, *CD36*, *GLUT4*) were decreased in visceral adipose tissue of steers from nutrient restricted heifers: however, DMI was not measured. Hence, it is reasonable to speculate that prenatal nutrient restriction might influence postnatal nutrient utilization by the offspring and consequently alter feed efficiency. In the present study, there were differences between subspecies in RFI and feed to gain ration. Although AN heifers had similar ADG during the feed efficiency test compared to BN heifers, average daily DMI was in AN heifers. Consequently, BN heifers were less feed efficient than AN heifers and had lower RFI and greater feed to gain ratio. Opposing our hypothesis, there were no effects of cow breed or cow diet in feed efficiency or any other growth related variables measured in this study, indicating that the genotype of the offspring might have a greater influence in postnatal feed efficiency than the genotype of the dam.

Age at puberty was also assessed in the present study. Puberty achievement is a complex physiological process that is severely impacted by nutrition in mammals (Amstaden et al., 2014). Nutritional approaches that promote high rates of body weight gain alters the metabolic profile of heifers, increasing adiposity and circulating concentrations of hormones such as insulin-like growth factor 1, insulin and leptin (Allen et al., 2002; Cardoso et al., 2014b). These metabolic changes can influence hypothalamic function, thus hastening puberty achievement in heifers (reviewed by Cardoso et al., 2018). Because differences in fetal development were associated changes in glucose metabolism and adiposity in the offspring, we hypothesized that the prenatal treatments could influence

age at puberty in the present study. Angus heifers reached puberty earlier than BN heifers, as expected. Yet, there were no effects of cow breed or diet in offspring age at puberty.

In summary, cow breed and diet did not alter any of the postnatal outcomes evaluated in the present study. Instead, the observed differences were associated with the genotype of the offspring. There were no differences in growth throughout the study: however, *B. taurus* heifers were more feed efficient than *B. indicus*-influenced heifers, consuming less and having similar rates of BW gain. In addition, *B. taurus* heifers reached puberty earlier compared with *B. indicus*-influenced counterparts. The results from the present experiment do not support our hypothesis and indicate that breed of the offspring might play a greater role in the postnatal performance differences between *B. indicus* and *B. taurus* cattle than uterine environment changes related to the breed and diet of the dam.

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5. CONCLUSIONS

5.1. Impacts of the Nutrient Restriction, Fetal and Maternal Subspecies and Their Respective Interactions on Prenatal Development of the Offspring

Data from the present study indicates that the inclusion *Bos taurus* genetics in either maternal or fetal systems decreased the probability of pregnancy when nutrient restriction was superimposed, indicating that *B. taurus* cattle are more susceptible to experiencing early pregnancy failure when exposed to a negative plane of nutrition. In addition, the present corroborates with previously reported differences in fetal growth rate and circulating concentrations of PAGs between *B. indicus* and *B. taurus* cattle. The differences observed between subspecies on plasma concentrations of PAGs were always associated with the conceptus breed. Therefore, the differences observed previously across the literature, where *B. indicus* and *B. taurus* females have different plasma concentrations of PAGs (Mercadante et al., 2013), are likely associated with the conceptus genotype rather than the maternal genotype. Furthermore, using a reciprocal embryo transfer approach, our results indicated that the maternal environment, to some extent, controls fetal growth rate differences between these subspecies.

The present study also explored potential differences in the metabolic profile between *B. indicus*-influenced and *B. taurus* cows. No clear differences were observed in plasma concentrations of glucose, NEFA and BHB. However, the results of the present study indicate that there is a more pronounce difference in circulating concentrations of insulin and IGF-1 between MAINT and REST cows that are *B. taurus*. These results corroborate with the differences in pregnancy failure between subspecies observed in cows under energy restriction.

5.2. Impacts of the Nutrient Restriction, Fetal and Maternal Subspecies and Their Respective Interactions on Postnatal Growth, Feed Efficiency and Puberty Achievement

Although the present study provided insights into differences in pregnancy establishment and early fetal development between *B. indicus* and *B. taurus* cattle, there were no effects of maternal genotype or diet during early gestation on any of the response variables evaluated in postnatal life. Because of the previously reported metabolic changes observed in ruminants that undergo intrauterine growth restriction during the first trimester of gestation, we expected that our prenatal treatments could influence post-weaning feed efficiency in the offspring. However, there was only an effect of heifer breed on postnatal response variables. Angus heifers consumed less feed to gain similar weights and reached puberty earlier than BN heifers. It is important to acknowledge that the unexpected differences in pregnancy establishment observed in this experiment resulted in a suboptimal number of offspring to explore potential interaction proposed in this study with adequate statistical power. Hence, further research is required to better explore the potential interactions between fetal development differences and postnatal growth in different subspecies of cattle.

