



LUCCA GABRIEL BATISTA PEREIRA

**ASSESSMENT OF PERFORMANCE AND PHYSIOLOGICAL
PARAMETERS OF BEEF COWS AND THEIR OFFSPRING AS
A FUNCTION OF PRODUCTION SYSTEM
INTENSIFICATION LEVEL**

**LAVRAS, MG
2025**

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Master thesis presented to the Universidade Federal de Lavras, as part of the requirements of the Animal Science Graduate Program, in the area of Ruminant Nutrition and Production, to obtain the title of "Master in Animal Science".

Advisor: Prof. Dr. Mateus Pies Gionbelli

Co-advisor: Dra. Karolina Batista Nascimento

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**AVALIAÇÃO DO DESEMPENHO E DOS PARÂMETROS FISIOLÓGICOS DE
VACAS DE CORTE E DA PROGÊNIE EM FUNÇÃO DO NÍVEL DE
INTENSIFICAÇÃO DO SISTEMA DE PRODUÇÃO**

Master thesis presented to the Universidade Federal de Lavras, as part of the requirements of the Animal Science Graduate Program, in the area of Ruminant Nutrition and Production, to obtain the title of "Master in Animal Science".

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Dedicate

ABSTRACT

The intensification of production systems for breeding herds has the potential to mitigate the effects of seasonality on forage availability and quality, contributing to the economic and environmental sustainability of beef cattle production. This study aimed to evaluate the productive parameters of beef cows and their offspring in response to different levels of production system intensification during gestation, considering the influence of fetal sex, and to analyze maternal physiological and gestational variables throughout pregnancy. A total of 72 pregnant Tabapuã cows (*Bos taurus indicus*) were used and allocated to three treatments: Control (CON) – unfertilized pasture with mineral supplementation; Intensive (INT) – nitrogen (N)-fertilized pasture with protein-energy supplementation during the dry season; and Superintensive (SUP) – fertilized pasture with total mixed ration during the dry season. A significant interaction between maternal nutrition and fetal sex was observed for Longissimus muscle area ($P = 0.049$), with a trend for greater area relative to body weight in cows from the intensive system carrying male fetuses ($P = 0.102$). CON cows showed greater rump fat thickness ($P = 0.033$), while pregnancy rates and hemodynamic parameters were not affected by the systems. Regarding offspring, male calves showed higher average daily gain, weaning weight, and ribeye area, whereas female calves demonstrated greater rump fat thickness. Calf vigor at birth was not significantly affected; however, a trend toward reduced vigor was observed in calves from SUP cows. The results indicate that, although production system intensification did not significantly enhance offspring performance under nutritional differences, sex-based differences in performance traits were evident. In addition, the analysis of maternal and gestational variables contributes to understanding the interactions between maternal nutrition during pregnancy and productive performance in different systems of intensification in beef cattle production.

Keywords: Doppler; fetal programming; gestational nutrition; maternal tissue; offspring sex.

RESUMO

A intensificação dos sistemas de produção para rebanhos de cria tem o potencial de mitigar os efeitos da sazonalidade na disponibilidade e qualidade das forragens, contribuindo para a sustentabilidade econômica e ambiental da pecuária de corte. Este estudo teve como objetivo avaliar os parâmetros produtivos de vacas de corte e seus descendentes em resposta a diferentes níveis de intensificação do sistema de produção durante a gestação, considerando a influência do sexo fetal, além de analisar variáveis fisiológicas e gestacionais maternas ao longo da gestação. Foram utilizadas 72 vacas prenhes da raça Tabapuã (*Bos taurus indicus*), distribuídas em três tratamentos: Controle (CON) – pastagem sem fertilização com suplementação mineral; Intensivo (INT) – pastagem fertilizada com nitrogênio (N) e suplementação proteico-energética durante a estação seca; e Superintensivo (SUP) – pastagem fertilizada com ração total misturada durante a estação seca. Foi observada uma interação significativa entre nutrição materna e sexo fetal para a área de olho de lombo ($P = 0,049$), com uma tendência de maior área relativa ao peso corporal em vacas do sistema intensivo que gestavam fetos machos ($P = 0,102$). As vacas CON apresentaram maior espessura de gordura na anca ($P = 0,033$), enquanto as taxas de prenhez e os parâmetros hemodinâmicos não foram afetados pelos sistemas. Quanto as progênes, bezerros machos apresentaram maior ganho médio diário, peso à desmama e área de olho de lombo, enquanto as fêmeas demonstraram maior espessura de gordura na garupa. O vigor dos bezerros ao nascimento não foi significativamente afetado, no entanto, uma tendência de redução do vigor foi observada em bezerros provenientes de vacas do tratamento SUP. Os resultados indicam que, embora a intensificação do sistema de produção não tenha melhorado significativamente o desempenho dos descendentes sob diferenças nutricionais, diferenças baseadas no sexo em características de desempenho foram evidentes. Além disso, a análise das variáveis maternas e gestacionais contribui para a compreensão das interações entre a nutrição materna durante a gestação e o desempenho produtivo em diferentes sistemas de intensificação na pecuária de corte.

Palavras-chave: Doppler; nutrição gestacional; programação fetal; sexo da progênie, tecido materno.

Impactos sociais, tecnológicos, econômicos e culturais

A ampliação de sistemas de produção para rebanhos de cria, conforme analisado neste estudo, gera efeitos consideráveis nas áreas social, tecnológica, econômica e ambiental, favorecendo a sustentabilidade da pecuária de corte em regiões tropicais e subtropicais. Socialmente, os resultados ajudam na segurança alimentar, ao aumentar a eficiência produtiva de sistemas de pecuária e ampliar a disponibilidade de carne bovina de qualidade para satisfazer a crescente demanda global. O estudo também ressalta seu potencial extensionista, ao reforçar práticas sustentáveis e propagar estratégias nutricionais eficientes durante a gestação. No contexto tecnológico, a pesquisa aprofunda a compreensão sobre os impactos da nutrição da mãe na fisiologia dela e no desempenho dos filhos, produzindo informações que podem fundamentar a criação de tecnologias e métodos inovadores direcionados à programação fetal e ao manejo reprodutivo. Esses avanços também possibilitam melhorar a utilização de recursos, como fertilizantes e suplementos, promovendo uma maior eficiência na produção animal. Economicamente, os resultados indicam a diminuição das despesas de produção e o incremento do retorno financeiro para os produtores, ao aprimorar indicadores como ganho de peso e rendimento de carcaça dos bezerros. Ademais, a valorização de sistemas intensivos pode promover investimentos em tecnologias de manejo, auxiliando no desenvolvimento econômico regional. Culturalmente, a pesquisa gera uma transformação de paradigma na pecuária, ao incorporar métodos inovadores e ecológicos na rotina dos criadores, aprimorando a percepção da pecuária brasileira como uma atividade sustentável. Do ponto de vista ambiental, os sistemas de intensificação sugeridos mostram capacidade para minimizar os efeitos da sazonalidade forrageira, aprimorar a utilização de áreas agrícolas e diminuir a pressão por conversão de novas terras, convergindo para os Objetivos de Desenvolvimento Sustentável (ODS) da ONU, em particular os ODS 2 (Fome Zero e Agricultura Sustentável), 12 (Consumo e Produção Responsáveis) e 13 (Ação Contra a Mudança Global do Clima). Os efeitos potenciais do trabalho abrangem áreas significativas da produção pecuária no Brasil, beneficiando comunidades rurais e mercados consumidores, ao mesmo tempo que fortalecem a importância da ciência na formulação de soluções inovadoras para desafios atuais. Através da junção de produtores e regiões à adoção de técnicas mais eficazes e sustentáveis, a pesquisa estimula o fortalecimento socioeconômico e ambiental de regiões chave para a pecuária de corte.

Social, technological, economic and cultural impacts

The expansion of production systems for breeding herds, as analyzed in this study, generates significant effects in the social, technological, economic, and environmental domains, promoting the sustainability of beef cattle production in tropical and subtropical regions. Socially, the results contribute to food security by increasing the productive efficiency of livestock systems and enhancing the availability of high-quality beef to meet the growing global demand. The study also highlights its extension potential by reinforcing sustainable practices and disseminating efficient nutritional strategies during gestation. In the technological context, the research deepens the understanding of maternal nutrition's impacts on maternal physiology and offspring performance, providing information that can support the development of innovative technologies and methods aimed at fetal programming and reproductive management. These advancements also enable improved use of resources such as fertilizers and supplements, promoting greater efficiency in animal production. Economically, the results suggest a reduction in production costs and an increase in financial returns for producers by improving indicators such as weight gain and carcass yield in calves. Furthermore, the valorization of intensive systems can drive investments in management technologies, aiding in regional economic development. Culturally, the research fosters a paradigm shift in livestock production by integrating innovative and eco-friendly methods into farmers' routines, enhancing the perception of Brazilian cattle farming as a sustainable activity. From an environmental perspective, the proposed intensification systems demonstrate potential to mitigate the effects of forage seasonality, optimize the use of agricultural areas, and reduce pressure for the conversion of new lands, aligning with the United Nations Sustainable Development Goals (SDGs), particularly SDG 2 (Zero Hunger and Sustainable Agriculture), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action). The potential impacts of this work encompass key areas of livestock production in Brazil, benefiting rural communities and consumer markets while reinforcing the importance of science in crafting innovative solutions to contemporary challenges. By integrating producers and regions into the adoption of more effective and sustainable techniques, the research fosters the socioeconomic and environmental strengthening of strategic areas for beef cattle production.

