MATHEUS CASTILHO GALVÃO

EFFECTS OF CRUDE PROTEIN SUPPLEMENTATION DURING BEEF COW'S MID-GESTATION ON THE COWS PERFORMANCE, MILK PRODUCTION AND METABOLISM

LAVRAS – MG
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Thesis presented to the University of Lavras, as part of the Animal Science Graduate Program requirements, in Ruminant Nutrition and Production, to obtain the Ph.D. title in Animal Science.

Advisor

Professor Dr. Mateus Pies Gionbelli

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EFEITOS DA SUPLEMENTAÇÃO DE PROTEINA BRUTA EM VACAS DURANTE O TERÇO MÉDIO DA GESTAÇÃO NO DESEMPENHO, PRODUÇÃO DE LEITE E METABOLISMO.

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LAVRAS – MG
2022

A toda minha familia, em especial meus pais José e Silvia e meu irmão Bruno

Dedico

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RESUMO

O objetivo deste estudo foi avaliar o efeito da restrição proteica durante o terço médio da gestação de vacas de corte e seu efeito durante a fase pós-parto das vacas. O experimento foi conduzido por 2 anos consecutivos. Foram utilizadas 43 vacas da raça Tabapuã (*Bos taurus indicus*) separadas em dois tratamentos: restrição proteica (RES; *n* = 24) e suplementadas (SUP; *n* = 19), os tratamentos foram aplicados dos 100 aos 200 dias de gestação. O grupo restrito recebeu uma dieta basal composta por silagem de milho + bagaço de cana. O grupo suplementado recebeu a mesma dieta basal com suplementação proteica (3.5 g/kg do PV, ~40% de proteina bruta). Dos 200 dias até o parto as vacas foram alocadas em pastagem de *Brachiaria brizantha* cv. Marandu até o parto. Foram realizadas ordenhas aos 7, 30, 60, 120 e 210 dias pós parto para a obtenção da produção de leite das vacas, as ordenhas foram feitas de forma manual logo após a aplicação de ocitocina. O leite foi pesado, o volume foi medido e cerca de 50 mL foram enviados para um laboratório comercial para a analise da composição do leite e vacas e bezerros foram pesados logo após a ordenha. Aos 30 dias de lactação foram feitas coletas de sangue das vacas, e posteriormente centrifugadas e o plasma separado para a analise de BHBA, NEFA, glicose e nitrogenio ureico no sangue. A avaliação de consumo e ensaio de digestibilidade foram realizados aos 120 e 200 dias pós-parto. OS dados foram avaliados utilizando o PROC MIXED do SAS, considerando tratamento materno, sexo do bezerro e sua interação como efeito fixo e ano como aleatório. Vacas que SUP foram 11% mais pesadas aos 7 e 30 dias pós-parto quando comparadas as vacas RES ($P < 0.05$), porém, essa diferença não foi mantida ao longo da lactação. A produção de leite das vacas SUP foi 23% superior as vacas RES aos 7 dias de lactação ($P < 0.05$), essa diferença não foi mantida ao longo da lactação ($P > 0.05$). Não houve diferença significativa para consumo, digestibilidade e composição do leite durante a fase de lactação ($P > 0.05$). Em conclusão, o uso de suplementação proteica no terço médio da gestação resultou em melhoria na produção de leite das vacas no ínicio da lactação, porém, essa diferença foi sumindo com o tempo.

Palavras-chave: Dimorfismo Sexual. Nutrição Gestacional. Programação Fetal. Produção de Leite. Vacas de Corte.

ABSTRACT

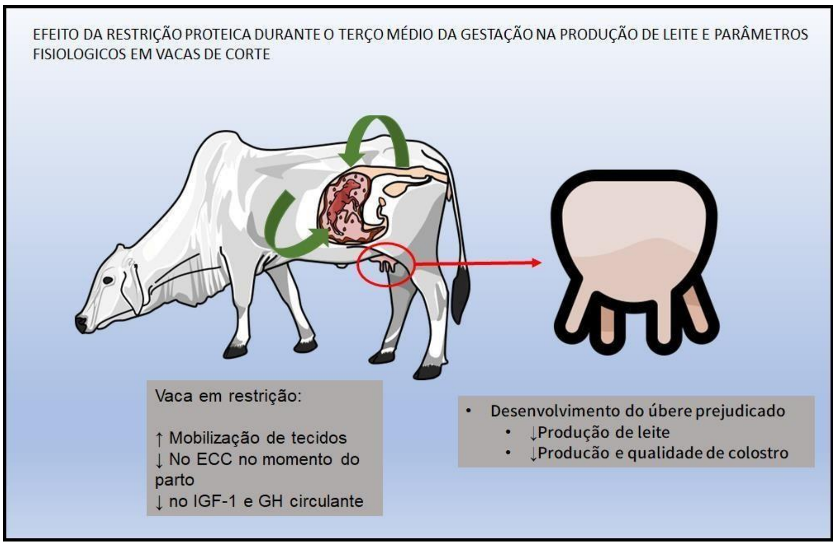
The aim of this study was to evaluate the effect of protein restriction during the middle third of pregnancy in beef cows and its effect during the postpartum phase of the cows. The experiment was conducted for 2 consecutive years. Forty-three Tabapuã cows (Bos taurus indicus) were used, separated into two treatments: protein restriction (RES; n = 24) and supplemented (SUP; n = 19), treatments were applied from 100 to 200 days of gestation. The restricted group received a basal diet composed of corn silage + sugarcane bagasse. The supplemented group received the same basal diet with protein supplementation (3.5 g/kg BW, ~40% crude protein). From 200 days until calving, the cows were allocated to Brachiaria brizantha cv. Marandu until partum. Milking were carried out at 7, 30, 60, 120 and 210 days postpartum to obtain the milk production of the cows, the milking were done manually right after the application of oxytocin. The milk was weighed, the volume was measured and about 50 ml were sent to a commercial laboratory for analysis of the milk composition and cows and calves were weighed right after milking. At 30 days of lactation, blood samples were collected from the cows, which were later centrifuged, and the plasma separated for analysis of BHBA, NEFA, glucose and BUN. Intake assessment and digestibility assay were performed at 120 and 200 days postpartum. Data were evaluated using PROC MIXED from SAS, considering maternal treatment, calf sex and their interaction as fixed effect and year as random. Cows of treatment SUP were 11% heavier at 7 and 30 days postpartum when compared to RES cows ($P < 0.05$), however, this difference was not maintained throughout lactation. Milk production of SUP cows was 23% higher than RES cows at 7 days of lactation ($P < 0.05$), this difference was not maintained throughout lactation ($P > 0.05$). There was no significant difference for milk intake, digestibility and composition during lactation ($P > 0.05$). In conclusion, the use of protein supplementation in the middle third of pregnancy resulted in an improvement in the milk production of cows in early lactation, however, this difference disappeared over time.

Keywords: Beef Cows. Fetal Programming. Gestational Nutrition. Milk Production. Sexual Dimorphism.

Informe Gráfico

Elaborado por **Matheus Castilho Galvão** e orientado por **Mateus Pies Gionbelli**

Muitas vezes os pecuáristas negligenciam o que é a principal etapa do sistema produtivo de bovinos de corte, a fase de cria. É nessa fase que se tem origem o principal produto comercializado, que é o bezerro que está sendo gerado, e muitas vezes essas vacas na fase de gestação passam por restrição nutricional tanto no terço médio quanto no terço final da gestação. Vacas gestantes apresentam exigencia nutricional mais elevada quando comparada com vacas não gestantes, isso se dá devido ao feto que está sendo gerado com o adicional no desenvolvimento da glandula mamária que irá fornecer os nutrientes nescessários para o crescimento do bezerro. Quando as vacas passaram por restrição no terço médio da gestação, foi observado a redução de aproximadamente 1 kg de leite durante a fase inicial da lactação, assim como, seu peso no momento do parto foi inferior aos animais que não passaram por restrição nutricional. No ponto de vista prático, vacas que não apresentam suas exigencias nutricionais atendidas durante a gestação produzem menos leite quando comparadas com vacas sem restrição, e isso vai refletir diretamente no desempenho da progenie, já que no inicio da vida do bezerro o leite materno é responsável em fornecer todos os nutrientes para o desenvolvimento da cria. Assim como a produção de leite, o desempenho das vacas também é afetado no pós-parto e vacas que apresentam baixo peso ao parto tendem a ter problemas em retorno de cio e consequentemente aumenta seu intervalo entre partos, situação indesejada no sistema de cria.