APPENDIX A

FIGURES

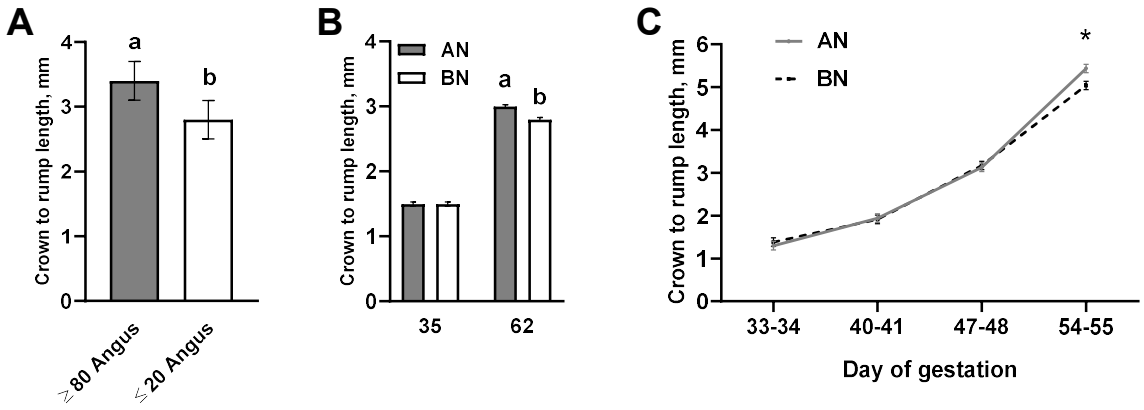

Figure A1. Adapted from Mercadante et al., (2013). Fetus size during early pregnancy in *Bos indicus* and *Bos taurus* cattle. Transrectal ultrasonography was completed to measure crown rump length (CRL). On or after day 54, crown to nose length was measured and data were converted to CRL (Riding et al, 2008). **Panel A**: The study was completed on a multi-breed herd (Gainesville, FL) containing various amounts of Angus and Brahman genetics. A single measurement was completed. Day of gestation ranged from 48-56 days and averaged 53 days. Day of measurement was used as a covariate in the analysis. **Panel B**: Ultrasonography was completed at day 35 and 62 of pregnancy in Angus (**AN**; n = 17) and Brangus (**BN**; n = 25) cows (Marianna, FL). **Panel C**: Ultrasonography was completed on two consecutive days each week for 4 weeks between day 33 to 55 of pregnancy in AN (n = 43) and BN (n= 33) cows (Alachua, FL).
* and ^{a,b} superscripts indicates differences between breeds within day ($P < 0.05$).

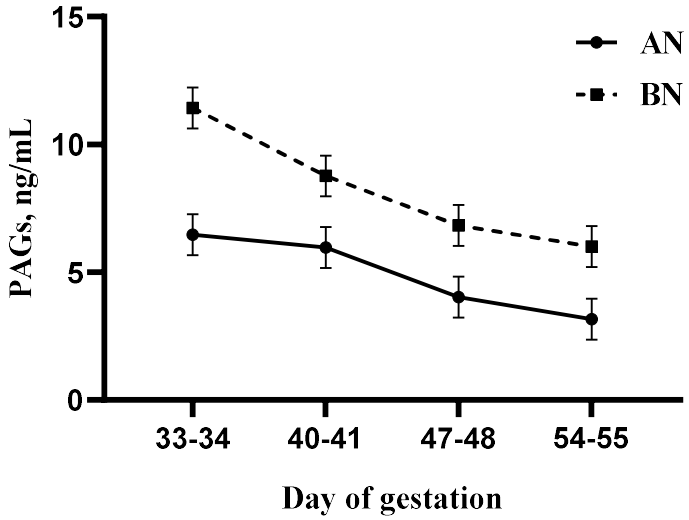

Figure A2. Adapted from Mercadante et al., 2013. Plasma concentrations of pregnancy associated glycoproteins (**PAGs**) concentrations in Angus (**AN**) and Brangus (**BN**) cows during early pregnancy. Cows were sampled on one of two days each week from week 4 to 7 of gestation. A main effect of breed was evident across all weeks ($P < 0.01$).