LIST OF FIGURES

Figure 1. Gestational components (PREG) of pregnant beef cows concerning fetal sex and varying levels of intensification.....	50
Figure 2. Variation in crude protein in forage during production cycles.	50
Figure 3. Variation of Neutral Detergent Fiber (NDF) in forage during production cycles.	51

LIST OF TABLES

Table 1. Concentration of nutrients in silage and supplement.....	47
Table 2. Influence of intensification levels during mid and late gestation on muscle and adiposity accretion in beef cows.....	48
Table 3. The influence of intensification levels in beef cows during the mid and late gestation periods on pulsatility indices (PI) resistance indices (RI) and systolic/diastolic ratios in beef cows.	48
Table 4. Influence of intensification levels during mid and late gestation on birth weight, weaning weight, average daily gain, and vigor of calves.....	49
Table 5. Influence of intensification levels during mid and late gestation on skeletal muscle and adiposity of calves.	49

SUMMARY

FIRST SECTION.....	6
1.0 INTRODUCTION	13
2. BACKGROUND	14
2.1 Brazilian Beef Cattle Production	14
2.2 Seasonality	15
2.3 Fetal Programming and Gestational Nutrition.....	16
2.4 Intensification Strategies in Beef Cattle Production	18
3.0 REFERENCES.....	23
SECOND SECTION – ARTICLE.....	29
ARTICLE 1 - Impact of Production System Intensification on Maternal Tissue Dynamics, Uterine Hemodynamics, and Skeletal Muscle Development in Beef Cows and Their Progeny.....	29
1.0 INTRODUCTION	30
2. MATERIAL AND METHODS	31
2.1 Animals, treatments, feeding, and experimental design	31
2.2 Maternal weight gain, and body score condition	34
2.3 Maternal nutrient accretion and mobilization.....	34
2.4 Uterine hemodynamics	35
2.5 Evaluation Offspring.....	35
2.6 Statistical Analyses	36
3. RESULTS	39
3.1 Skeletal muscle and adiposity, and pregnant compounds in beef cows	39
3.2 Hemodynamic parameters.....	39
3.3 Offspring’s vigor score and performance.....	40
3.4 Skeletal muscle and adiposity in calves	40
4. DISCUSSION	40
5. CONCLUSION	43
6. REFERENCES.....	44

1.0 INTRODUCTION

Pastures are the primary feed source for beef cattle, particularly in the Midwest, due to their low cost and ease of implementation. However, these systems are highly susceptible to climatic variations, resulting in marked seasonal fluctuations in forage availability and quality (Paula et al., 2010). For cow-calf systems, the breeding season typically aligns with the rainy season, meaning that cows are often in the mid to late stages of pregnancy during the dry season, a period characterized by limited forage availability and reduced nutritive value. This scenario exacerbates nutritional deficiencies, imposing metabolic challenges on pregnant cows and compromising fetal development (Rodrigues et al., 2020).

Nutritional deficits during gestation often force pregnant cows to mobilize body reserves, especially lean tissue, to support fetal development (Lopes et al., 2020). Consequently, peripheral mobilization of body reserves becomes a key mechanism, particularly during periods of forage scarcity (Costa et al., 2021).

Enhancing livestock productivity requires targeted nutritional interventions that prioritize efficiency and cost reduction, ultimately contributing to economically viable and environmentally sustainable production systems. Nutrition also plays a pivotal role in beef cattle reproduction. Nutritional inadequacies can lead to significant reproductive inefficiencies, resulting in substantial financial losses for the meat industry. These include reduced reproductive rates, delayed breeding, increased veterinary expenses, and decreased calf birth rates (Fontes et al., 2020).

In addition, recent studies have highlighted the negative effects of maternal undernutrition during gestation on offspring outcomes. These include reduced birth weights (Caton et al., 2019; Funston et al., 2010), lower calf survival rates (Monteiro et al., 2016), and decreased weaning weights (Marquez et al., 2017). These findings underscore the importance of investigating how maternal nutrition impacts both the progeny's productivity and the dams' physiological parameters.

While intensification strategies can mitigate the effects of forage scarcity, their implementation requires careful management to optimize resource use and minimize costs. Furthermore, the principles of fetal programming suggest that maternal nutrition during critical gestational periods can have lasting effects on the offspring's growth, metabolism, and productivity.

Based on this, the objectives of the present study were:

- To evaluate the productive parameters of beef cows and their offspring in response to varying levels of production system intensification during gestation, accounting for the influence of calf sex.
- To analyze the maternal physiological and gestational variables, including Longissimus muscle area (LMA), subcutaneous fat thickness in the LMA, rump muscle length, rump fat thickness, and gestational components throughout pregnancy.

2. BACKGROUND

2.1 Brazilian Beef Cattle Production

Beef cattle production systems in Brazil are pivotal for the national economy. In 2023, these systems contributed approximately 7% to the country's Gross Domestic Product (GDP) (CEPEA, 2023). Between 2010 and 2020, the contribution of beef cattle agribusiness to the national GDP fluctuated between 7.5% and 10%, peaking in 2020 (ABIEC, 2021). This reflects Brazil's global leadership as the largest exporter of beef, possessing the second-largest herd worldwide and ranking among the top producers (USDA, 2022).

In the first quarter of 2023, beef carcass production totaled 2.14 million tons, representing a 12.6% increase compared to the same period in the previous year. In the second quarter of 2023, 8.25 million cattle were slaughtered under sanitary inspection, resulting in an 11.0% rise compared to the second quarter of 2022 and a 12.3% increase compared to the first quarter of 2023. Moreover, beef carcass production in the second quarter of 2023 increased by 9.5% relative to the same quarter of the previous year (IBGE, 2023).

For tropical pastures continuously grazed by cattle, the recommended stocking rate ranges from 5.0 to 6.0 Animal Units per hectare (AU/ha) (Costa et al., 2021). Maintaining this rate is crucial to balance pasture productivity with cattle performance and prevent both undergrazing and overgrazing. However, achieving and sustaining this rate poses challenges for producers as it enables increased beef production without expanding pasture areas (Michalk et al., 2019).

Analysis of production systems in Brazil highlights a stark contrast between the country's vast potential and the rudimentary practices still prevalent on many farms. This discrepancy underscores the pressing need to adopt more efficient practices.

The drive to maximize productivity in livestock systems amplifies the challenges posed by the dry season. Characterized by climatic variations, this period negatively impacts forage quality, leading to reduced productivity (Ivo, 2021). Furthermore, reproductive inefficiencies are key limiting factors, undermining herd productivity and profitability (Bergamaschi; Machado; Barbosa, 2010).

Despite its position as a leading beef producer, Brazil still holds significant untapped potential. Average production could be substantially improved through the adoption of straightforward techniques such as pasture subdivision, restoration and fertilization, enhanced management practices, supplementation during critical periods, more effective sanitary control, and genetic improvement of herds (Kichel; Miranda; Zimmer, 1999). Addressing these areas provides a pathway to improving production systems.

2.2 Seasonality

The Brazilian beef production system typically initiates the reproductive season between November and January. During this time, the second trimester of gestation coincides with the dry season, creating dietary challenges for pregnant cows and posing critical risks for beef production (de Almeida et al., 2022). To enhance reproductive efficiency, most cows should conceive as early as possible, reducing the calving interval and increasing calving rates. Considering the 9.5-month gestation period, the mating season should ideally last no more than 90 days, ensuring a calving interval (CI) of 12 months, or one calf per cow per year (Ferreira et al., 2018).

Du et al. (2010) observed that animal performance is heavily influenced by annual variations in protein availability. In tropical regions, the crude protein limitations of dry-season pastures compromise rumen energy-protein balance, reducing fiber digestion and overall energy intake (Sampaio et al., 2009). Moreover, Renquist et al. (2006) noted that body condition score (BCS) variations at calving—from 3.5 to 4.5 (on a 1-to-9 scale)—were associated with shorter CI. Cows with moderate BCS (4.5–5.5) at weaning also tended to produce heavier calves compared to those with either low or high BCS. Similarly, BCS during the second trimester of gestation correlated with higher birth weights.

Another crucial factor is the annual fluctuation in pasture protein levels, which directly impacts reproductive performance. Higher protein levels during the rainy season improve the nutritional value of pastures and positively influence mating outcomes. According to Gionbelli; Valadares Filho; Duarte (2016), this condition aligns the

concentration of calvings with the end of the dry season and the onset of the rainy season, synchronizing lactation with periods of greater forage availability. Aligning lactation with nutritional requirements during abundant periods can improve BCS and enhance pregnancy rates, potentially reducing reliance on artificial insemination protocols and limiting exposure time to bulls (Marquez et al., 2017).

2.3 Fetal Programming and Gestational Nutrition

The concept of fetal programming involves the response triggered in offspring due to the dam's exposure to specific conditions during gestation (Zhu et al., 2004). Initial studies on this topic emerged from epidemiological observations in humans that linked low birth weight to mothers with inadequate diets during pregnancy, resulting in a higher incidence of various severe diseases in these affected individuals (Dj, 2002). Research on fetal programming in animals is relatively recent, demonstrating the detrimental effects of protein restriction on offspring performance in several species (Ford et al., 2007; Zambrano et al., 2006).

During the formation of fetal tissues, there is a hierarchy in the allocation of energy from maternal nutrients. Skeletal muscle tissue has a lower priority compared to other tissues, such as cerebral, cardiac, or hepatic tissues. This makes skeletal muscle tissue particularly vulnerable to any changes in nutrient supply from the mother, resulting in impaired development and increased susceptibility to malnutrition (Zhu et al., 2006). In the early stages of gestation, however, maternal nutrition has limited influence on the formation of skeletal muscle, as only a limited number of muscle fibers are generated (Rissell and Oteruelo, 1981; Underwood et al., 2010). Most muscle fibers develop between the 2nd and 7th months of gestation. Therefore, if this potential for muscle fiber formation is not utilized during this phase, it will result in lasting effects and irreversible physiological consequences for the offspring throughout their life (Du et al., 2010).

The intermediate and late stages of gestation are critical periods, given that dietary restrictions during these phases may induce irreversible impairments in muscle cell generation. Research indicates that, besides a decline in muscle fiber hyperplasia, detrimental outcomes extend to muscle hypertrophy as well (Russel and Oteruelo, 1981; Zambrano et al., 2006), impacting calf performance and meat quality (Du et al., 2010; Zambrano et al., 2005).

This decline in muscle fiber hyperplasia results in its replacement by collagen (Costa et al., 2021), leading to diminished quality and increased toughness of meat (Dos

Santos Fontes et al., 2021). Collagen serves as the primary constituent of connective tissue and acts as the foundational unit of the extracellular matrix (Du et al., 2015).

Connective tissue assumes a pivotal role as a supportive framework, contributing to the resilience and integrity of muscle tissue (Santos et al., 2022). Consequently, a reduction in muscle fiber hyperplasia can instigate heightened deposition of connective tissue, aiming to uphold the structural robustness and functionality of muscle tissue.