CONTENTS

FIRST SECTION **10**

1. INTRODUCTION **10**

2. BACKGROUND **11**

2.1. FETAL PROGRAMMING AND MUSCLE AND ADIPOSE TISSUE DEVELOPMENT **11**

2.2. NUTRITIONAL RESTRICTION DURING THE MIDDLE THIRD OF PREGNANCY AND ITS EFFECT ON MILK PRODUCTION **14**

2.3. NUTRITIONAL RESTRICTION DURING THE MIDDLE THIRD OF PREGNANCY AND ITS EFFECT COW PERFORMANCE POST-PARTUM **16**

3. REFERENCES **20**

SECOND SECTION **23**

1. INTRODUCTION **24**

2. MATERIALS AND METHODS **25**

2.1. *EXPERIMENTAL DESIGN AND MANAGEMENT* **25**

2.2. *MEASUREMENTS* **27**

2.2.1. *PERFORMANCE MEASUREMENTS* **27**

2.2.2. *DIGESTIBILITY ASSAYS AND FEEDSTUFFS CHEMICAL ANALYSIS* **27**

2.2.3. *MILK YIELD AND COMPOSITION* **28**

2.3.4. *BLOOD HORMONE AND METABOLITES* **29**

2.3.5. *STATISTICAL PROCEDURES* **29**

3. RESULTS **30**

3.1. *BODY WEIGHT AND BODY WEIGHT GAIN* **30**

3.2. *MILK YIELD* **31**

3.3. *MILK COMPOSITION* **32**

3.3. *BLOOD PARAMETERS* **34**

3.4. *VOLUNTARY INTAKE AND TOTAL TRACT DIGESTIBILITY* **34**

4. DISCUSSION **36**

5. CONCLUSIONS **39**

6. REFERENCES **40**

First Section

1. Introduction

The Brazilian livestock system is considered, for the most part, pasture. Data from ABIEC (2022) show that only 17.19% of the animals slaughtered come from confinement, with that, 82.81% of the animals are produced on pasture, and the breeding system, which comprises the pair cow:calf, is exclusively to pasture. However, in countries with a tropical climate there is a seasonality of forage production which, due to the breeding season, coincides with the middle third of pregnancy (NASCIMENTO et al., 2022).

During the middle third of pregnancy, muscle hyperplasia is occurring, that is, an increase in the number of muscle fibers and the nutritional deficit during this phase of pregnancy directly influences the transcription factors that will compromise the undifferentiated cells of the mesoderm to follow the lineage myogenic (CARDOSO et al., 2022). In addition to the problems with fetal development, the nutritional deficit during pregnancy causes problems mainly in the development of the mammary gland, since it develops more markedly during the gestation period (SEJRSEN, 1994), this development is mainly due to the action of GH and IGF-1 hormones (NEVILLE et al., 2002) and when cows undergo nutritional restriction, plasma levels of these hormones tend to be lower than in cows that did not undergo nutritional restriction (MENESES et al., 2022) , and the poor development of the mammary gland, as well as the nutritional deficit, directly affects the production of colostrum and milk, as well as their qualities (MELLOR et al., 1985; MELLOR et al., 1987; BARCELOS et al., 2022).

Nutritional restriction, in addition to harming the fetal development of the progeny and the development of the mammary gland, has been shown to be harmful to the body condition score (BCS) of the cows. The BCS ranges from 1 to 9, with 1 = thin and 9 = obese (WAGNER et al., 1988). Several studies have shown that cows that during parturition had an adequate BCS, around 5, showed a reduction in the postpartum interval. childbirth (RICHARDS et al., 1986; YUSUF et al., 2010) and consequently in the subsequent pregnancy rate. Thus, the aim of this study was to evaluate the effect of protein restriction during the middle third of pregnancy in Zebu beef cows regarding the performance, physiology and metabolism of beef cows in the postpartum period, as well as milk production and quality.

2. Background

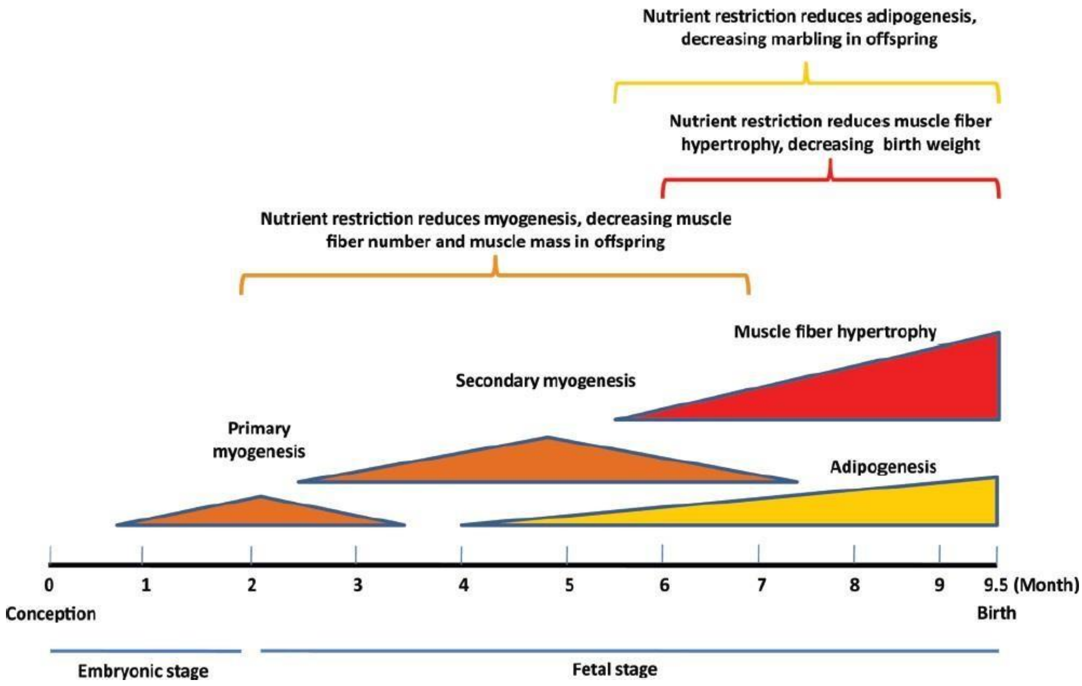
2.1. Fetal programming and muscle and adipose tissue development

The concept of fetal programming began with studies done in humans, this concept came up with Dr. David Barker (Barker et al., 1990; Barker, 2004) it was observed that mothers who went through some type of nutritional restriction during the gestation period had children with a high incidence of metabolic disorders, low birth weight. Fetal programming, or also called gestational nutrition, directly affects the development of the individual throughout his life. After all, many of the changes that occur in embryonic development last throughout life (DU et al., 2010).

Studies with production animals are relatively recent, in ruminants the first studies were carried out in the 1950s and 1960s in order to understand how fetal programming affects the productive characteristics of animals (SHORT, 1955; TAPLIN; EVERITT, 1964). Since then, several studies have been conducted to assess how the effect of undernutrition (Gionbelli et al., 2015; Meneses et al., 2022; Nascimento et al., 2022; Cardoso et al., 2022) and the effect of overnutrition (Sanl et al., 2019; Sartori et al., 2020; Sartori et al., 2022) affects embryonic development.