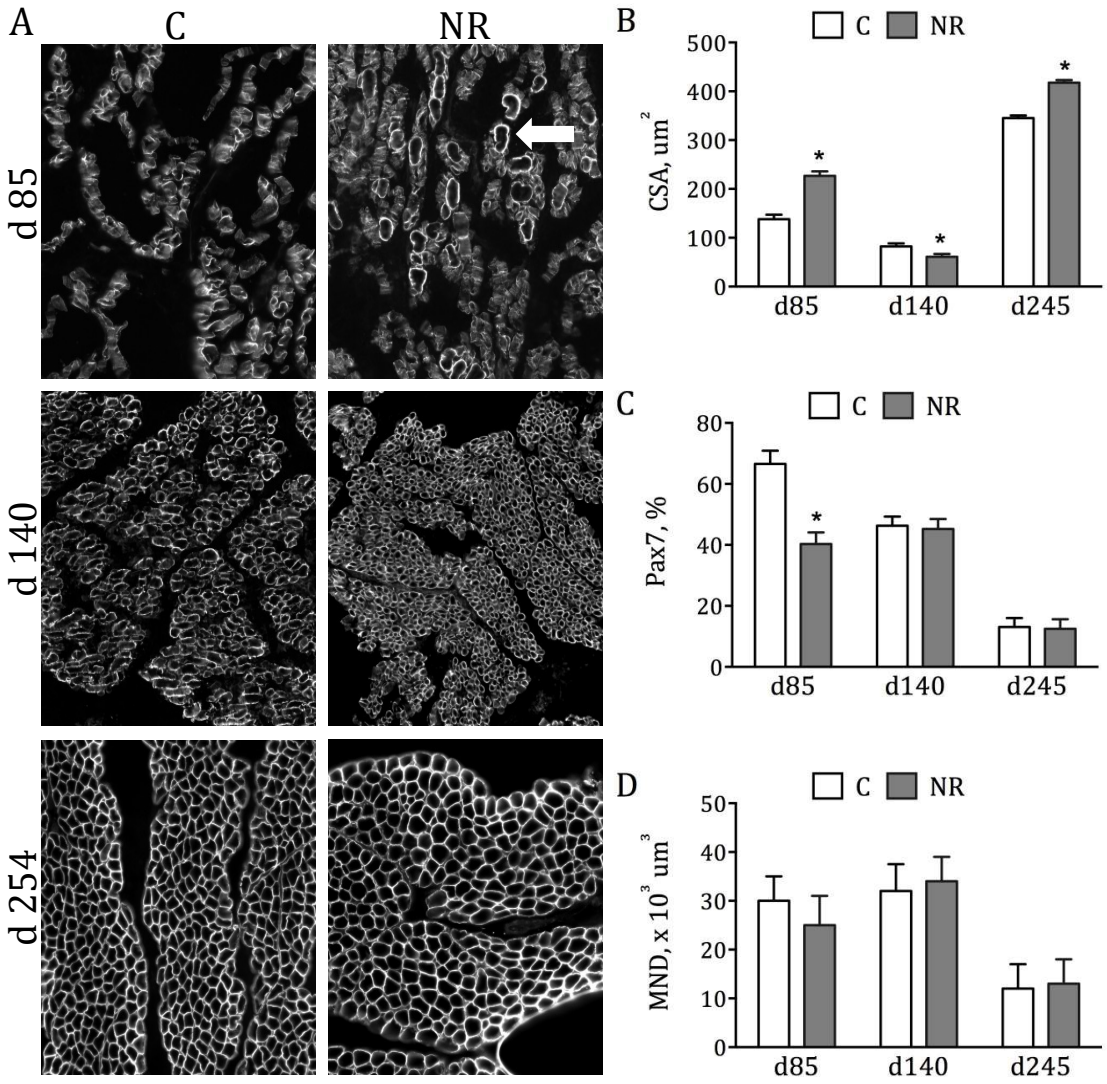

Figure A3. Adapted from Gonzales et al., (2013). Gestational undernutrition impacts fetal skeletal muscle growth**.** Control (**C**) and nutrient restricted (**NR**) cows were slaughter at d 85 of gestation, the end of the NR period, and at d 140 and d 254 gestation. Fetuses were collected and a portion of the forelimb infraspinatus was removed for analysis. Cryosections were immunostained for dystrophin (Panel A) and cross-sectional area (CSA) was measured (Panel B). Arrow indicates a primary muscle fiber. Myogenic progenitors were enumerated following Pax7 immunostaining (Panel C). Myonuclear domain (MND) was calculated as nuclei/CSA volume (D). * represent significance at $P < 0.05$ between C and NR within day.

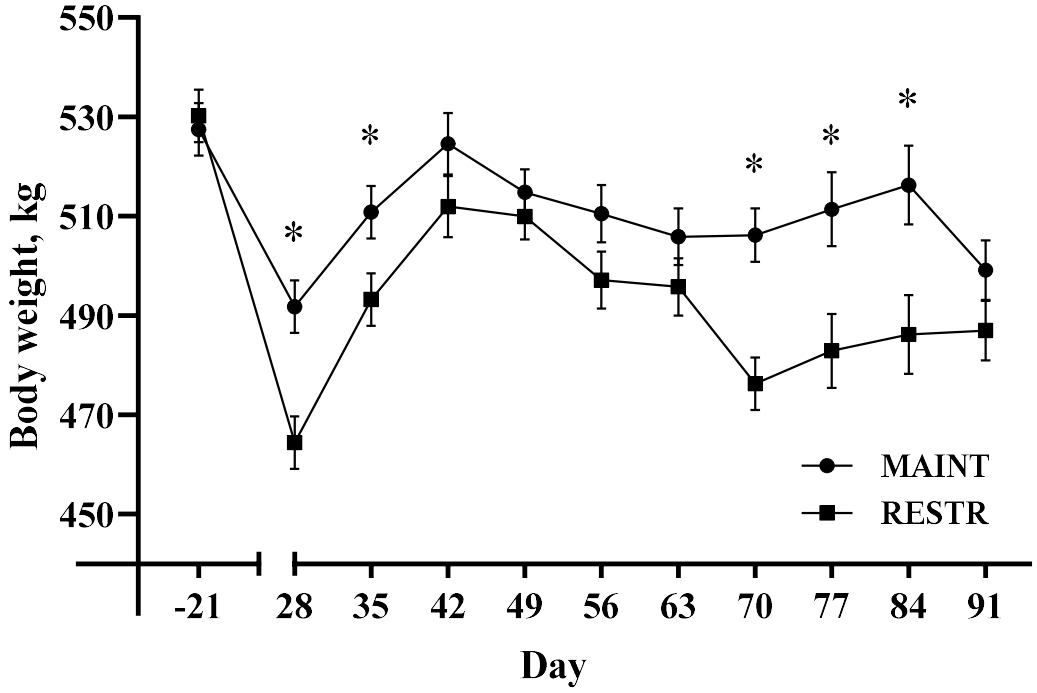

Figure A4. Effects of diet treatments on recipient body weight (**BW**)**. MAINT**: Diet formulated to meet the daily energy requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%); **RESTR**: Diet formulated to meet 70% of daily energy requirements, comprised of 70% pellets, 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: $P < 0.01$. Error bars represent the SEM. *Least square mean difference $P < 0.05$.

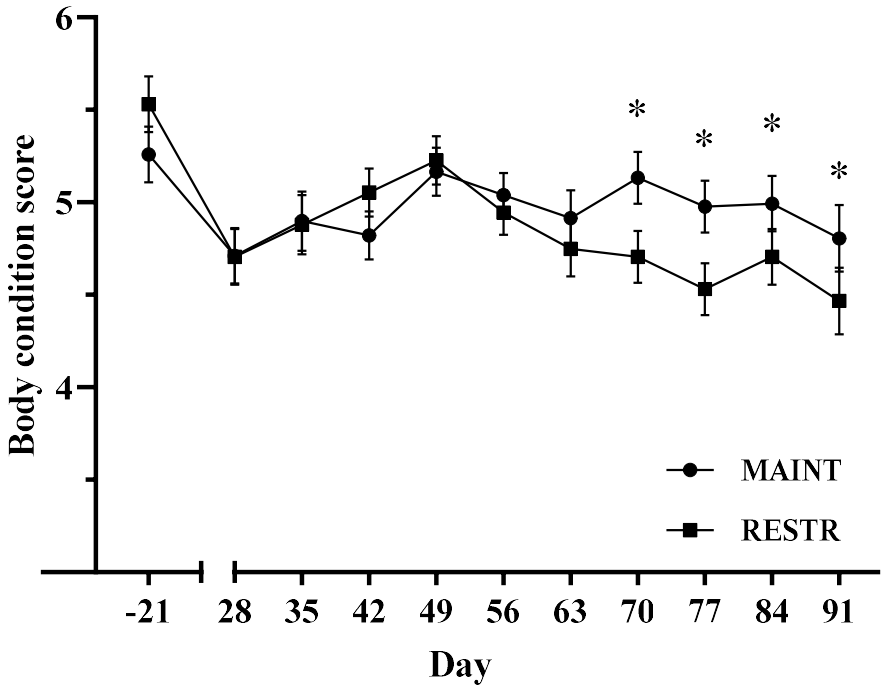

Figure A5. Effects of dietary scheme on recipient body condition score; **MAINT**: Diet formulated to meet the daily requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%) and **RESTR**: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pellets, 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: $P < 0.01$. Error bars represent the SEM. *Least square mean difference $P < 0.05$.