Another important aspect to consider is adipogenesis, which initiates during the mid-third of gestation and becomes more pronounced in intramuscular adipose tissue from late gestation to 250 days of age (Du; Ford and Zhu, 2017). Consequently, restrictions during this period can impede the hyperplasia of these adipocytes, reducing marbling (Du et al., 2015). To enhance meat quality and juiciness, a greater quantity of intramuscular fat is necessary, influenced by both the number and size of intramuscular adipocytes, originating from the same pool of mesenchymal stem cells as skeletal muscle cells (Du et al., 2010).

During the final stage of gestation, pregnant cows experience significantly heightened nutritional demands, essential for meeting the cow's requirements and facilitating continual fetal development (Ferrell; Garrett and Hinman, 1976). Maternal nutrient constraints during late gestation, while not impacting fiber numbers, do affect their size (Du et al., 2010). However, limitations during this gestational phase can decrease satellite cell density, impeding postnatal muscle growth and regeneration (Woo et al., 2011).

Nonetheless, nutrition in late gestation enhances the physical condition of cows during the early postpartum period, a vital factor in optimizing reproductive performance in subsequent breeding seasons. Improved nutrient intake during the third trimester of gestation enhances calf production per cow within the herd, thereby increasing the economic returns from the operation (Klein et al., 2021).

Literature findings suggest that fetal programming via maternal nutrition exerts indirect influences on meat quality. For instance, cows provided with enhanced pasture (9.4% protein) during the mid-third of gestation yielded progeny with increased weights, higher feedlot weight, and enhanced meat attributes compared to cows grazed on native pasture (Underwood et al., 2010).

Costa et al. (2021) demonstrated that calves born to cows experiencing protein restriction during the latter half of gestation exhibited reduced hyperplasia at 30 days of age, persisting until 450 days, in contrast to calves born to cows receiving protein

supplementation during the same period. Moreover, offspring from restricted cows displayed higher collagen content at 30 days compared to those from supplemented cows. However, by 450 days, no disparity was observed between the treatments.

Nascimento (2021), in a study assessing calf performance based on maternal protein supplementation during the mid-third of gestation, reported that calves from supplemented mothers exhibited greater birth and weaning weights, with increases of 3.8 kg and 16.5 kg, respectively, compared to calves from mothers subjected to protein restriction during the same gestational phase. Moreover, throughout all weighings during each experimental phase, calves from supplemented mothers consistently maintained higher weights, averaging approximately 7.8 kg more. By the conclusion of the study, at 445 days of age, calves from supplemented mothers outweighed their counterparts by 30.8 kg.

In evaluating dry matter intake and efficiency of calves from cows subjected to protein restriction or supplementation during the mid-third of gestation, Nascimento et al. (2022) utilized forty-three Tabapuã cows, with treatments consisting of restricted diets containing 5.5% total crude protein or control diets with 10% total CP. The authors observed a trend towards higher dry matter and pasture intake for supplemented cows. Additionally, feed efficiency was notably improved in male calves, indicating a potential influence of sex on fetal programming.

Considering the detrimental effects of malnutrition in pregnant cows, it underscores the importance of employing nutritional strategies to optimize breeding system outcomes, offering substantial positive impacts and significant enhancements in results.

2.4 Intensification Strategies in Beef Cattle Production

Currently, various intensification strategies are implemented in beef cattle production, including nitrogen fertilization, deferred grazing, TMR (Total Mixed Ration), protein supplementation, and protein-energy supplementation. These strategies offer distinct advantages to cow-calf operations, each with its specific characteristics. When combined, they have the potential to generate significant positive effects on the system, particularly concerning the nutrition of pregnant cows. The primary goal is to reduce fluctuations in weight and BCS (Body Condition Score) while enhancing the performance of their progeny.

2.4.1 Nitrogen Fertilization

Nitrogen is the primary nutrient for plant development, promoting growth and influencing plant height, tiller number, and consequently, forage production (Irving, 2015). Therefore, its use is highly relevant when aiming to increase the forage production potential of tropical pastures. Nitrogen has a direct effect on biomass production, increasing area productivity when applied, thus allowing for higher stocking rates (Gomide, Paciullo, and Martins, 2020).

After correcting soil acidity and increasing phosphorus and potassium levels, nitrogen becomes the essential nutrient for promoting plant growth. Its application in tropical grasses results in significant increases in biomass production and carrying capacity (Lugão et al., 2003).

De Oliveira and Rebello (2020) observed an increase in stocking rate in response to nitrogen fertilization in *Megathyrsus maximus* cv. Mombaça pastures under grazing by Nelore bulls (9 months; 173 kg). They assessed the effects of increasing nitrogen levels (150, 300, and 450 kg ha⁻¹ per year) and found that the highest level of nitrogen fertilization resulted in increases in dry matter forage production, leaf mass, ground cover height, and forage accumulation rate (8068.2; 7204.7; 6767.5 kg ha⁻¹), leaf mass (5754.2; 5144.7; 4302.1 kg ha⁻¹), ground cover height (82.0; 79.3; 70.9 cm), and forage accumulation rate (254.7; 211.7; 188.4 kg ha⁻¹ day⁻¹).

Chemical composition analysis of the leaves showed an increase in crude protein content (204.1; 192.5; 128.9 g kg⁻¹) and a reduction in neutral detergent fiber (NDF) (675.1; 686.2; 709.8 g kg⁻¹), acid detergent fiber (ADF), and lignin. These findings led to a higher stocking rate and total weight gain per hectare over 112 days (906.61; 799.24; and 587.21 kg/ha), highlighting the potential of nitrogen fertilization for intensifying production systems.

Furthermore, the study indicated that the amount of supplement offered linearly increased forage digestibility. This improvement in nutrient digestion was attributed to the increased supply of nitrogen and energy, meeting the requirements of ruminal microorganisms and consequently enhancing the activity of cellulolytic bacteria in the rumen.

One of the key effects of nitrogen on grasses is related to tillering. This positive effect is associated with increased leaf appearance rate (Cruz and Boval, 2000), with linear increments in tiller density (Faria et al., 2018; Silva et al., 2009). Nitrogen fertilization increased tillering in pasture, resulting in a higher proportion of leaves

compared to other morphological components. In addition to increasing forage production, nitrogen fertilization offers several benefits to plants by ensuring that, when applied to pastures, the number of live leaves per tiller reaches its peak early, ideally applied when forage is at its maximum accumulation rate (Boin, 1986).

In more intensive animal production systems based on pasture, higher stocking rates are assumed, leading to increased defoliation of the pasture. This condition results in greater nitrogen demand by the plant (Martha Júnior et al., 2004). Therefore, in management strategies aiming for higher pasture utilization efficiency, characterized by lower post-grazing heights and shorter defoliation intervals, there is a higher demand for nutrients, which is met by fertilization.

In summary, nitrogen fertilization is essential in intensification strategies, aimed at optimizing the use of pasture as the primary feeding source in extensive systems, achieving greater forage production, reducing pasture degradation, and ensuring adequate forage supply for the soil-plant-animal system.

2.4.2 Deferred Grazing

Pasture deferment can be employed independently or in conjunction with other intensification strategies, such as nitrogen fertilization and protein supplementation (Beleossoff, 2009).

Santos et al. (2010) evaluated the relationship between tiller number, forage mass, and morphological components in *Urochloa decumbens* cv. Basilisk pastures during the pasture deferment period. The study found a linear reduction in the number of vegetative tillers as pasture deferment continued. Most of these vegetative tillers transformed into reproductive tillers, which eventually became dead tillers, following the natural phenological cycle of the grass. Smaller vegetative tillers likely died due to shading and competition for light with the larger tillers during the deferment period.

Additionally, the mass of green stems increased linearly with longer deferment periods (Santos et al., 2010). During pasture deferment, the grass canopy began to intercept approximately 95% of the incident light, reaching the critical leaf area index (LAI). The critical LAI represents the point at which competition for light among tillers increases, leading to excessive accumulation of stems and dead material within the canopy. Managing pasture conditions that exceed the critical LAI may negatively impact the productive system.

In a study conducted by Gimenes et al. (2011), an increase in stocking rate (3.13 and 2.85 AU ha⁻¹) and average daily gain (ADG) (0.629 and 0.511 kg day⁻¹) was

observed in Nelore cattle grazing *Brachiaria brizantha* cv. Marandu grass at a maintained height of 25 cm (which results in 95% light interception), compared to pasture heights above the critical LAI of 35 cm. Beyond this critical LAI, light competition becomes significant, leading to stem elongation and exposing leaves only at the top of the canopy (Carnevalli et al., 2006).

This suggests that while pasture deferment can be a beneficial strategy for improving forage production, careful management is essential. When the deferment period exceeds the critical LAI, there is an increased risk of competition among tillers, leading to a reduction in leaf-to-stem ratio, which can impair the overall productivity of the pasture system. Therefore, the optimal deferment period must be carefully balanced to prevent excessive stem growth and degradation of pasture quality.

2.4.3 Supplementation

Another strategy to be employed in intensifying cattle production systems is animal supplementation, which can involve protein, energy, or a combination of both. In many production systems, the crude protein content of pastures becomes a limiting factor for animal performance, particularly during the dry season, when pastures experience reduced leaf content and increased lignification of stems (Detmann et al., 2014). Under these conditions, supplementation should aim to ensure a minimum ammonia nitrogen concentration of 8 mg/dL, which can be achieved by diets with approximately 100 g/kg of crude protein (CP) on a dry matter (DM) basis (Detmann et al., 2014). The availability of nitrogen for ruminal microorganisms in such scenarios stimulates enhanced microbial growth and enzymatic activity, influencing the digestion of forage carbohydrates and subsequently increasing pasture dry matter intake (Sampaio et al., 2010).

Research in Brazil has demonstrated that supplementing cattle results in higher weaning weights for both male and female animals, reduced time spent in the rearing phase, and increased performance during the rearing and finishing stages, irrespective of the supplementation period (Carvalho et al., 2019; de Almeida et al., 2022). These findings suggest that strategic supplementation can improve production efficiency and performance, regardless of seasonal variations.

In the first third of gestation, Gionbelli et al. (2015) noted that the protein requirement for a pregnant Nelore cow is 1,036 g/day. This period coincides with the dry season, during which pastures composed of tropical grasses typically contain between 6 to 10% CP. Even when cows consume pasture at 1.8% of their body weight, they experience a significant CP deficit. Low CP intake impairs bacterial growth in the rumen,

consequently reducing the digestion of neutral detergent fiber (NDF) and organic matter in the pasture. As a result, these cows face not only a protein nutritional restriction but also an energy deficiency.