The gestation period can be divided into three phases: initial third, middle third and final third. In each of these stages, something different is happening in embryonic development. During the initial third of gestation, the development of the central nervous system and organs occurs mainly, and this is where secondary myogenesis begins. The final third is where we have muscle hyperplasia and an increase in the accumulation of adipocytes (DU et al., 2010) (Figure 1).

Figure 1.1: Effects of maternal nutrition on bovine fetal skeletal muscle development

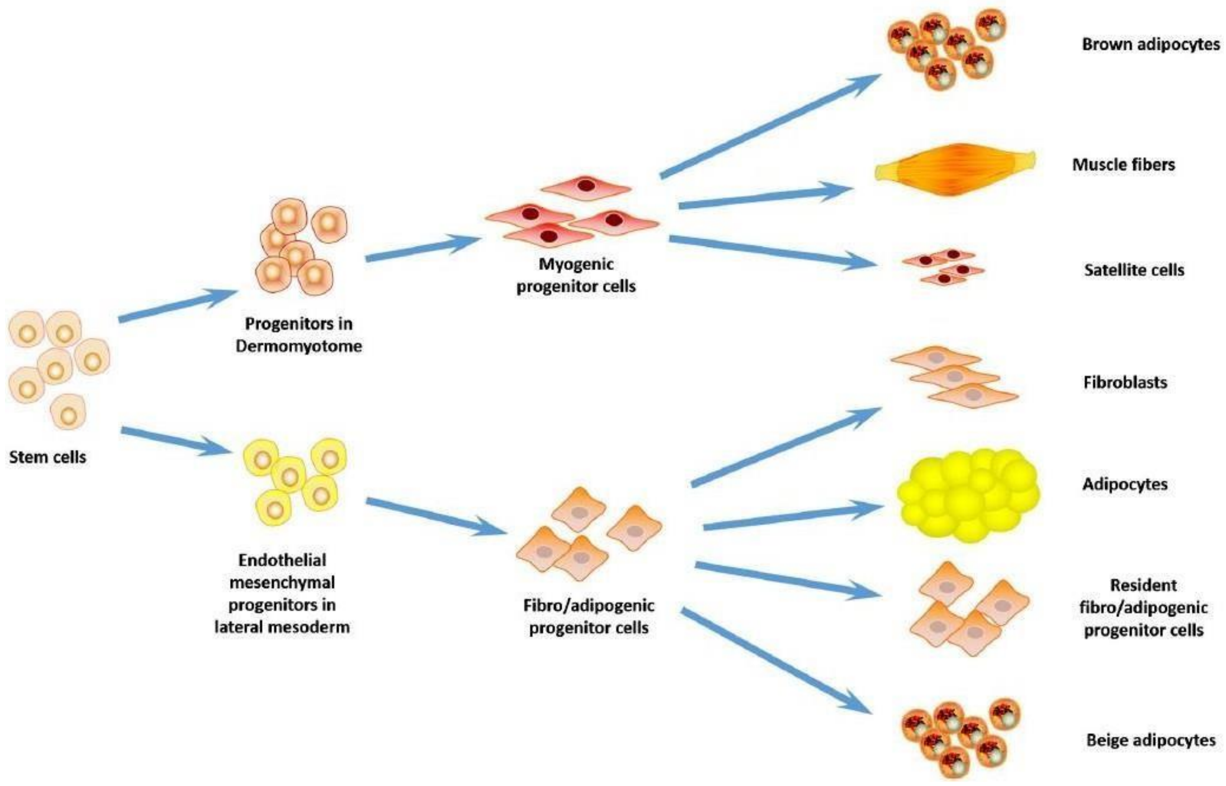


Source: Du et al., 2010

Thinking about production animals, mainly animals whose main product is meat, it is in the middle third of pregnancy that you need to pay more attention. It is during this phase that muscle hyperplasia occurs, that is, an increase in the number of muscle fibers, however, the emergence of fibers occurs during embryonic development when the undifferentiated cells undergo a process called commitment (BARCELOS et al., 2022). This impairment begins even in the embryonic phase, at this stage, the portion of the mesoderm cells express Pax3 and Pax7 and then there is the expression of Myf5 and MyoD, these are responsible for the differentiation of mesoderm cells to the muscle lineage (DU et al. al., 2010). Up to this point we have the muscle cells formed, however they are not muscle fibers, for the fibers to be formed it is necessary the action of Myogenin, it is responsible for stimulating the muscle cells to unite in multinuclear cells, called muscle fibers.

However, mesenchymal cells can also follow other destinations, not just the formation of muscle tissue. Depending on the stimulus that the cell receives, it can follow the myogenic or fibro-adipogenic lineage,

Figure 2.1: Early mesoderm development and the commitment of mesenchymal progenitors cells into myogenic and fibro-adipogenic cell lineage during fetal development.



Source: Du et al., 2015

Briefly, the development of adipose tissue takes place in two distinct phases, determination, and differentiation (DU et al., 2015). In the determination phase, a part of the fibro-adipogenic cells is determined for the formation of pre-adipocytes, this is done by the expression of the transcription factor Zfp423 (Zync finger protein 423), which induces the expression of PPARG, and they also convert pre-adipocytes into mature adipocytes (ZAMUDIO et al., 2022).

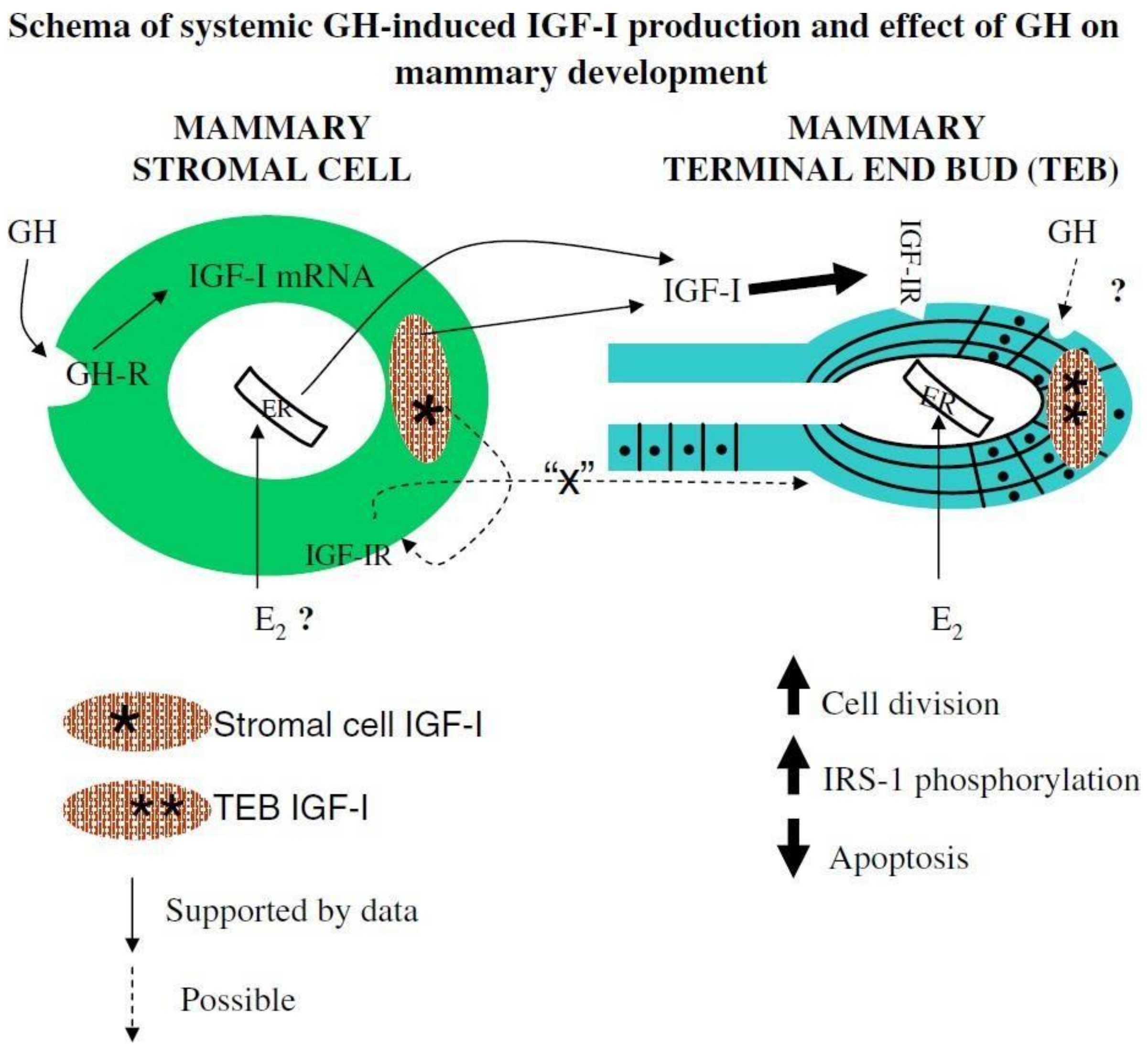
After the formation of pre-adipocytes, the maturation of these cells begins, this is due to the beginning of the normal metabolism of the cell, which includes its filling with lipids and its morphological change to a spherical shape (ZAMUDIO et al, 2022). As in the pre-adipocyte phase, PPARG plays a key role in cell maturation along with CCAAT/Protencier Binding Proteins (C/EBPs). The transient expression of C/EBPβ and C/EBPδ activates the expression of PPARG and C/EBPα, and both act synergistically to activate genes that will interrupt the cell cycle and induce adipocyte maturation (ZAMUDIO et al, 2022).