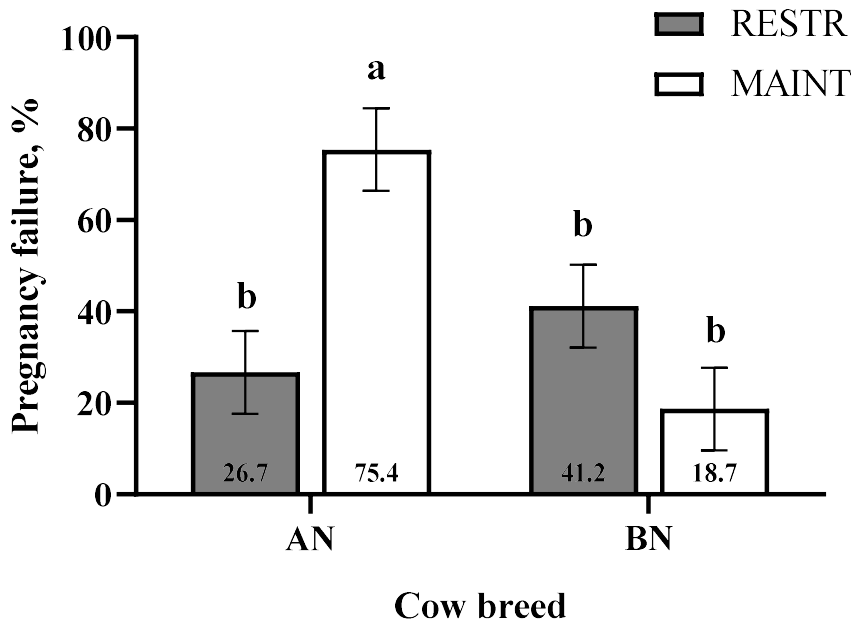

Figure A6. Effects of recipient breed on pregnancy failure at d 28 of gestation. Explanatory variables: **MAINT**: Diet formulated to meet the daily requirements of a 550 kg of body weight suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%); **RESTR**: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%;); **AN**: Angus recipients; **BN**: Brangus recipients. Recipient breed × diet interaction: $P < 0.01$. Error bars represent the SEM. [a,b] Significant least square mean difference ($P < 0.05$).

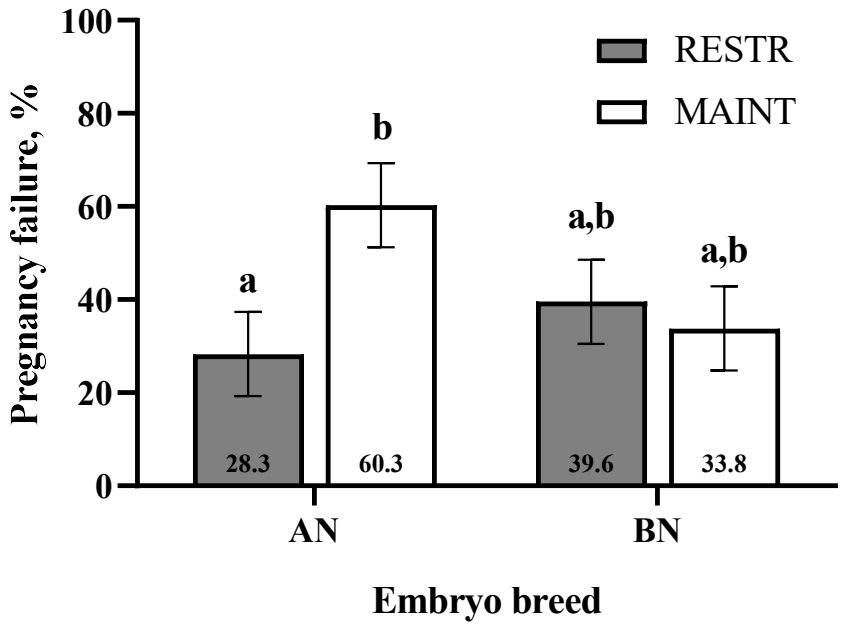

Figure A7. Effects of embryo breed on pregnancy failure at d 28 of gestation. Explanatory variables: **MAINT**: Diet formulated to meet the daily requirements of a 550 kg of body weight suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (CP = 12.5%, TDN = 46%); **RESTR**: Diet formulated to meet 70% of daily requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (CP = 11.2%, TDN = 37%); **AN**: Recipient cows receiving Angus embryos; **BN**: Recipient cows receiving Brangus embryos. Embryo breed × diet interaction: $P = 0.03$. Error bars represent the SEM. [a,b]Significant least square mean difference ($P < 0.05$).

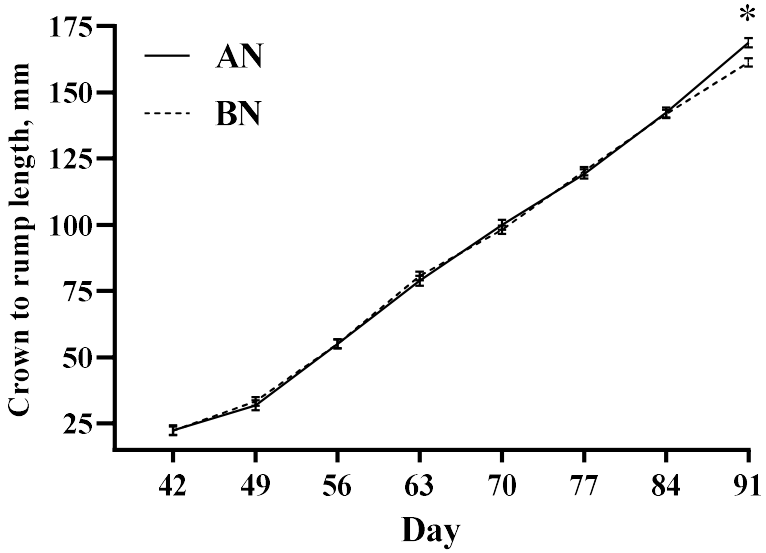

Figure A8. Effects of recipient breed on fetal crown-to-rump length (**CRL**). Transrectal ultrasonography was performed weekly from d 42 to d 91, and fetal CRL was assessed. At d 63, 70, 77, 84 and 91, crown-to-nose length was used to estimate CRL as previously described (Riding et al., 2008). **AN**: Angus recipient and **BN**: Brangus recipient. Recipient breed × Day: $P < 0.01$. Error bars represent the SEM. *Least square mean difference $P < 0.05$.