Adjusting the diet during gestation is critical to ensure adequate nutrition for the pregnant cow. However, short-term adjustments are often impractical. Gionbelli, Valadares Filho, and Duarte (2016) proposed a stepwise model for adjusting the diet, with specific recommendations for each gestational phase (early, middle, and late). This model standardizes the energy and protein requirements to be met throughout pregnancy.

In situations of nutritional restriction, the carbon-to-nitrogen ratio in ruminal substrates becomes high, which negatively impacts nitrogen compound availability for the synthesis of microbial enzymes responsible for forage fiber degradation (Detmann et al., 2009). Therefore, supplementing protein during the dry season is vital to improve or maintain productive system indices. Protein deficiency in pastures during this period coincides with high nutritional demands for pregnant cows, creating a vulnerable situation for the production system (Detmann et al., 2009).

To address these challenges, it is essential to develop supplementation strategies and consider system intensification, aimed at optimizing food resource use to achieve better outcomes. Protein supplementation plays a critical role in optimizing pasture utilization and maintaining the appropriate body condition of pregnant cows.

Another strategy for overcoming seasonal feed scarcity is the use of silage, which involves preserving the nutritional value of feed by utilizing surplus production and providing feed to ruminant animals during periods of food scarcity (Mezzomo, Rêgo, and Vargas, 2023). In recent years, silage production has evolved beyond being merely an alternative to feeding cattle during dry or less rainy seasons. It has become a key component of high-productivity systems, significantly enhancing agricultural system efficiency for properties seeking to intensify production (Mezzomo, Rêgo, and Vargas, 2023). Silage production reduces agricultural dependence on climatic conditions, enabling producers to meet animal nutritional requirements during forage shortages or when seeking to intensify production systems.

The production of grass silage, whether made from deferred areas or as a supplement during the dry season, represents an excellent alternative for sustaining cattle production. However, precautions must be taken due to the low dry matter content and soluble carbohydrate levels in plants (Gusmão, 2017).

Additionally, the production of Total Mixed Ration (TMR) provides an alternative strategy for more technified systems, ensuring a more complete diet for animals. This approach allows for the utilization of surplus pasture production and ensures a continuous feed supply during the dry season without compromising nutritional quality. TMR typically involves incorporating ingredients into silage to increase dry matter content and improve nutritional value. For instance, Dried Distillers Grains (DDG), a byproduct from corn grain ethanol production, is high in protein and energy. DDG contains 30% protein, 10% fat, 33.7% NDF, 89% total digestible nutrients (TDN), and a net energy value of 1.5 g/Mcal (NASEM, 2016).

However, the environmental impact of TMR use should be considered. A study by Hünenberg et al. (2014) showed a 7.8% increase in nitrous oxide (N₂O) emissions from beef produced with TMR, compared to beef from TMR made with barley grains as the main energy supplement. This study reported a corresponding increase in Greenhouse Gas (GHG) emissions, from 14.1 to 15.2 kg of CO₂ equivalent per kilogram of live weight.

General recommendations suggest providing only essential nutrients, such as crude protein, to beef cattle, avoiding excesses. It is also advisable to apply manure from DDG-fed cattle to the soil in amounts that meet the crop's nitrogen needs, contributing to sustainability. Despite the promising potential of TMR, it is important to consider its limitations, particularly with regard to sustainable agricultural practices, to avoid adverse environmental impacts.

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SECOND SECTION – ARTICLE

ARTICLE 1 - Impact of Production System Intensification on Maternal Tissue Dynamics, Uterine Hemodynamics, and Skeletal Muscle Development in Beef Cows and Their Progeny.

Article formatted according to Animal guidelines

Abstract

This study evaluated the productive parameters of beef cows and their offspring based on the production cycle stage and the levels of intensification applied. Seventy-two pregnant purebred Tabapuã cows (*Bos taurus indicus*) with an average weight of 500 ± 72 kg, age of 4.5 ± 2.5 years, and body condition score of 5.5 ± 0.69 (scale of 1 to 9) were used in a randomized block design. Cows were distributed across three pasture blocks: *Urochloa decumbens*, *Urochloa brizantha* cv. *Marandu*, and *Urochloa hybrid* cv. *Sabiá*, with two paddock repetitions per treatment within each block. Treatments included: Control (CON) - non-fertilized pasture with mineral supplementation; Intensive (INT) - pasture fertilized with 165 kg of nitrogen (N) during the rainy season, deferred 50% of the area, and provided protein-energy supplementation during the dry season; and Superintensive (SUP) - fertilized pasture with 165 kg of N per hectare in the rainy season and total mixed ration during the dry season from silage of 30% deferred pasture and protein concentrate. A significant interaction ($P = 0.049$) between maternal nutrition and fetal sex was observed for longissimus muscle area (LMA), with a trend for ribeye area relative to body weight ($P = 0.102$). Cows in intensive systems carrying male fetuses exhibited larger LMA than those carrying females. However, no significant interactions were noted for subcutaneous fat thickness or rump fat thickness. Among treatments, CON cows showed greater rump fat thickness ($P = 0.033$). There were no differences in pregnancy rates or hemodynamic parameters. Offspring vigor at birth showed no significant interaction but tended to decrease in calves from cows in super-intensive systems. Male calves presented higher average daily gain and weaning weights, with higher LMA, whereas females had greater rump fat thickness. In conclusion, while production system intensification for breeding herds did not significantly enhance offspring performance under discrete nutritional differences, the study highlights the sex-based differences in beef cattle performance characteristics.

1.0 INTRODUCTION

The efficiency of beef cattle production systems is shaped by a combination of genetic factors, management practices, and the prenatal environment to which animals are exposed. Among these, the gestational phase has emerged as a pivotal period influencing fetal development and cellular growth. Maternal nutrition during gestation plays a fundamental role in shaping offspring performance and lifetime productivity.

Evidence indicates that maternal diet significantly affects fetal development, with skeletal muscle formation being particularly responsive to nutritional inputs (Meneses et al., 2022). For instance, protein supplementation during gestation has been shown to enhance the nutritional status of pregnant cows, as demonstrated by increased blood glucose, insulin, and IGF-1 concentrations. Furthermore, this supplementation supports hepatic gluconeogenesis from amino acids, reducing maternal tissue mobilization (Meneses et al., 2022). These processes underscore the concept of fetal programming or developmental programming, whereby the intrauterine environment induces changes in the fetus that impact its subsequent performance and the quality of animal products.

In tropical and subtropical regions, where nutritional challenges are common during late gestation, mid-gestation protein supplementation has been shown to increase gestational component weight (PREG) and result in heavier calves at birth (Nascimento et al., 2022; Nascimento et al., 2024). Understanding the mechanisms governing these outcomes is critical, as fetal skeletal muscle development is a multifaceted process influenced by both genetic and environmental factors, including maternal diet (Du et al., 2010). The progression of gestation involves dynamic changes in placental components and uterine blood flow, both of which are essential for fetal growth and health (Wathes et al., 2020).

Studies have highlighted that nutrient availability and maternal resource allocation significantly influence fetal muscle development (Zhu et al., 2006). For example, nutrient-restricted dams experience reduced body weight, chest girth, and backfat thickness, along with elevated NEFA concentrations and decreased circulating glucose, urea N, and triglycerides during late gestation (Refider et al., 2023). These findings emphasize the need for strategies that ensure consistent maternal nutrition to support fetal development while minimizing excessive maternal tissue mobilization.

As global demand for beef rises, sustainable production strategies must balance the need for intensified productivity with ecological and economic constraints. Although

gestational nutrition strategies show promise for improving productivity indices in beef cattle (Larson et al., 2012), their full potential under varying pasture conditions remains uncertain. Advanced management practices offer an opportunity for sustainable intensification without expanding land use (OECD, 2022).

Thus, further investigation is required to unravel the interplay between maternal nutrition, gestational components, and fetal muscle development across the different stages of pregnancy. Identifying these relationships could provide essential insights for refining nutritional strategies, minimizing maternal tissue loss, and supporting sustainable production. The findings of this study are expected to contribute to the scientific basis for developing more effective management practices and advancing the efficiency of beef cattle production systems.

2. MATERIAL AND METHODS

This study was performed in the Beef Cattle Facilities of the Department of Animal Science at the Federal University of Lavras (UFLA – Lavras, Minas Gerais, Brazil – Latitude: 21°13'54.0''S Longitude: 44°58'02.5''W). The experiment lasted 24 months. During this period, the cows received nutritional supplementation only during the second and third thirds of gestation, remaining in the treatment throughout the entire year. Additionally, their calves were monitored throughout the entire productive year.

This study was approved by the Ethics Committee on Animal Use of UFLA (CEUA/UFLA – protocol 009/21), considering the ethical principles of animal experimentation established by the Brazilian National Council for the Control of Animal Experimentation (CONCEA).

2.1 Animals, treatments, feeding, and experimental design

Seventy-two pregnant Tabapuã breed cows (*Bos taurus indicus*), purebred, with an average weight of 500 ± 72 kg, 4.5 ± 2.5 years of age, and a body condition score of 5.5 ± 0.69 (on a scale of 1 to 9), were used in a randomized block design experiment.

The cows were initially subjected to a fixed-time artificial insemination (FTAI) protocol. Pregnancy diagnosis was conducted using rectal ultrasonography with an ultrasound device (model SSD-3500; Aloka ProSound Alpha 6) in B-mode and Doppler (Color and Spectral), equipped with a 7.0 MHz linear transrectal probe (Aloka UST-672; Aloka, Wallingford, CT, USA) at 30 days of gestation. Fetal sexing was performed 60 days after conception.

After pregnancy confirmation, the cows were distributed into experimental units (paddocks) based on weight, age, number of calves, genetic merit, and calf sex, and remained in their respective units throughout the experiment. At the end of the first year, the cows underwent the reproductive protocol again, and those that did not conceive were replaced by other pregnant cows and redistributed into their respective experimental units.

The experimental units were arranged in blocks, with two replications of each treatment within each block to account for environmental effects, as each block was characterized by different pastures (Block 1: *Brachiaria Decumbens*, Block 2: *Brachiaria Brizantha* cv. Marandu, Block 3: *Brachiaria Hybrid* cv. Sabiá).