2.2. Nutritional restriction during the middle third of pregnancy and its effect on milk production

The production of milk and colostrum is essential for the survival of mammalian animals. The development of the mammary gland begins during fetal development; however, it is during pregnancy that the greatest development of the mammary gland occurs (SEJRSEN, 1994). It is at this stage that the mammary ducts develop into alveolar lobules, differentiating into cells capable of producing and excreting milk, and the number of cells in the mammary gland directly influences milk production in cows. These cells are found in the parenchymal region of the mammary gland, and throughout pregnancy the adipose tissue is gradually replaced by milk-secreting alveoli.

One of the main factors that affect the growth and development of the mammary gland is nutrition, mainly the energy intake of cows (SEJRSEN, 1994). This occurs because there is an increase in the deposition of adipose tissue in the mammary gland and this impairs the formation of the alveoli, thus reducing the milk production capacity. The same is observed when cows undergo nutritional restriction during pregnancy. Hormones such as GH and IGF-1 are essential for the development of mammary glands (NEVILLE et al., 2002), as shown in the work by Meneses et al., 2022, where Zebu beef cows undergoing protein restriction during the middle third of pregnancy observed a reduction in the concentration of circulating IGF-1 in cows that suffered restriction. In addition, IGF-1 is essential for the development of the mammary gland, it works in synergy with other hormones (epidermal growth factor (EGF), amphiregulin and TGF-B) to stimulate the growth and development of the mammary gland.

Figure 3.1: Schematic representation of the interaction between growth hormone (GH), IGF-1 and stadiol (E2) in the stromal and glandular compartment of the mammary gland



Source: Kleiberg et al., 2008

Like GH and IGF-1, prolactin is one of the main hormones related to milk production. Its production is directly related to the production of TRH, vasopressin, and oxytocin (NEVILLE et al., 2002). In general, prolactin has been shown to stimulate the metabolism of the epithelial cells of the mammary gland tissue to maintain the production of milk protein, such as α-lactalbumin, which is important for the synthesis of milk protein (SVENNERSTEN - SJAUNJA, 2005).

In addition to the hormonal problems that directly affect the alveolar development of the mammary glands, nutritional restriction during the prenatal period directly affects milk production and colostrum quality (BARCELOS et al., 2022). During the lactation period, the nutritional requirements of cows are met by a combination of diet plus tissue mobilization

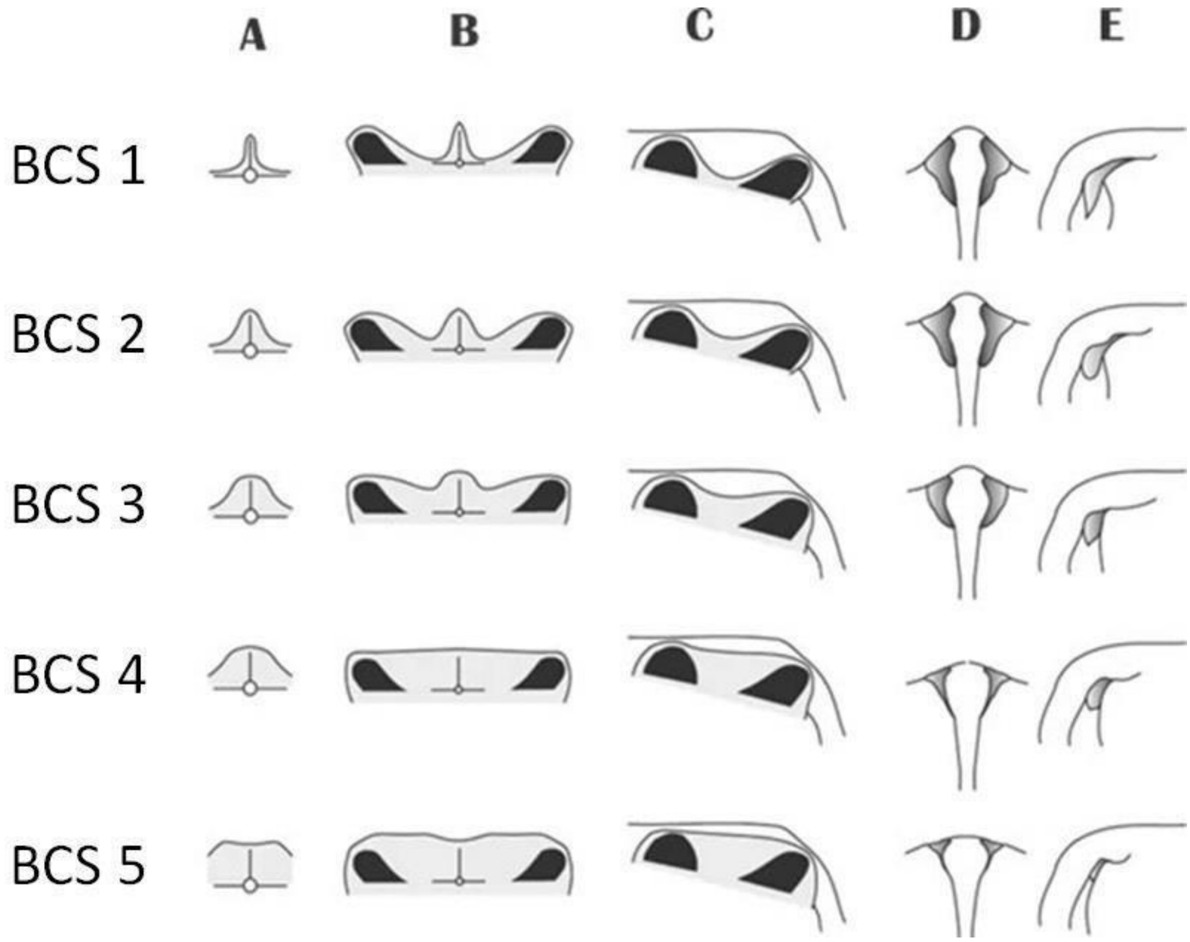
(BUTLER & SMITH, 1989) and this energy used in mobilization comes from fat. According to Rennó et al., 2006, in the first months of lactation, body reserves can contribute with about 33% of cows' milk production. As a result, animals that undergo nutritional restriction during the gestation period need to mobilize their tissues for fetal development, causing their reserve for milk production to be scarce at the time of delivery.