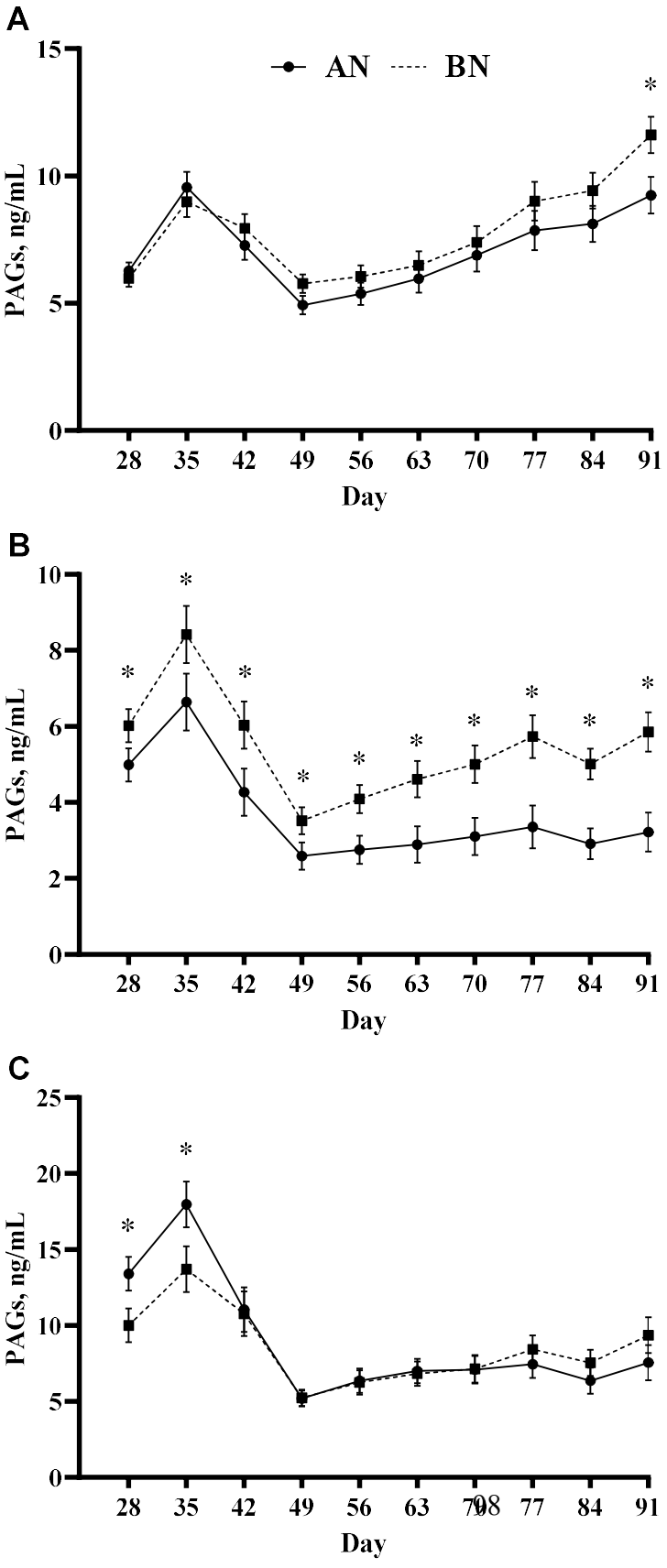
A
AN
BN
PAGs, ng/mL
Day
B
PAGs, ng/mL
Day
C
PAGs, ng/mL
Day

Figure A9. Effects of embryo breed (Angus = **AN**; Brangus = **BN**) on recipient plasma concentrations of pregnancy-associated glycoproteins (**PAGs**). Blood samples were collected weekly from d 28 to 91 of gestation and plasma concentrations of PAGs were measured using 2 commercial PAGs ELISA antibody combinations (A1 [Panel A] and A2 [Panel B]), and an in-house monoclonal-based PAG 63 ELISA (Panel C) as described by Green et al. (2005) and modified by Reese et al., (2017). A1: embryo breed x day ($P < 0.01$). A2: Main effect of embryo breed ($P < 0.01$). In house: embryo breed × day ($P < 0.01$). Error bars represent the SEM. *Least square mean difference $P < 0.05$.