The animals were allocated to three treatments: control (1.68 ha), intensive (0.95 ha), and superintensive (0.68 ha). Each treatment consisted of two cows per experimental unit, which were monitored throughout the production year along with their calves after birth. Replacement animals were introduced as needed to optimize the consumption of surplus pasture, maintaining similar pasture heights across all treatments. This adjustment was made every 14 days to keep the stocking rate consistent in each experimental unit, depending on the pasture used. The animals had ad libitum access to water via one water trough for every two paddocks, separated by the paddock's fence.

In the control treatment, cows were kept on pasture without nitrogen fertilization, receiving only mineral supplementation ad libitum throughout the production cycle. The mineral supplement, Bellnutri 90 Dry from Trouw Nutrition, was provided once a week to all animals at 7 a.m., using a commercial product [Assurance levels: 122.10g Ca (max), 96.40g Ca (min), 81.80g P (min), 178.6g Na⁺ (min), 11.1g Mg²⁺ (min), 29.6g S²⁻ (min), 1111.1mg Co²⁺ (min), 185.6mg Cu²⁺ (min), 275.6mg I⁻ (min), 71.7mg Mn²⁺ (min), 355.6mg Se²⁺ (min), and 229.6mg Zn²⁺ (min)]. These supplements were offered in strategically positioned feeders in each paddock across all treatments, respecting the recommended 5 cm linear spacing. The objective of this treatment was to simulate typical beef cattle farms in Brazil, characterized by extensive systems, low animal stocking rates per hectare, low investment, and poor feeding management, resulting in limited animal performance. Additionally, pasture height management was conducted every 14 days for adjustments in paddock stocking rate when necessary.

In the intensive treatment, paddocks received nitrogen fertilization with 165 kg of N per year, divided into 4 applications during the rainy season. For deferral, fifty percent of the total paddock area was reserved for forage storage during the dry season, being deferred at the end of the rainy season (late February to early March) and opened at the

peak of the dry season (July). During this system, a commercial protein-energy supplement was provided during the dry period, from 200 days of gestation until calving, along with mineral supplementation throughout the year. The protein-energy supplement used was Lambisk S Cria (Table 1), ready-to-use, intended for beef cattle, and was exclusively provided to pregnant cows in the intensive treatment, with ad libitum consumption from 200 days of gestation until calving. For the rest of the production cycle, they received the mineral supplementation Bellnutri 90 Dry from Trouw Nutrition. Additionally, pasture height management was conducted every 14 days for adjustments in paddock stocking rate when necessary. This treatment aimed to represent common intensification options and the technological level employed in the system.

In the superintensive treatment, paddocks designated for this treatment also received 165 kg of N per year, divided into 4 applications during the rainy season. Thirty percent of the paddock area was deferred during the wet season for grass silage production. Areas designated for silage production underwent a deferral period during the rainy season to accumulate forage in an area not accessible and not interfering with animal grazing. The criteria for initiating the silage-making process were determined according to the time of year, with all stages occurring at the end of the rainy season, approximately 45 days before the end of precipitation.

To achieve the desired crude protein concentration, mixing calculations were performed considering the nutritional composition of the ingredients (Table 1). Near the end of the rainy season, grass harvesting was carried out using a forage harvester (model JF 1300 AT) attached to a tractor. The cutting was done at the lowest viable height, aiming for maximum utilization of the available forage mass. Subsequently, the resulting mass was quantified using a forage wagon, and 8.1% of DDG (Dried Distillers Grains) was incorporated to ensure the homogeneity of the mixture.

Once homogenized, the silage in the wagon was ensiled in six "bag" type silos (one for each experimental unit) lined with a tarp, using a forage compactor (JF Silo Master Forage). These silos remained sealed for a minimum period of 45 days, favoring the occurrence of the fermentative process that preserves the nutritional qualities of the silage. This resulted in a TMR (Total Mixed Ration) silage with improvements in digestibility and nutrient preservation (Ramos et al., 2021).

After opening the silos, supplementation was exclusively provided to the cows in the superintensive treatment group, starting from 200 days of gestation until calving. Daily offerings were placed in the feeders positioned in the paddocks designated for this

treatment, respecting the recommended spacing of 40 cm. The control was conducted by weighing both the provided food and leftovers, allowing for the monitoring of the average daily consumption for each experimental unit.

Deferral was performed in October, allowing for silage-making in March. Afterward, it was deferred again to be used in July, representing, in our tropical conditions, the peak of the dry season. Subsequently, the paddock was deferred again during the wet season and opened according to the need for food for the cows. In this treatment, pasture height management was also conducted every 14 days for possible adjustments in the paddock stocking rate. e fluctuations in crude protein and neutral detergent fiber in the pastures are shown in graphs 1 and 2, respectively.

2.2 Maternal weight gain, and body score condition

The animals were weighed every 30 days throughout the experimental period. The body weight collected for a pregnant cow was defined as the pregnant body weight (BWp). The BWp was used to estimate the empty body weight of the pregnant cow (EBWp) using the S1 spreadsheet and the models described by Gionbelli et al. (2015). The EBWp value was then separated into non-pregnant EBW (EBWnp) and the weight relative to pregnant compounds (PREG) using the spreadsheet and models also described by Gionbelli et al. (2015). To establish a better relationship between maternal body tissue, gain, and gestating tissue, the authors (Gionbelli et al., 2015) determined the pregnancy component (PREG), mathematically estimated as an extra component of the cow ($EBWp = EBWnp + PREG$). The PREG component takes into account the increase in uterine weight caused by pregnancy (GUdp: gravid uterus increases during pregnancy) plus the increase in udder weight due to pregnancy. GUdp represents the weight of the pregnant uterus minus the weight of the cow's uterus in a non-pregnant state.

Body condition score assessments were carried out at 30-day intervals throughout the experimental period. The scores were represented on a scale ranging from 1 to 9, where a score of 1 indicated a state of severe emaciation, while a score of 9 indicated pronounced obesity (Nicholson and Butterworth, 1986; Richards et al., 1986). The assessment was carried out by three trained assessors who made observations and palpated the animals. The resulting final score was calculated as the average of the anatomical points assessed by the evaluators.

2.3 Maternal nutrient accretion and mobilization

Ultrasound measurements of the maternal carcass were used to estimate the dynamics of the maternal muscle and fat tissues resulting from the application of the

treatments. Two measurements of each tissue (muscle + fat) were recorded. Ultrasonic measurements of the Longissimus muscle area (LMA) between the 12th and 13th ribs, as well as the depth of the rump muscle, were used as indicators of muscle measurements. The thickness of the subcutaneous fat between the 12th and 13th ribs, as well as the thickness of the rump fat, were used as adipose tissue measurements. All measurements were also taken throughout the experimental period at intervals of every 30 days. The measurements were taken by placing the ultrasound transducer directly on the skin using mineral oil as a coupling agent, oriented perpendicular to the spine. Insonification was carried out using an Aloka 500-V device (Corometrics Medical Systems, Wallingford, CT), equipped with a 3.5 MHz, 17.2 cm linear array transducer. The images were analyzed using ImageJ (version [1.8.0], National Institutes of Health, Bethesda, MD, EUA).

2.4 Uterine hemodynamics

To assess the hemodynamic behavior of the uterine artery, Doppler measurements were taken in the prepartum period (270 days of gestation) using an ultrasound device with B and Doppler modes (Color and Spectral) and a 7.0 MHz transrectal linear probe (model UST-5813-5, Corometrics Medical Systems, Wallingford, CT). After inserting the probe through the rectum and activating digital mode B, the pregnant uterine body and uterine artery were located according to Bollwein et al. (2002). The uterine artery ipsilateral to the corpus luteum was examined using a method with high intra-observer reproducibility. The indices of resistance (RI), pulsatility (PI), systolic/diastolic ratio (S/D), and blood velocity were visualized after activating the spectral Doppler function in the uterine artery. The RI was obtained as follows: $RI = (\text{peak systolic velocity} - \text{end-diastolic velocity}) / \text{peak systolic velocity}$ (Camacho et al., 2014). The PI, in turn, was obtained as $PI = (\text{peak systolic velocity} - \text{end-diastolic velocity}) / \text{average velocity}$ (Camacho et al., 2014). Both hemodynamic index calculations were obtained automatically by a pre-programmed Doppler program. The uterus was not palpated before the evaluations so as not to influence blood flow through transrectal manipulation. The Doppler indices were analyzed using the equipment's analysis software.

2.5 Evaluation Offspring

Calves were weighed from birth to weaning at 30-day intervals using a calibrated scale. The average daily gain (ADG) for each calf was calculated using the formula: $ADG = (\text{Final weight} - \text{Initial weight}) / \text{Number of days}$. At weaning, which took place at 210 days of age, carcass ultrasonography was conducted to evaluate the Longissimus muscle

area (LMA, cm²), subcutaneous fat thickness (SFT, mm), rump muscle length (RML, cm), and rump fat thickness (cm). The ultrasonographic images were obtained from the right side of the calves using an Aloka 500-V system (Corometrics Medical Systems, Wallingford, CT), equipped with a 17.2 cm, 3.5 MHz linear array transducer. LMA and SFT measurements were taken between the 12th and 13th ribs, covering three-quarters of the ventral length of the Longissimus muscle. RML and rump fat thickness were measured at the junction of the biceps femoris and gluteus medius, between the ischium and ilium, parallel to the spinal column. Image analysis was performed using ImageJ software (version 1.8.0, National Institutes of Health, Bethesda, MD, USA).

Newborn calf vigor was assessed by a single observer, with scores assigned based on the following criteria:

1 - Calf that did not survive the neonatal period due to extreme weakness, showing signs such as difficulty breathing, inability to stand or nurse, severe dehydration, and lack of responsiveness.

2 - Calf that was weak and lethargic at birth, struggling to stand and ingest colostrum promptly. This calf required frequent human assistance for nursing, showing clear signs of needing intervention, such as reduced sucking reflexes, low muscle tone, and delayed responsiveness to stimuli.

3 - Calf that appeared normal, showing no signs of weakness and requiring no special care. This calf demonstrated typical reflexes at birth, standing, and nursing without difficulty, with good muscle tone, healthy coloration, and regular breathing patterns.

4 - Strong and alert calf that stood within minutes of birth and exhibited high vitality throughout the neonatal period. This calf showed quick sucking reflexes, active movement, and no need for human intervention, indicating excellent health and robust development.