Another factor can influence the milk production of cows based on the sex of the progeny. According to the theory proposed by Trivers-Willard (1973), the sex of the calf directly influences the milk production of cows. In this theory, these authors observed that cows pregnant with females tend to produce more milk during the lactation period, this is due to the fact that cows “prioritize” the gestation of an individual who will have a greater possibility of perpetuating the species. Another point to highlight is the gestation requirement of males when compared to females. The nutritional requirement of cows pregnant with males is greater than that of females (BARCELOS et al., 2022), this is due to the fact that males tend to be larger and heavier than females at the time of delivery, therefore, the difference is more pronounced in cows pregnant with males, as they will prioritize their metabolism over their progeny.

2.3. Nutritional restriction during the middle third of pregnancy and its effect cow performance post-partum

One of the main indicators that the animals in a herd are in good body condition is the assessment of their score. When we think of a score for reproduction of beef cows, the recommended value would be around 3.5, remembering that the score ranges from 1 to 5, with 1 being extremely thin and 5 being extremely obese (Figure 4).

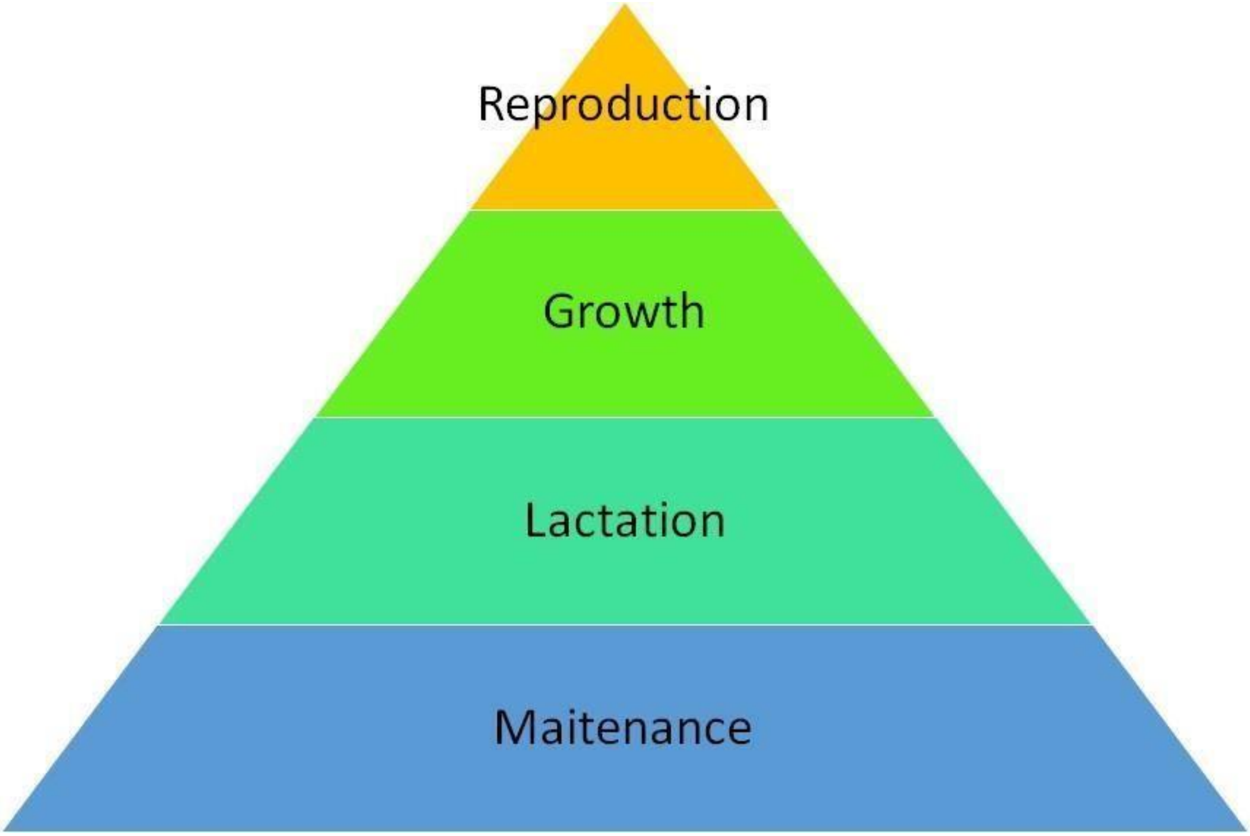
Figure 4.1: Points to evaluate the body condition score (BCS) in cattle. A – middle back vertebra; B – posterior view of the pelvic bone (transversal); C – Lateral view of the line between the ilium and ischium; D – Insertion point of tail (back view); E – Insertion point (lateral view).



Source: Edmondson et al., 1989

Cows whose body condition score is very high have serious reproductive problems, such as problems getting pregnant and problems during calving, and cows with a very low score have serious problems returning to heat (CHEBEL et al., 2018). This is because, when talking about energy partitioning, animals prioritize their basal metabolism rather than reproduction. According to NRBC, 2016, the use of energy for reproduction is the last option for animals (Figure 5).

Figure 5.1: Nutritional preference according to the physiological state of the cow



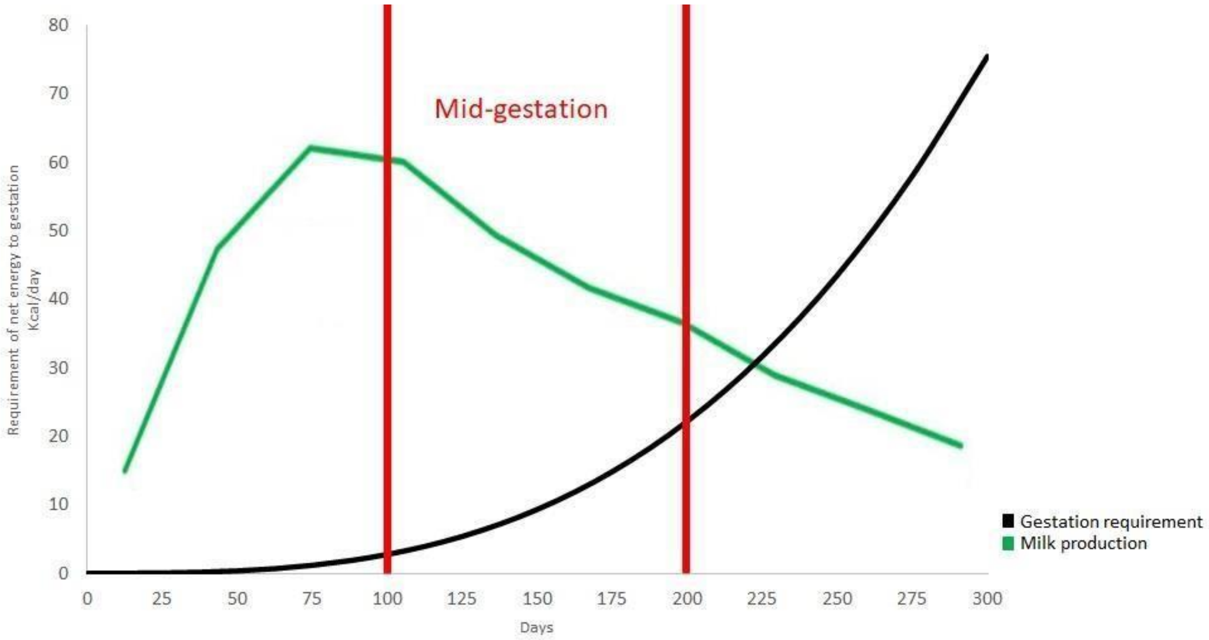
Adapted from: NRBC, 2016.

In their work with restricted cows during the middle third of gestation, Meneses et al., (2022) observed a reduction of approximately 1 point in the BCS of cows that underwent restriction, indicating that these cows were mobilizing tissues for the maintenance of pregnancy and this can directly interfere with the reproduction of cows, however, it was not possible to evaluate the effects on reproduction due to the number of animals in the project. In the same work, the authors reported that restricted cows were lighter at parturition and this difference in weight was observed up to 30 days postpartum.