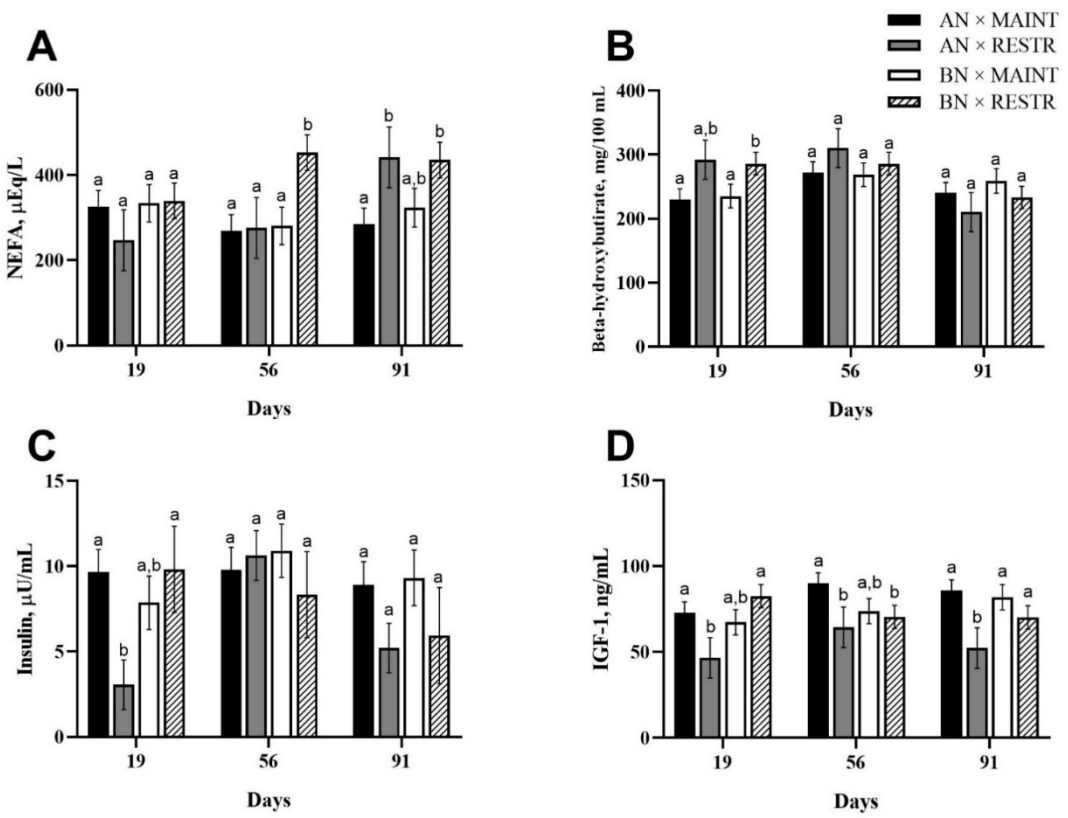

Figure A10. Effects of cow subspecies (**AN**: Angus [*Bos taurus*] and **BN**: Brangus [*Bos indicus*-influenced]) and dietary scheme (**MAINT**: diet formulated to meet the energy requirements of a 550 kg of body weight suckled beef cow [NASEM, 2016]; and **RESTR**: diet formulated to meet 70% of the energy requirements) on plasma concentration of non-esterified fatty acids (**NEFA**; Panel A), beta-hydroxybutirate (**BHB**; Panel B), insulin (Panel C), and insulin-like growth factor 1 (**IGF-1**; Panel D). Panel A: diet × day: P = 0.01. Panel B: diet × day: P = 0.005. Panel C: breed × diet × day: P = 0.04. Panel D: breed × diet: P =0.03, breed × day: P = 0.02, diet × day: P = 0.06. The day in which ovulation was induced (7 days prior to embryo transfer) was considered d 0 of the experiment. Pregnancy diagnosis was performed at d 28.
[a,b]Least square mean difference: $P < 0.05$.

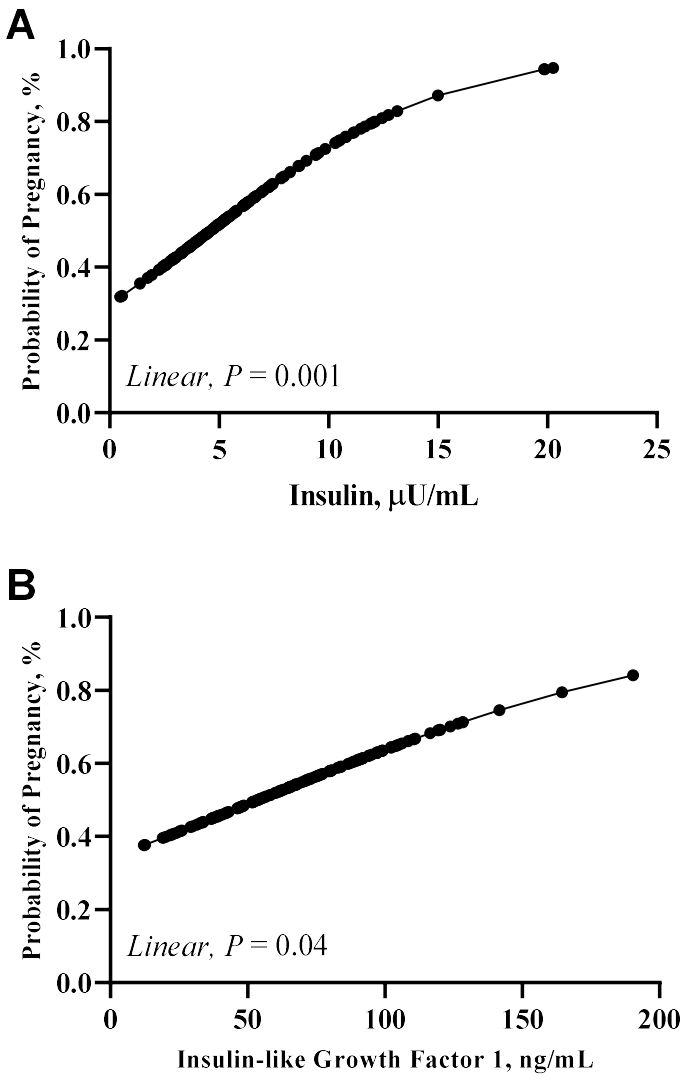

Figure A11. Probability of pregnancy to fixed-time embryo transfer in suckled beef cows according to the plasma concentrations of insulin at d -21 (**A**) and insulin-like growth factor 1 at d 19 of gestation (**B**). Pregnancy status was assessed through transrectal ultrasonography at d 28 of gestation.