2.6 Statistical Analyses

Carcass data. The carcass data were analyzed according to the following linear mixed model:

$$\begin{aligned}
 y_{ijklmnop} = & \mu + MN_i + S_j + MN_i * S_j + PG_k + T_l + \sum_{m=1}^2 \beta_m GD_p^m \\
 & + \sum_{i=1}^3 \sum_{m=1}^2 \beta_{im} GD_p^m * MN_i + \sum_{m=1}^2 \beta_m adjMG_p^m + \beta_{iBW} iBW_p \\
 & + \beta_{iBCS} iBCS_p + B_n T_l + P_o(T_l) + C_P(T_l) + C_P + e_{ijklmnop}
 \end{aligned}$$

[Eq. 1]

where $y_{ijklmnop}$ is the observed value; μ is the intercept; MN_i is the fixed effect of the i^{th} level of Maternal Nutrition, with $j = 1$ to 3; S_j is the fixed effect of the j^{th} level of Sex of the progeny, with $j = 1$ or 2; $MN_i * S_j$ is the fixed effect of the interaction between MN and S ; PG_k is the fixed effect of the k^{th} level of Pregnancy Group, with $k = 1$ to 3; T_l is the fixed effect of l^{th} level Year, with $l = 1$ or 2; $\beta_m GD_p^m$ is the fixed effect of the m^{th} partial regression coefficient (β) for the fixed effect of Gestation Day (GD) at collection, where GD_p^m is the m^{th} -order polynomial of GD for the p^{th} cow, with $m = 1$ to 2 and $p = 1$ to 51; $\beta_{im} GD_p^m * MN_i$ is the fixed effect of the interaction between MN and GD ; $\beta_{jm} GD_p^m * S_j$ is the fixed effect of the interaction between S and GD ; $\beta_{ijm} GD_p^m * MN_i * S_j$ is the fixed effect of the interaction between MN , S , and GD ; $\beta_m GM_p^m$ is the m^{th} β for the fixed effect of the adjusted Maternal Tissue Gain ($adjMG$) at collection (see below), where $adjMG_p^m$ is the m^{th} -order polynomial of $adjMG$ for the p^{th} cow; $\beta_{iBW} iBW_p$ is the fixed effect of the partial regression coefficient of initial Body Weight (iBW) of the cow at the beginning of the trial, and iBW_p is the iBW of the p^{th} cow; $\beta_{iBCS} iBCS_p$ is the fixed effect of the partial regression coefficient of initial Body Condition Score ($iBCS$) of the cow at the beginning of the trial, and $iBCS_p$ is the $iBCS$ of the p^{th} cow; $B_n(T_l)$ is the fixed effect of the n^{th} level of Block within T , with $n = 1$ to 3; $P_o(T_l)$ is the random effect of the o^{th} level of paddock within T , with $o = 1$ to 18, assuming $P(T) \sim N(0, \mathbf{I}\sigma_P^2(T))$, where \mathbf{I} represents the identity matrix and $\sigma_P^2(T)$ the paddock within Year variance; $C_p(T_l)$ is the random effect of Cow within T , assuming $C(T) \sim N(0, \mathbf{CSH}\sigma_C^2(P))$, where \mathbf{CSH} represents the heterogeneous compound symmetry covariance structure and $\sigma_C^2(T)$ represents the Cow within Year variance; C_p is the random effect of the Cow, assuming $C \sim N(0, \mathbf{I}\sigma_C^2)$, where σ_C^2 represents the Cow variance; and $e_{ijklmnop}$ is the random error associated with $y_{ijklmnop}$, assuming $e \sim N(0, \mathbf{I}\sigma_e^2)$, where σ_e^2 represents the residual variance. Prior to the analysis, the observed Maternal Tissue Gain (MG) pre-adjusted for fixed effects. The model used in this step was the same described above, but without $\sum_{m=1}^2 \beta_m adjMG_p^m$.

Doppler Data. Before the analysis, the Doppler data was averaged within each individual for each trait. This was done within each year for cows with data for two years. After taking the average values, the data were analyzed according to the following mixed model:

$$y_{ijklmnop} = \mu + MN + S_j + MN_i * S_j + PG_k + T_l + \beta_{iBW}iBW_p + \beta_{iBCS}iBCS_p + B_nT_l + P_o(T_l) + e_{ijklmnop}$$

[Eq. 2]

where all terms are as defined in Eq. [1].

Calf data. The data measured in the calves were analyzed with a modified version of the model used for the Doppler data (Eq. [2]). The model was the same as in Eq. [2] but with the addition of two fixed effect covariates, one for the effect of genetic potential for growth of the calf and the other for the calf's birth weight.

Assessment of Normality

Before final analyses, residuals were evaluated for outliers and normality. Observations with absolute Studentized residuals greater than 3 were removed one at a time while normality was assessed in parallel based on Shapiro-Wilk's test at $P > 0.01$. In the cases of PREG for the Carcass data, and Pulsatility Index (PI) and Volume Flow (VF) for the Doppler data, these were log-transformed before analyses and then back-transformed for the presentation of results. In the presence of significant effects ($P < 0.05$), expected means were generated for each level of the fixed effects and compared amongst each other using Fisher's LSD test ($P < 0.05$). All the data were analyzed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS

3.1 Skeletal muscle and adiposity, and pregnant compounds in beef cows

A significant interaction between maternal nutrition and sex (MN \times S: $P = 0.049$) was observed for ribeye area (expressed in cm²), with a trend towards interaction for ribeye area relative to body weight (expressed in cm²/100 kg; MN \times S: $P = 0.102$). Cows managed in an intensive system carrying male fetuses had a larger LMA than those carrying female fetuses within the same system. Additionally, CON cows carrying male fetuses showed a smaller LMA than those in the INT group carrying calves of the same sex (Table 2). No significant interactions between maternal nutrition and sex ($P \geq 0.617$) were observed for subcutaneous fat thickness in the LMA or rump fat thickness (Table 2). While subcutaneous fat thickness in the LMA was similar between cows managed in different production systems ($P = 0.697$), rump fat thickness was greater ($P = 0.033$) in CON cows than in INT or SUP cows. CON cows tended to have greater rump muscle length than other production systems ($P = 0.087$). Neither adiposity measurements differed according to the sex of the gestated fetus ($P \geq 0.625$; Table 2).

No NM \times S interaction existed for PREG ($P = 0.34$). Furthermore, the weight of the gestational components was similar among cows managed in CON, INT, or SUP systems ($P = 0.70$) and between cows pregnant with male and female fetuses ($P = 0.34$) (Figure 1).

3.2 Hemodynamic parameters

No MN \times S interaction was observed for pulsatility and resistance indexes, or systolic-to-diastolic ratio ($P \geq 0.492$; Table3). Additionally, these parameters did not differ between cows in the CON, INT, or SUP groups ($P \geq 0.129$). Although the pulsatility and resistance indexes were similar between cows carrying male and female fetuses ($P \geq$

0.113), the systolic-to-diastolic ratio tended ($P = 0.107$) to be higher in cows carrying males compared to those carrying females (Table 3).

3.3 Offspring's vigor score and performance

No $MN \times S$ interaction was found for the vigor score at birth ($P = 0.700$; Table 4). However, a trend ($P = 0.085$) indicated that calves born to cows managed in a super-intensive system had lower vigor scores than those born to CON and INT cows. No $MN \times S$ interaction was observed for birth weight, weaning weight, or ADG ($P \geq 0.362$). Moreover, the different production systems applied during pregnancy had no significant effects on the performance variables of the offspring ($P \geq 0.290$). Although birth weight did not differ significantly between males and females ($P = 0.328$), males exhibited greater ADG and higher weaning weights compared to females ($P \leq 0.014$; Table 4).

3.4 Skeletal muscle and adiposity in calves

No $MN \times S$ interaction was observed for LMA, LMA per 100 kg (LMA/100 kg), subcutaneous fat thickness, rump muscle length, and rump fat thickness ($P \geq 0.565$) (Table 5). Additionally, these outcomes were similar among calves from CON, INT, and SUP dams ($P \geq 0.153$). A trend towards a sex effect was verified for LMA ($P = 0.080$), with males showing higher values than females. Conversely, rump fat thickness was greater in females than in males ($P = 0.044$).

4. DISCUSSION

Maternal nutrition during pregnancy can influence the postnatal development of offspring through epigenetic mechanisms (Santos et al., 2022), acting as a memory of environmental exposure (Wu et al., 2006). Dietary adjustments during pregnancy offer opportunities to enhance offspring performance and meat quality, but they can also have irreversible effects on these traits. It is well-established in the scientific literature that nutritional restriction during the second third of pregnancy can impair cell proliferation (Jennings et al., 2016), reducing the number of muscle fibers in the offspring (Costa et al., 2021). This, in turn, promotes reduced performance throughout the post-natal productive life of these animals (Nascimento et al., 2024). Moreover, since adipocyte development partially takes place during gestation, fat deposition in the offspring may also be affected by the maternal feeding regimen during gestation (M Du et al., 2010).

Given this context, we hypothesized that calves from cows under an intensive or super-intensive nutritional regime would exhibit enhanced performance and muscle development compared to those from cows in a less advanced system. However, this anticipated outcome was not observed.

Based on this, a meta-analysis (Barcelos et al., 2022) which analyzed data from 35 studies involving 3,854 animals, showed that the degree to which the protein and energy requirements of pregnant cows were met significantly influenced the body weight of their offspring. The authors also reported that when nutritional requirements were exceeded, the magnitude of the offspring's response diminished. This may account for the absence of observed differences between the production systems in which the pregnant cows were managed. Despite our initial aim to simulate an extensive system, the quality of the paddocks in this treatment remained satisfactory, probably meeting the cows' nutritional requirements. Additionally, despite the different production strategies in the intensive and super-intensive systems, the variation in meeting maternal nutritional requirements probably was not substantial. This condition is in line with the lack of observed effects on the LMA and subcutaneous fat thickness in the cows, suggesting that the treatments produced comparable outcomes in terms of muscularity and adiposity measurements, which in turn is associated with their nutritional status.