A way to recover the score of these cows during lactation is to provide supplementation during this period. Supplementation aims to overcome the nutritional deficit that forage is often unable to supply, in addition to improving the digestibility of forage dry matter (MOURA et al., 2020).

Supplementation is indicated during the "critical" phases of lactation, which would be in the initial third and middle third of pregnancy. In the initial third of lactation, milk production of cows and milk consumption of calves is high, which means that cows need more nutritional support to meet the demand of milk production (GALVÃO, 2018) while in the final third of pregnancy, the greatest requirement is for the growth and development of the fetus (Figure 6).

Figure 6: Milk production and net energy requirement of gestation



Source: adapted from BR-Corte 2016

In the middle third of gestation there is a "gap" in the demand for fetal growth and milk production, in this period the demand for milk production, as well as for feal growth are low and this makes the cow's gains during this period low. more pronounced.

One way to save the cows at this stage would be to provide supplementation, via creep-feeding, for the calves, Galvão, 2018 observed in his study a reduction of approximately 1 liter of milk from cows whose calves received supplementation and this directly affects energy use by the cows, according to the BR-Corte system (2016) it is necessary 1.07 Mcal to produce 1 liter of milk, that is, the supplementation of the calves helps in the recovery of the score and consequently in the performance of these cows.

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Second Section

Article

Carryover effects of protein supplementation over pregnancy on performance, milk yield, nutritional and metabolic parameters of Zebu beef cows at subsequent lactation

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Abstract: This study aimed to access the effects of protein supplementation (PS) during gestation and its interaction with the calf sex (CS) on the beef cows' performance, physiology and metabolism at subsequent lactation. From 100 to 200 days of gestation, 43 purebred Tabapuã beef cows, were randomly assigned into 2 groups: Protein restricted (RES; $n = 24$) and Supplemented (SUP; $n = 19$). The RES cows were fed a basal diet. The SUP cows received the same RES basal diet with an additional supplementation (3.5 g/kg of BW, ~40% of crude protein). From day 200 of gestation from weaning all cows and their calves were equally fed. The SUP beef cows were 14% and 13% heavier ($P < 0.01$) at day 270 of gestation (pre-calving period) and 30 days in milk (DIM) than RES. They also tended ($P = 0.08$) to be ~15 kg heavier at 210 DIM (end of lactation period). The SUP cows produced ~2 additional kg of milk at 7 and 30 DIM than RES ($P \leq 0.02$). From 30 days in milk (DIM) until the end of lactation (210 DIM) SUP and RES cows presented similar milk production. Milk fat and total solids percentages were lower for RES cows sucking female calves ($P \leq 0.04$) at 60 DIM. The SUP cows had greater BHBA levels than RES at 30 DIM ($P = 0.03$). No PS × CS interactions ($P \geq 0.40$) was verified for non-esterified fat acids, glucose or ureic nitrogen blood parameters No difference was observed in intake and digestibility of DM and nutrients ($P > 0.05$). In conclusion, the protein restriction during mid-gestation affect the milk production and metabolic parameters in beef cows.

Keywords: calf sex; gestational nutrition; homeorhesis, milk composition, Zebu.

1. Introduction

Strategic supplementation programs is an effective practice to alleviate the negative nutritional impacts of lower natural pastures allowance on the beef cattle raised in tropical regions (POPPI et al., 2018). This management is especially important for pregnant beef cows under pasture systems (RODRIGUES et al., 2020; LOPES et al., 2020), commonly exposed to a protein restriction from mid- to late-gestation (COSTA et al., 2021). Recent findings from our lab Meneses et al (2022) e Meneses et al., (2022), demonstrated that protein supplementation (PS) during mid-gestation for pregnant beef cows fed low quality forage induced positive associative effects on maternal voluntary feed intake, which in turn enhanced maternal nutritional status through hepatic gluconeogenesis from AA substrates. Strategic protein supplementation for only 100 days of gestation in the second trimester of gestation, also promoted a greater tissues reserves to be mobilized in late pregnancy in supplemented than in unsupplemented beef cows (MENESES et al., 2022). This in turn, increased the weight of pregnant compounds (gravid uterus and udder accretion promoted by pregnancy), and the calf birth weight (MENESES et al., 2022; NASCIMENTO et al., 2022). In addition, the protein supplementation program utilized, was able to produce beneficial effects on the offspring performance, physiology and metabolism in a long term (COSTA et al., 2021; MENESES et al., 2022; MENESES et al., 2022; NASCIMENTO et al., 2022). Therefore, based on our previously responses and aiming to proceed our researches focused on gestational nutrition effects on Zebu beef cattle, we hypothesized that PS during pregnancy is also able to promote carryover effects on the beef cows at subsequent lactation.

Poor dietary plans during the prenatal period may impair the cows' colostrum and milk production and the synthesis of its components (BARCELOS et al., 2022). This is partially attributed to the lower contribution of body reserves to supply the mammary gland demand (MEYER et al., 2011; RENNÓ et al., 2006). Nevertheless, some scientific evidences Banchero et al., (2006) using ruminant animals as a model, also demonstrated that prenatal plane are associated with endocrine changes related to lactogenesis onset. This, in turn, may impair the udder development, the prenatal accumulation of colostrum and its subsequent production (MELLOR et al., 1985; MELLOR et al., 1987). Other available evidence Swanson et al., 2008 also shows potential effects of prenatal maternal nutritional plan on matrices alveolar secretory epithelial cell proliferation index of mammary tissue, clearly demonstrating that improper prenatal nutrition may affects the lactation outcomes. Based on the aforementioned, increasing nutritional status and body condition of beef cows through supplementation during prenatal period, may provide an opportunity to enhance beef cows' performance and milk production at subsequent lactation. This condition may improve the calf postnatal performance at the cow-calf phase, beneficing the matrices' longevity in the herd and favoring the financial viability of beef cattle operations.

Moreover, some studies suggested that there is a calf sex-biased for maternal resources allocation during gestation (Ithurralde et al., 2019; Gionbelli et al., 2018; Copping et al., 2014; Nugent et al., 2015) and for milk production programmed during pregnancy in ruminants (ITHUTTALDE et al. 2019; HINDE et al., 2014). In other words, the offspring sex being gestated may affected the milk production in the subsequently lactation, suggesting an *in utero* programming of mammary gland (HINDE et al., 2014). Thus, over gestational period, seems that dams 'may sense' their offspring sex to promote physiological adjustments through a biological negotiation involving hormonal and bioactive molecules signals to promote a 'safe bet' regarding the maternal resources allocation (BARCELOS et al., 2022). Despite this propose had been received little attention to date, especially on beef cattle, the other hypothesis of this study is that milk production will differ between cows nursing males and females, and that this response will occur in a dependent manner of maternal nutritional background.

Therefore, this study aimed to evaluated with strategic protein supplementation for pregnant beef cows fed low quality forage during mid-gestation may be an effective practice to promote carryover effects on the beef cows' performance, physiology and metabolism at subsequent lactation. We also aimed to assess if there are associative effects between prenatal nutrition and calf sex on these interest outcomes.

2. Materials and Methods

All project procedures were performed in accordance with the Universidade Federal de Lavras (UFLA) Ethics Committee on Animal Use (Protocol No. 015/17).