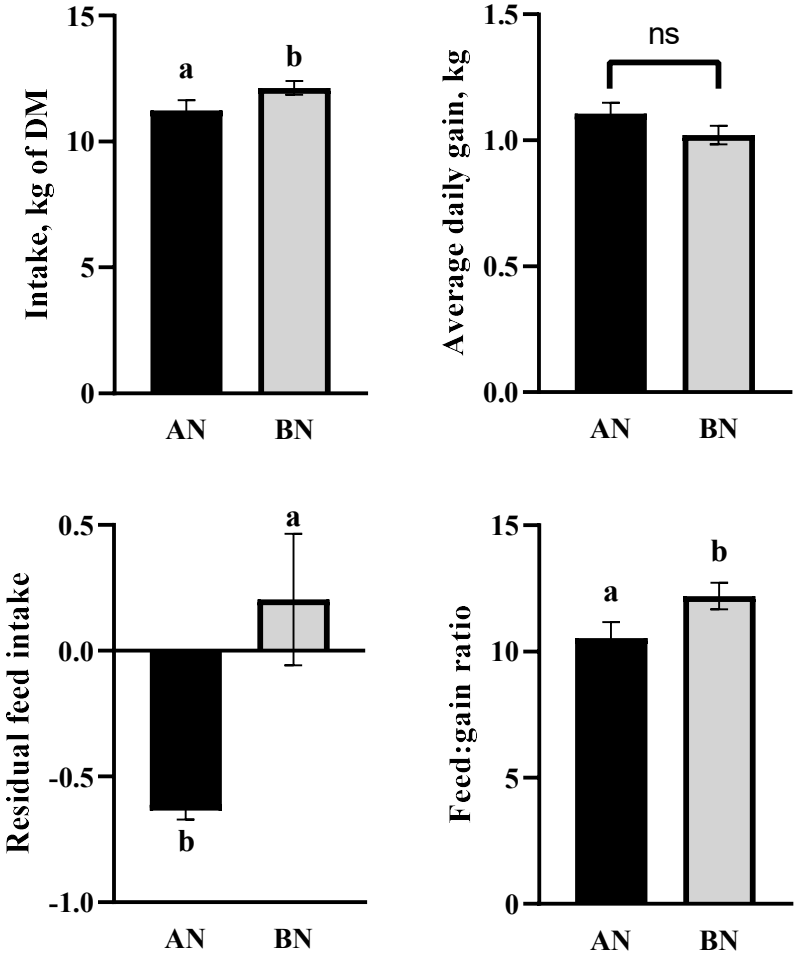

Figure A12. Effects of heifer genotype on dry matter (**DM**) intake, average daily gain, residual feed intake, and feed to gain ratio during a 70-d feed efficiency evaluation. Heifers were generated through a reciprocal embryo transfer, where Angus (**AN**) or Brangus (**BN**) embryos were transferred to AN or BN recipients exposed to either a diet formulated to meet the energy maintenance requirements (**MAINT**) or to restrict energy intake to 70% of the maintenance requirements (**RESTR**) during the first trimester of gestation. There were no effects of breed or diet of the dam on postnatal performance ($P > 0.10$). ^{a,b} uncommon superscript represent an effect of heifers breed ($P < 0.05$).^{ns} represent no statistical difference ($P > 0.10$).

APPENDIX B

TABLES

Table B1. Composition and nutrient analysis[2] of dietary treatments.

Item	REST	MAINT
Ingredient, % (as-fed basis)		
Fiber pallets	70.0	60.0
Soybean hulls	10.0	30.0
Bermudagrass hay	5.0	5.0
Peanut hulls	15.0	5.0
Nutrient profile		
DM, %	92.3	94.8
CP, %	11.2	12.5
NDF, %	69.2	67.9
ADF, %	63.0	56.2
TDN, %	37	46
NE_l, Mcal/kg	0.73	0.97
NE_m, Mcal/kg	0.68	0.98
NE_g, Mcal/kg	0.15	0.43

[1]**MAINT**: Diet formulated to meet the daily requirements of a 550 kg of body weight suckled beef cow (NRC, 2000), comprised of 60 % fiber pallets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls; **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.

[2]Analyzed by a commercial laboratory using a wet chemistry package (Dairy One, Ithaca, NY).

Table B2. Nucleotide sequence of bovine-specific primers used in the quantitative real-time reverse transcription PCR to determine expression of target genes.

Target gene	Gene name	Accession no.	Primer	Primer sequence
ISG15	Interferon stimulated gene 15	NM_174366	Forward	5' – GGTATCCGAGCTGAAGCAGTT – 3'
			Reverse	5' – ACCTCCCTGCTGTCAAGGT – 3'
PPIA	Peptidylprolyl Isomerase A	BF230516	Forward	5' – GCCATGGAGCGCTTTGG – 3'
			Reverse	5' – CCACAGTCAGCAATGGTGATCT – 3'
MX2	Myxovirus resistance 2	NM_173941	Forward	5' – CTTCAGAGACGCCTCAGTCG – 3'
			Reverse	5' – TGAAGCAGCCAGGAATAGT – 3'
OAS1	2'-5'-oligoadenylate synthetase 1	NM_001040606	Forward	5' – TAGCCTGGAACATCAGGTC – 3'
			Reverse	5' – TTTGGTCTGGCTGGATTACC – 3'

Table B3. Effects of dietary treatments[1] on recipient body weight (**BW**) and body condition score (**BCS**).

Item	RESTR	MAINT	SEM	*P* - value
NE_m requirements met[2], %	83.2	108.7	2.49	< 0.01
Initial BW, kg	532.1	514.9	8.11	0.11
BW at d 28, kg	466.2	475.7	3.68	0.08
BW change from d -21 to d 28, kg	-57.6	-45.3	3.90	0.03
BW change from d -21 to d 91, kg	-47.7	-27.2	6.30	0.02
Initial BCS	5.4	5.2	0.07	0.16
BCS change from d -21 to d 90, kg	-1.0	0.4	0.12	<0.01

[1]**MAINT**: Diet formulated to meet the daily energy requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (crude protein = 12.5%, total digestible nutrients = 46%); **RESTR**: Diet formulated to meet 70% of daily energy requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (crude protein = 11.2%, total digestible nutrients = 37%).

[2]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, according to the NASEM (2016).

Table B4. Effects of recipient subspecies, diet[1] and subspecies × diet on parameters associated with cow nutritional status.