The pulsatility index, resistance index, and systole/diastole ratio were originally developed to assess growth, development, and predict responses in growth-restricted fetuses (Burton; Fowden, 2012; Lekatz et al., 2015; Maršál, 2009; Owens; Falconer; Robinson, 1986; Vonnahme et al., 2015). Some studies suggest that placental programming effects may occur, involving compensatory mechanisms in response to nutritional restriction (Ford et al., 2007). In such cases, the placenta may act as a sensor for maternal stress factors, undergoing modifications to protect fetal development (Meneses et al., 2024). However, in our study, no significant interactions were observed between the production system used during pregnancy and fetal sex. Additionally, these parameters were consistent across CON, INT, and SUP cows. The lack of intensified placental mechanisms could be attributed to the adequate nutritional levels provided to the cows, making additional nutrient delivery to the fetus unnecessary.

Camacho et al. (2011) subjected their cows to different durations of restriction (Control, no restriction; Short period, 55 days; or 100 days—60% restriction of intake compared to the control). They observed effects on a general decrease in the resistance index (RI) compared to animals subjected to short-term restriction and control cows, which did not differ. Interestingly, the RI decreased after refeeding in cows that underwent the longest duration of the restriction, which may be attributed to the ability of the uteroplacental to compensate for the fetus (Ford et al., 2007). This supports the results obtained in the present study, as it is likely that the animals did not experience

severe restriction. Hassan et al. (2020) evaluated hemodynamic changes and their relationships between ipsilateral and contralateral uterine arteries during different stages of gestation in *Bos indicus* cows. However, the pulsatility index (PI) and RI did not differ between uterine arteries in the cows. Another point may be attributed to the maternal cardiovascular capacity to adapt during pregnancy, reducing systemic blood pressure and vascular resistance, and promoting cardiac output, heart rate, and systolic blood volume (Duvekot and Peeters, 1994), potentially masking differences between treatments.

There was a trend related to maternal nutrition for offspring vigor score. In addition to the criteria used in human medicine (heart rate, respiration, response to stimuli, and muscle tone), other common criteria used to assess neonatal vitality include mucous membrane color, coat appearance, rectal temperature, attempts to stand within 15 minutes, standing at 1 hour, and suckling within 2 hours (Veronesi et al., 2009). In a study involving calves, the sucking reflex was assessed through responses to orofacial stimuli, and calf vigor was measured by the duration of time spent standing or attempting to stand during the first hour after birth and while suckling colostrum. The amount of colostrum consumed was best predicted based on a combination of birth weight, vigor during colostrum feeding, and vigor in the first hour of life. Meal duration was predicted by ambient temperature and maternal suckling interaction. Smaller calves and those with lower vigor had reduced colostrum intake. Additionally, variations in sucking reflex intensity were not associated with colostrum intake (Vasseur; Rushen; De Passillé, 2009). In a study by Pires et al. (2020), the characteristics of maternal-offspring behavior in Guzerá cattle (*Bos indicus*) and the influence of this behavior on herd losses were assessed. Calf vigor was influenced by the duration of cow-calf contact, with less vigorous calves requiring more time with their mothers. This may explain the lack of difference between treatments, as all calves spent equal time with their mothers after birth.

The data from this study confirm differences in development between males and females. However, our results indicate that the nutritional conditions tested through different production systems did not show associative effects with the offspring's sex on the target outcomes, despite evidence from other studies suggesting a sexual bias in fetal programming (Copping et al., 2014; Gionbelli et al., 2018).

The PREG represents the actual quantities of components directly associated with gestation. This includes the gravid uterus minus the estimated weight of the non-gravid uterus, plus the gestation-related growth of the mammary gland (Gionbelli et al., 2015). In a study by Meneses et al. (2024), maternal supplementation with crude protein for beef

cows consuming low-quality forage during the middle third of gestation was found to improve the cows' body condition score in this phase. Consequently, in the final third of gestation, when cows experienced nutritional restriction, those that had received supplementation exhibited a higher intensity of maternal tissue mobilization, leading to increased weight of gestational components and, therefore, a higher calf birth weight. However, contrary to the findings of Meneses et al. (2024), the present study observed no effects of breeding system intensification on gestational components (PREG). This lack of effect may be attributed to similar nutrient availability for fetal development across treatments, which is consistent with the absence of differences in calf birth weight.

In the present study, differences in the weaning weight, ADG, and LMA between males and females indicate a significant impact of sex on muscle development. Such differences (where favorable responses were verified for males), can be attributed to the higher number of muscle fibers in males (Barcelos et al., 2022). Additionally, these findings may be linked to testosterone, an anabolic hormone that significantly influences mesenchymal stem cells, promoting increased muscle fiber development (Gionbelli et al., 2018; Baatar and Hwang, 2020). Moreover, testosterone also stimulates the *Mammalian target of the rapamycin complex 1* (mTORC1) signaling pathway via insulin-like growth factor (IGF), enhancing the synthesis of myofibrillar proteins (Sullivan et al., 2010; White et al., 2013).

Rump fat thickness showed a significant difference between the sexes, with females exhibiting greater measurements than males. This disparity can be attributed to the physiological maturity of females, who reach sexual maturity earlier than males (NRC, 2000). Thus, females begin to accumulate body fat earlier due to earlier onset of puberty compared to males. They may also initiate muscle fiber hypertrophy sooner, as adipose tissue deposition generally follows the deceleration of lean mass accumulation (Peng et al., 2020). Consistent with this, Nascimento et al. (2024) observed that females exhibited greater expression of lipogenic markers in skeletal muscle tissue at similar ages compared to males, supporting the observed differences in growth curves between the sexes.

5. CONCLUSION

In conclusion, while maternal nutrition during pregnancy has the potential to influence offspring, this study found no significant effects of breeding system intensification on gestational components or calf performance. This lack of impact may be attributed to similar nutrient availability across treatments. Although different

production systems did not show associative effects on fetal sex, significant differences in muscle development and fat deposition between male and female offspring were observed, likely due to hormonal influences such as testosterone and physiological maturity.

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TABLES

Table 1. Concentration of nutrients in silage and supplement.

	CP (%)	DM (%)	NDF (%)	MM DM (%)	OM DM (%)
Silage	11,0	33,0	66,3	7,7	92,3
Supplement¹	30,0	91,7	-	47,9	52,1

The average values are expressed as percentages of each component (%): CP: Crude Protein; DM: Dry Matter; NDF: Neutral Detergent Fiber; MM: Mineral Matter and OM: Organic Matter.

¹Supplement provided for intensive treatment during the middle and final thirds of pregnancy. Daily reference value for the maintenance of an animal weighing 450 kg: Crude Protein (g/day): 7.27; Total Digestible Nutrients (g/day): 0.85; Macrominerals (g/day): Calcium (Ca²⁺): 37.10-75; Phosphorus (P): 13.64; Sodium (Na): 57.14; Magnesium (Mg): -; Sulphur (S): 11.11; Potassium (K): -; Microminerals (mg/day): Cobalt (Co): -; Copper (Cu): 166.67; Iodine (I): 28.89; Manganese (Mn): 42.22; Selenium (Se): 11.11; Zinc (Zn): 55.56; Iron (Fe): 35.56; Vitamins (IU/day): Vitamin A: 14; Vitamin D: 11; Vitamin E: 7.86; Monensin sodium (mg/kg): 200.

Table 2. Influence of intensification levels during mid and late gestation on muscle and adiposity accretion in beef cows.

Trait	CON		INT		SUP		P-value		
	F	M	F	M	F	M	MN	S	MN*S
Longissimus muscle area, cm^2	59.15 ^{AB} (1.42)	56.70 ^B (1.72)	56.96 ^B (1.62)	61.8 ^A (1.74)	60.23 (1.80) ^{AB}	58.56 (1.53) ^{AB}	0.879	0.867	0.047
Longissimus muscle area, $cm^2/100kg$	10.82 (0.23)	10.33 (0.28)	10.62 (0.27)	11.24 (0.28)	10.65 (0.30)	10.49 (0.25)	0.192	0.946	0.102
Fat thickness in LMA, mm	14.3 (1.7)	13.5 (2.1)	11.7 (2.0)	11.6 (2.1)	10.5 (2.2)	9.0 (1.9)	0.697	0.625	0.950
Rump muscle length, cm	18.01 (0.46)	18.21 (0.57)	17.92 (0.52)	17.60 (0.55)	17.20 (0.59)	18.16 (0.50)	0.087	0.537	0.477
Rump fat thickness, mm	24.6 ^A (2.8)	26.3 ^A (3.5)	21.7 ^B (3.2)	17.4 ^B (3.4)	21.2 ^B (3.6)	21.4 ^B (3.0)	0.033	0.780	0.617

^{A-B} Means showing different superscripts are statistically significant based on Tukey's test ($P < 0.05$) and the standard error of each mean is shown in parentheses. Abbreviations: CON = Cows subjected to the lowest technological level. without fertilization. low stocking rate. and only mineral supplementation; INT = Cows subjected to a medium technological level. with nitrogen fertilization. higher stocking rate compared to CON. protein supplementation during mid and late gestation. and deferred grazing; SUP = Highest technological level employed. highest stocking rate. nitrogen fertilization. deferred grazing. and provision of TMR during mid and late gestation. MN = Maternal Nutrition; S = Sex.

Table 3. The influence of intensification levels in beef cows during the mid and late gestation periods on pulsatility indices (PI) resistance indices (RI) and systolic/diastolic ratios in beef cows.

Trait	CON		INT		SUP		P-value		
	F	M	F	M	F	M	MN	S	MN*S
PI	0.532 (0.082)	0.719 (0.123)	0.660 (0.116)	0.704 (0.131)	0.677 (0.106)	0.618 (0.094)	0.831	0.452	0.395
RI	0.371 (0.037)	0.434 (0.041)	0.422 (0.043)	0.468 (0.045)	0.401 (0.039)	0.433 (0.038)	0.565	0.113	0.896
Systolic/diastolic	1.60 (0.32)	1.89 (0.340)	2.05 (0.37)	2.80 (0.37)	1.73 (0.34)	1.82 (0.33)	0.129	0.107	0.492

Abbreviations: CON = Cows subjected to the lowest technological level. without fertilization. low stocking rate. and only mineral supplementation; INT = Cows subjected to a medium technological level. with nitrogen fertilization. higher stocking rate compared to CON. protein supplementation during mid and late gestation. and deferred grazing; SUP = Highest technological level employed. highest stocking rate. nitrogen fertilization. deferred grazing. and provision of TMR during mid and late gestation. MN = Maternal Nutrition; S = Sex.