2.1. Experimental design and management

A 2-yr study, comprising 2 repetitions, was conducted at the Beef Cattle Facilities of the UFLA (Minas Gerais, Brazil). Each year of study was performed considering the same experimental procedures. Details of the experimental design were previously described by Meneses et al (2022) and Meneses et al., 2022. Briefly, forty-three Tabapuã (*Bos taurus indicus*) multiparous beef cows (3 ± 2 parities) were used. In y2, some cows used in y1 were re-used, considering a randomly dietary treatments designation. During early gestation (conception to day 100 of gestation), all cows were managed as a single group in a grazing system. The fetal sex was determined at day 60 of gestation, through ultrasound scans performed by a trained professional. Were identified 20 and 23 beef cows carrying female (y1: *n* = 9; y2: *n* = 11) and male (y1: *n* = 15; y2: *n* = 8) fetuses, respectively. At 200 days of gestation, cows were allocated in individual roof covered pens, and the experimental feeding regimens were randomly assigned to the cows. The feeding regimens employed were: Restricted (RES; *n* = 24) – supply of basal diet (75% composed of corn silage and 25% of sugarcane bagasse, and mineral mixture provided *ad libitum*) and Supplement (SUP; *n* = 19) – basal diet with additional protein supplementation (Table 1). Supplement was formulated to contain 40% of crude protein (CP), and was provided at the level of 3.5 g per kg of body weight. From day 200 of gestation to parturition, all cows were equally fed with corn silage and mineral mixture (Table 1). During the second and third trimester of gestation, the feed was daily provided as total mixed ration, in the morning (0700 h) and afternoon (1300 h). Adjustments in the roughages quantity were periodic performed considering the feedstuffs dry matter (DM) content. Adjustments in the supplement quantity was also periodic done according to the beef cows body weight (BW).

After parturition, cows and their calves were allocated in an intensive pasture-grazing system. The herd was managed under a continuous stocking method. The pasture area (70.000 m² of *Brachiaria brizantha* cv. Marandu) was the same in y1 and y2 (Table 1) and was not subdivided in paddocks. Thus, all animals had equal access to the entire area during the cow-calf phase. The pasture height was weekly measured to stocking rate control based on the critical leaf area index [25]. During all the cow-calf phase, the lactating beef cows were *ad libitum* fed with a mineral mixture. All calves had *ad libitum* access to an energy-protein supplementation (Probeef maxima creep®, Cargill Nutrição Animal, Itapira, SP, Brazil). The supplement was daily provided at the level of 5 to 7 g per kg of BW, through the creep-feeding technique. Periodically, the calves were weighed to make adjustments in the amount of supplement provided. Calves were weaned at 210 days of age.

Table 1. Average of chemical composition of the experimental diets used at years 1 and 2 on a DM basis (mean ± standard deviation).

Item	Gestational Period			Lactation
	Day 100 to 200 of gestation		Day 100 of gestation to parturition	0 to 210 days in milk
	Basal diet[1]	Supplement[2]	Corn Silage	Pasture
DM	418 ± 5.8	881 ± 0.7	330 ± 2.9	297 ± 1.5
OM	951 ± 2.7	958 ± 0.9	941± 1.4	906 ± 1.4

CP	53.3 ± 2.3	400 ± 1.4	72.2 ± 0.4	130 ± 1.2
NDFap	631 ± 10.6	213 ± 0.2	549 ± 3.6	626 ± 1.5
NFC	242 ± 5.6	342 ± 2.2	291 ± 2.1	333 ± 2.2
EE	24.1 ± 1.1	41.2 ± 0.3	29.2 ± 0.4	27.4± 0.8

Abbreviations: DM = dry matter, OM = organic matter, CP = crude protein, NDFap = Ash and protein-free neutral detergent fiber, NFC = Non-fibrous carbohydrates, EE = Ether extract.

[1] Basal diet = 75% of corn silage + 25% of sugarcane bagasse.

[2] Probeef Proteinado Sprint®, Cargill Nutrição Animal, Itapira, SP, Brazil) (assurance levels per kilogram of product: 70 g Ca (max); 50 g Ca (min); 15 mg Co (min); 255 mg Cu (min); 15 g S (min); 2000 mg F (max); 20 g P (min); 15 mg I (min); 510 mg Mn (min); 340 NPN protein eq. (max); 450 g CP (min); 4 mg Se (min); 95 g Na (min); 850 mg Zn (min); 50 mg Flavomycin).

2.2. Measurements

2.2.1. Performance measurements

For phenotypic evaluation, cows were weighted at gestational period and during lactation. Throughout pregnancy, the cows body weight measurements were performed in the morning, after a 16 h fasting. Throughout lactation period, cows were weighed at 7, 30, 60, 120 and 210 days in milk, after udder milk depletion (to avoid milk weight counting). Data were presented as the beef cows BW, empty body weight (EBW) and shrunk body weight (SBW), all expressed in kg. The BW was considered as the value directly obtained from balance. The EBW and SBW were mathematically obtained according to spreadsheets proposed by Gionbelli et al 2015.

2.2.2. Digestibility assays and feedstuffs chemical analysis

Were performed two digestibility trials during lactation, at 120-and 200-days in milk. Each trial comprised 10 days. The dry matter intake (DMI) and the diets compounds digestibility were assessed using two previously well validated external markers in digestibility assays using livestock animals (DETMAN et al., 2021): the indigestible neutral detergent fiber (NDFi) (Valente et al., 2011) and the titanium dioxide (TiO_2) (FERREIRA et al., 2009; TITGEMEYER et al., 2001). The NDFi was used to estimate the pasture intake, while TiO_2 was used to fecal production mensuration. From day 1 to 10, 10 g of TiO_2 per animal was orally provided in the morning (0600 h). From day 6 to 9, pasture samples were collected through manual grazing simulation technique, in order to chemically characterize the forage consumed. The pasture area was stratified into 5 homogeneous plots, and the forage collection was concomitantly performed by 3 trained people, for representative samples collection. The fecal samples were obtained by the hand grab technique, in the morning (0600 h) and afternoon (1800 h) from day 7 to 10 of each digestibility assay.

All feedstuffs and fecal chemical analysis were performed at the Animal Research Laboratory of the Animal Science Department of UFLA. First, fecal and pasture samples were oven-dried under a 55º C for 72 hours. Subsequently, the dried samples were ground in a 1 and 2 mm porosity sieves in a Wiley mill (Wiley® TE-680). Ground composite samples were chemical analyzed considering the analytical guidelines of the Brazilian National Institute of Science and Technology (INCT-CA) (DETMANN et al., 2012). Feedstuffs and fecal materials were analyzed for moisture, ash, nitrogen, ether extract (EE), ash- and protein- free neutral detergent fiber (NDFap), NDFi and titanium contents considering the following references methods number: G-003/1; M-001/1; N-001/1; G-004/1; N-002/1; F-009/2 and M-007/2. The FDNap was determined through filtration in porous crucibles, using heat-stable α-amylase and sodium sulfite. The NDFi was determined using the 2 mm grounding samples in an autoclave, after *in situ* incubation (288 h) using rumen cannulated beef animals (DETMANN et al., 2012). To determine the titanium dioxide concentration, after the sulfuric digestion, addition of hydrogen peroxide, and filtering, the samples aliquots were determined through colorimetric method in a spectrophotometer (Multiskan™ GO Thermo Scientific) adjusted to a wavelength of 410 nm (DETMANN et al., 2012). The NFC was estimated according Detmann and Valadares Filho, 2010.

2.2.3. Milk yield and composition

The beef lactating cows were hand-milked on 7, 30, 60, 120 and 210 days in milk. For milk yield determination, the cow-calf pairs were separated and remained without physical contact for a 12 h period. The calves were separated from their dams at 1800 h and the milking started at 0600 h in the subsequent day. The milking procedure was stimulated through 2 mL of oxytocin (Ocitocina Forte UCB, Uzinas Chimicas Brasileiras S/A, Jaboticabal, Brazil) application in the abdominal subcutaneous vein. The time of the beginning and the end of the milking procedure were registered. After the total depletion of milk from the udder, the milk content was weighed, homogenized and sampled (~30 mL) using sterile vials with one bronopol tablet (D & F Control Systems Inc., San Ramon, CA). Samples were storage at 4°C until analysis.