	Angus		Brangus			*P*-value		
Item	**MAINT**	**RESTR**	**MAINT**	**RESTR**	**SEM**	**Subspecies**	**Diet**	**Subs × Diet**
Day -21								
Body weight, kg	512.1	533.7	516.8	531.4	11.69	0.918	0.120	0.763
Body condition score	5.2	5.3	5.3	5.5	0.19	0.230	0.157	0.533
Non-esterified fatty acids	547.8	512.2	628.2	669.9	285.57	0.015	0.949	0.424
β-hydroxybutyrate	244.0	279.0	297.6	286.4	57.26	0.014	0.335	0.062
Glucose	71.8	72.2	68.9	72.7	1.74	0.450	0.204	0.302
Insulin	6.4	6.1	5.5	6.7	0.95	0.797	0.489	0.278
Insulin-like growth factor 1	63.1	63.0	67.3	73.2	18.28	0.185	0.594	0.573
Day 19								
Body weight, kg	502.2	495.1	498.4	497.3	22.00	0.832	0.298	0.443
Body condition score	5.1	5.0	5.2	4.9	0.19	0.397	0.008	0.390
Non-esterified fatty acids	332.1	348.3	377.0	400.8	119.22	0.157	0.548	0.911
β-hydroxybutyrate	247.0	231.6	237.3	261.8	32.71	0.450	0.734	0.138
Glucose	72.3	70.8	72.4	73.8	3.92	0.214	0.959	0.260
Insulin	8.5[a]	6.2[b]	7.5[a]	8.9[a]	1.57	0.363	0.616	0.034
Insulin-like growth factor 1	74.5[a]	56.1[b]	71.7[a]	72.3[a]	5.05	0.135	0.049	0.034
Day -21 to d 91								
NE_m requirement met, %	95.6	79.1	92.9	75.9	13.14	0.288	<0.001	0.964

[1]MAINT: Diet formulated to meet the daily energy requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (crude protein = 12.5%, total digestible nutrients = 46%); RESTR: Diet formulated to meet 70% of daily energy requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (crude protein = 11.2%, total digestible nutrients = 37%). The day in which ovulation was induced (7 days prior to embryo transfer) was considered d 0 of the experiment.

[a,b] Least square mean difference: $P < 0.05$.

Table B5. Effects of recipient subspecies, diet[1] and subspecies × diet on cow body weight (BW), body condition score (BCS), and percentage of NE_m[2] met by the diet of pregnant cows only.

	Angus		Brangus			*P*-value		
Item	**MAINT**	**RESTR**	**MAINT**	**RESTR**	**SEM**	**Subspecies**	**Diet**	**Subs × Diet**
d -21								
BW, kg	511.5	584.0	545.0	554.3	21.72	0.931	0.066	0.152
BCS	5.2	5.8	5.4	5.6	0.21	0.911	0.036	0.424
d 91								
BW, kg	484.4	517.6	506.3	515.8	18.79	0.601	0.265	0.537
BCS	4.8	4.8	5.0	4.7	0.28	0.576	0.460	0.670
d -21 to d 91								
BW change, kg	-28.3[a]	-74.4[b]	-39.8[a,b]	-46.5[a,b]	15.18	0.464	0.025	0.084
BCS change	-0.4	-1.1	-0.4	0.9	0.20	0.547	0.002	0.807
Pecentage of NE_m, %	102.3	68.5	100.0	72.4	8.71	0.842	<0.001	0.445

[1]MAINT: Diet formulated to meet the daily energy requirements of a 550 kg of BW suckled beef cow (NASEM, 2016), comprised of 60 % fiber pellets, 30%, soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 5% peanut hulls (crude protein = 12.5%, total digestible nutrients = 46%); RESTR: Diet formulated to meet 70% of daily energy requirements, comprised of 70% fiber pellets, 10% soybean hulls, 5% bermudagrass (*Cynodon dactylon*) hay, and 15% peanut hulls (crude protein = 11.2%, total digestible nutrients = 37%). The day in which ovulation was induced (7 days prior to embryo transfer) was considered d 0 of the experiment.

[2]The percentages of NE_m met by the diets were calculated for each individual cow according to NASEM, 2016.

[a,b] Least square mean difference: $P < 0.05$.

Table B6. Composition and nutrient analysis[1] of heifer diet during feed efficiency evaluation.

Item	Year 1	Year 2
Ingredient, as-fed basis		
Corn gluten feed	22.5	22.5
Soybean hulls	22.5	22.5
Supplement pellets	5.0	5.0
Fiber pellets	50.0	50.0
Nutrient profile		
DM, %	94.9	92.1
CP, %	13.7	14.3
NDF, %	63.4	54.4
ADF, %	40.9	36.6
TDN, %	65	69
NE_m, Mcal/kg	0.62	0.71
NE_g, Mcal/kg	0.36	0.44

[1]Analyzed by a commercial laboratory using wet chemistry package (Dairy One, Ithaca, NY)

Table B7. Effects of recipient breed, embryo breed and diet on gestation length and postnatal performance of the offspring[1].

	AN Recipient				BN Recipient				
	AN Embryo		BN Embryo		AN Embryo		BN Embryo		
Item	REST	MAINT	REST	MAINT	REST	MAINT	REST	MAINT	SEM[2]
Gestation length, d	280.7	276.8	273.2	279.8	272.9	276.3	278.7	278.5	4.39
Birth weight, kg	64.3	64.0	58.3	62.5	54.9	60.2	67.0	64.1	11.58
Weaning weight, kg	224.3	240.1	232.8	225.2	232.3	236.2	240.1	241.4	26.78
205-d adj. WW, kg[3]	232.3	244.4	234.2	237.9	236.3	238.3	244.3	246.6	35.51
Initial test weight, kg	280.8	286.4	307.9	282.4	294.2	303.8	311.3	298.5	29.20
Final test weight, kg	352.5	364.8	383.7	358.7	372.3	370.7	383.1	366.8	33.43
Age at puberty, d	385[a]	366[a]	418[b]	418[b]	356[a]	386[a]	416[b]	486[b]	61.3

[1]Angus (**AN**) and Brangus (**BN**) recipients received AN or BN embryos. Recipient were exposed to 1 of the 2 diets: 1) **MAINT**: diet formulated to meet the energy requirements of a 550 kg of b suckled beef cow (NASEM, 2016); 2) **RESTR**: diet formulated to meet 70% of the energy requirements).
[2]Standard error of the means represents the largest standard error value. [a,b] Uncommon superscript represent significant differences within the same row ($P \leq 0.05$).
[3]Weaning weights were adjusted to 205 days of age (BIF, 2018)