Table 4. Influence of intensification levels during mid and late gestation on birth weight, weaning weight, average daily gain, and vigor of calves.

Trait	CON		INT		SUP		P-value		
	F	M	F	M	F	M	MN	S	MN*S
Vigor Score, <i>arbitrary units</i>	3.6 (0.4)	3.9 (0.4)	3.5 (0.4)	4.1 (0.4)	3.2 (0.4)	3.2 (0.4)	0.085	0.314	0.700
Birth weight, <i>kg</i>	35.6 (1.9)	34.1 (1.9)	34.1 (1.9)	37.6 (2.2)	34.8 (2.0)	37.5 (1.9)	0.780	0.328	0.362
Weaning weight, <i>kg</i>	210.8 ^B (10.2)	238.2 ^A (10.2)	213.7 ^B (10.6)	222.5 ^A (11.4)	206.7 ^B (11.3)	224.2 ^A (10.4)	0.577	0.014	0.557
ADG, <i>kg/day</i>	0.814 ^B (0.03)	0.944 ^A (0.03)	0.820 ^B (0.03)	0.865 ^A (0.03)	0.780 ^B (0.03)	0.882 ^A (0.03)	0.290	0.001	0.395

^{A-B} Means showing different superscripts are statistically significant based on Tukey's test ($P < 0.05$) and the standard error of each mean is shown in parentheses. Abbreviations: CON = Cows subjected to the lowest technological level, without fertilization, low stocking rate, and only mineral supplementation; INT = Cows subjected to a medium technological level, with nitrogen fertilization, higher stocking rate compared to CON, protein supplementation during mid and late gestation, and deferred grazing; SUP = Highest technological level employed, highest stocking rate, nitrogen fertilization, deferred grazing, and provision of TMR during mid and late gestation. MN = Maternal Nutrition; S = Sex.

Table 5. Influence of intensification levels during mid and late gestation on skeletal muscle and adiposity of calves.

Trait	CON		INT		SUP		P-value		
	F	M	F	M	F	M	MN	S	MN*S
Longissimus muscle area, <i>cm</i> ²	36.79 (1.97)	40.67 (2.15)	38.96 (2.20)	41.03 (2.44)	38.05 (2.53)	41.81 (2.05)	0.816	0.080	0.899
Longissimus muscle area, <i>cm</i> ² / <i>100kg</i>	17.62 (1.08)	17.77(1.14)	18.62 (1.15)	18.50 (1.25)	18.97 (1.31)	18.81 (1.13)	0.500	0.959	0.984
Fat thickness in LMA, <i>mm</i>	1.5 (0.2)	1.3 (0.2)	1.2 (0.2)	0.8 (0.2)	1.3 (0.2)	1.0 (0.2)	0.153	0.165	0.875
Rump muscle length, <i>cm</i>	5.72 (0.25)	6.2 (0.27)	5.76 (0.27)	5.75 (0.29)	5.58 (0.30)	5.85 (0.27)	0.539	0.178	0.503
Rump fat thickness, <i>mm</i>	2.2 ^A (0.3)	1.4 ^B (0.3)	1.7 ^A (0.3)	1.0 ^B (0.4)	1.3 ^A (0.4)	1.2 ^B (0.3)	0.448	0.044	0.565

^{A-B} Means showing different superscripts are statistically significant based on Tukey's test ($P < 0.05$) and the standard error of each mean is shown in parentheses. Abbreviations: CON = Cows subjected to the lowest technological level, without fertilization, low stocking rate, and only mineral supplementation; INT = Cows subjected to a medium technological level, with nitrogen fertilization, higher stocking rate compared to CON, protein supplementation during mid and late gestation, and deferred grazing; SUP = Highest technological level employed, highest stocking rate, nitrogen fertilization, deferred grazing, and provision of TMR during mid and late gestation. MN = Maternal Nutrition; S = Sex.

FIGURE

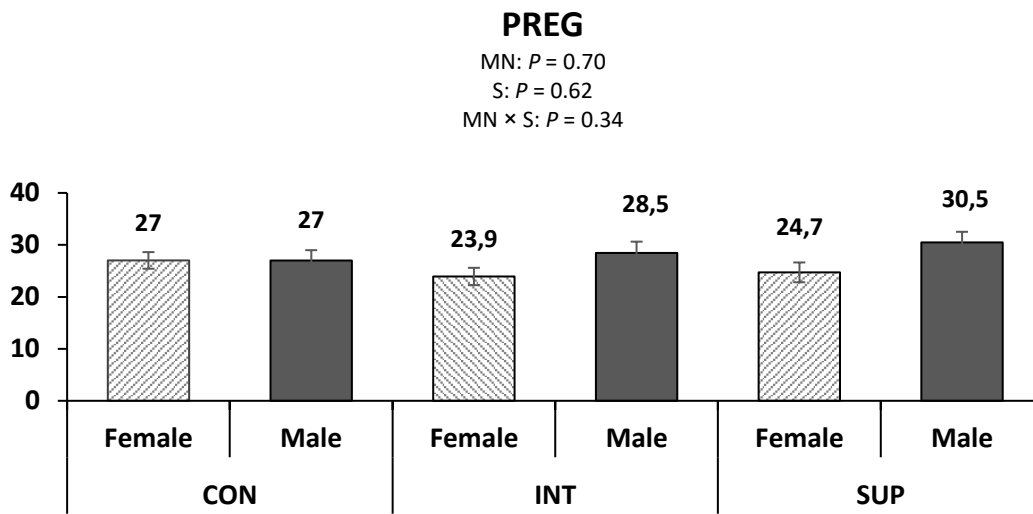


Figure 1. Gestational components (PREG) of pregnant beef cows concerning fetal sex and varying levels of intensification.

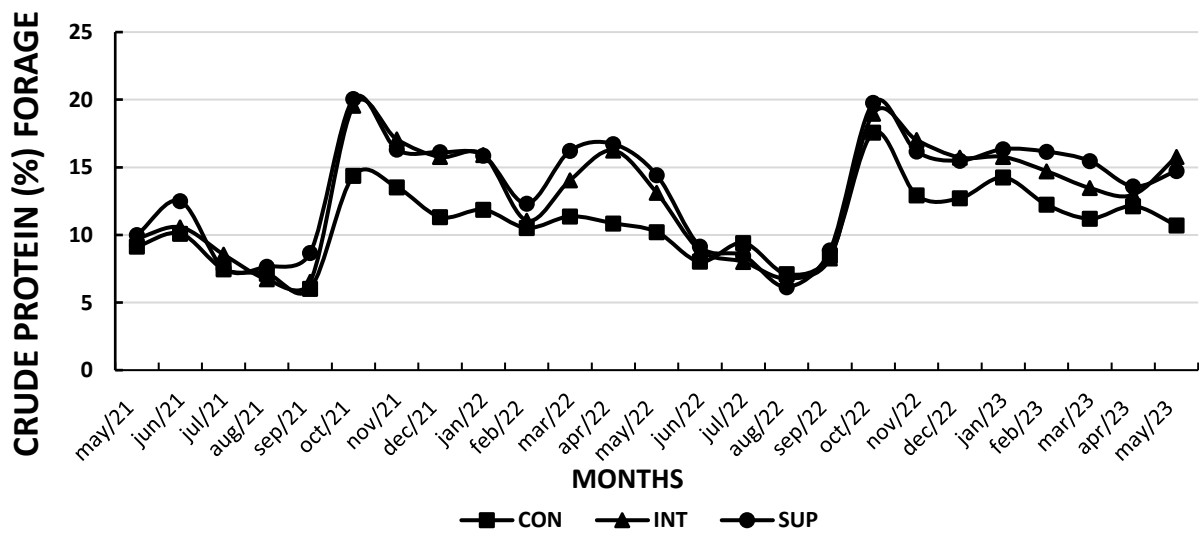


Figure 2. Variation in crude protein in forage during production cycles.

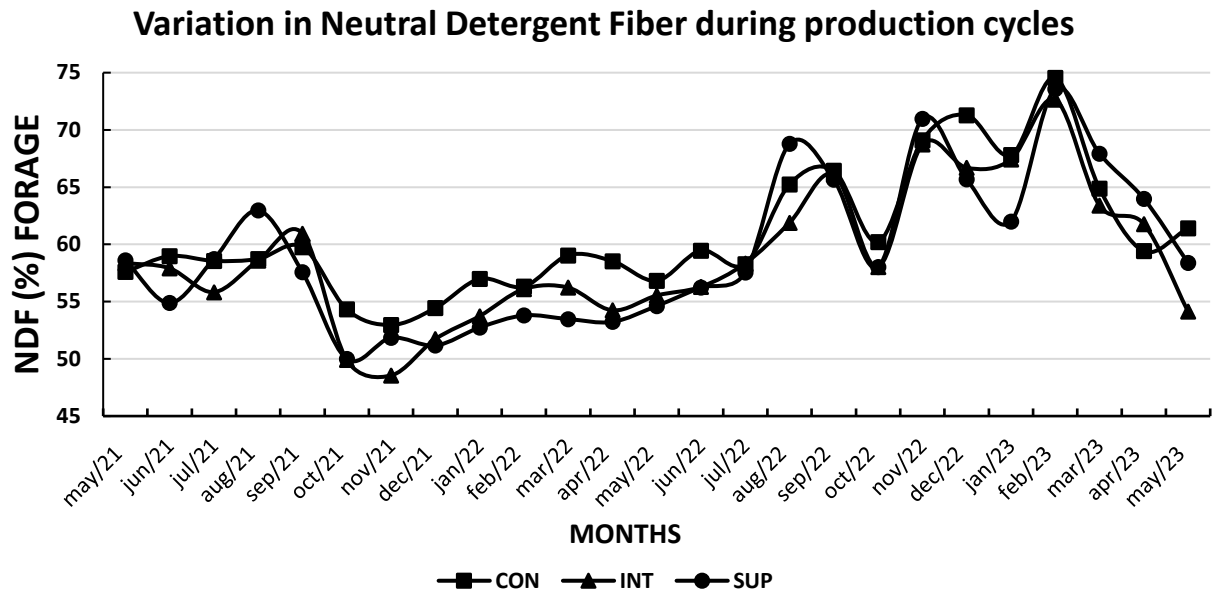


Figure 3. Variation of Neutral Detergent Fiber (NDF) in forage during production cycles.