The total daily milk yield (MY, expressed in kg) was estimated considering the morning milk yield [9,36] (Equation 1), and the corresponding times (expressed in arbitrary units) of milking extraction end (time 1) and of cow-calf pair physical isolation (time 2).

$$\text{Daily milk yield} = \frac{\text{Morning milk yield}}{(\text{Time 1} + 1) - \text{Time 2}} \qquad \text{Eq. (1)}$$

Milk composition of individual cows was analyzed by a milk quality specializing company (APCBRH/PARLPR, Paraná, Brazil). The 4% fat corrected milk (FCM) was calculated (Equation 2) according to NRC, 2016, considering the milk yield (kg per day) and the fat yield (%).

$$\textbf{4}\%\ \text{Fat-corrected milk} = (0.4 \times \text{MY}) + (0.15 \times \text{MY} \times \text{fat yield}) \qquad \text{Eq. (2)}$$

The Feed Conversion Efficiency (FCE) were calculated according to Arndt et al 2015, as the ratio between milk production (kg per day) and the DMI (kg per day; Equation 3).

$$\text{Feed conversion efficiency} = \frac{\text{MY}}{\text{DMI}} \qquad \text{Eq. (3)}$$

2.3.4. Blood hormone and metabolites

Blood samples were collected (7:00 a.m.) from the coccygeal vein at 30 days in milk. Plasma was isolated by centrifugation at 2700 G for 20 minutes, and then stored at -20°C, until analysis. Plasma β-hidroxybutyrate (BHBA; Randox Laboratories Ltd, Antrin, UK), non-esterified fatty acids (NEFA, Randox Laboratories Ltd., Antrin, UK), glucose (Labtest, Lagoa Santa, Brazil) and blood urea nitrogen (BUN; Labtest, Lagoa Santa, Brazil) levels were determined using the enzymatic calorimetric assay.

2.3.5. Statistical Procedures

Descriptive statistical analysis was performed using SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA). All outcomes were submitted to a mixed ANOVA model, considering the maternal feeding regimen during gestation (RES and CON), the calf sex (male and females) and its interactions as fixed effects, and the experimental year (y1 and y2) as random effect. When pertinent, the cow initial empty body weight, initial BCS, and lactation order were used as covariate. The following model was used to analyze the overall outcomes:

$$Y_{ijk} = \mu + D_i + S_j + Y_k + (DS)_{ij} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = is the observed measurement

μ = is the overall mean

D_i = is the fixed-effect of the i^{th} level of maternal feeding regimen (2 levels);

S_j = is the fixed effect of the j^{th} level of calf sex (2 levels)

Y_k = is the random effect or the Y^{th} level of the year (2 years)

DS_{ij} = is the D × S interaction

ε_{ijk} is the random error associated with Y_{ijk}, with $e_{ijk} \sim N(0,\sigma_e^2)$.

Prior the final analysis, all data were tested for normality distribution through Shapiro–Wilk test. The comparison between the means of the groups was performed using α = 10% of probability for type I error for all tests performed. Thus, results were deemed significant when P-value ≤ 0.05 and tendency when 0.05 < P-value ≤ 0.10.

3. Results

3.1. Body weight and body weight gain

Table 2: Performance of beef cows post-partum, supplemented or not, during mid-gestation.

Item	MN		CS		SEM	P-value		
	RES	CON	Male	Female		MN	CS	MN × CS
				7 days				
BW, *kg*	423	475	465	434	25.4	0.037	0.207	0.380
SBW, *kg*	410	463	451	422	26.1	0.032	0.241	0.448
EBW, *kg*	372	421	409	383	24.0	0.032	0.241	0.448
ADG, *kg/day*	-2.95	-2.51	-2.60	-2.86	1.84	0.708	0.897	.
				30 days				
BW, kg	440	491	476	455	25.0	0.035	0.370	0.375
SBW, kg	428	479	464	443	25.1	0.035	0.371	0.375
EBW, kg	388	435	422	402	23.1	0.035	0.372	0.375
ADG, kg/day	-0.77	-0.79	-1.32	-0.23	0.98	0.976	0.113	0.855

				60 days				
BW, kg	456	498	486	468	26.2	0.093	0.469	0.341
SBW, kg	444	486	474	456	26.4	0.096	0.478	0.337
EBW, kg	403	420	430	393	28.5	0.538	0.189	0.976
ADG, kg/day	-0.17	-0.18	0.06	-0.40	0.16	0.952	0.008	0.737
				120 days				
BW, kg	478	510	498	490	25.8	0.184	0.747	0.171
SBW, kg	466	499	487	479	25.7	0.184	0.752	0.180
EBW, kg	424	454	442	435	23.7	0.184	0.752	0.180
ADG, kg/day	0.06	-0.14	-0.26	0.18	0.31	0.304	0.021	0.594
				210 days				
BW, kg	502	522	519	504	27.0	0.427	0.564	0.284
SBW, kg	490	510	508	493	27.2	0.428	0.564	0.284
EBW, kg	445	464	461	448	25.0	0.429	0.564	0.283
ADG, kg/day	0.23	0.23	0.17	0.29	0.13	0.997	0.049	0.153

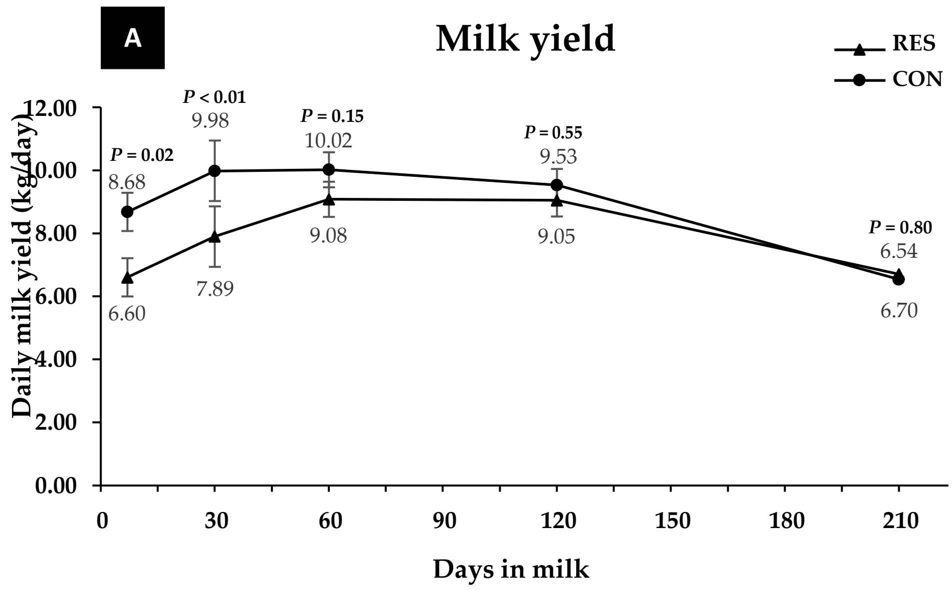
Abreviations: MN = Maternal nutrition; CS = calf sex; RES = offspring from unssuplemented cows; CON = offspring from supplemented cows from 102± 5 to 208 ± 6 days of gestation

Table 2 show the effect of the prenatal restriction on performance of beef cows. No interactions were observed between the maternal nutrition and calf sex in all parameters. Was observed difference between maternal nutrition pre-calving and 30 days in milking (DIM) (P < 0.05) during all these phases, supplemented cows were, approximately, 10% higher BW in compare with control group.

3.2. Milk yield

Cows from CON group produced around 2 additional kg of milk at 7 ($P < 0.01$) and 30 ($P = 0.02$) DIM compared to RES (Figure 1A). Nevertheless, maternal prenatal nutrition treatment did not influence the milk yield at 60, 120, or 210 DIM ($P \geq 0.15$). The milk yield was similar between dams of female and male calves ($P \geq 0.12$; Figure 1B) throughout overall lactation period. There was no MN × CS interaction for milk yield ($P = 0.16$) during the experimental period.

There was no difference in milk efficiency in 120 days post-partum and 200 days post-partum (P > 0.05) to MN, CS, and the interaction MN × CS.



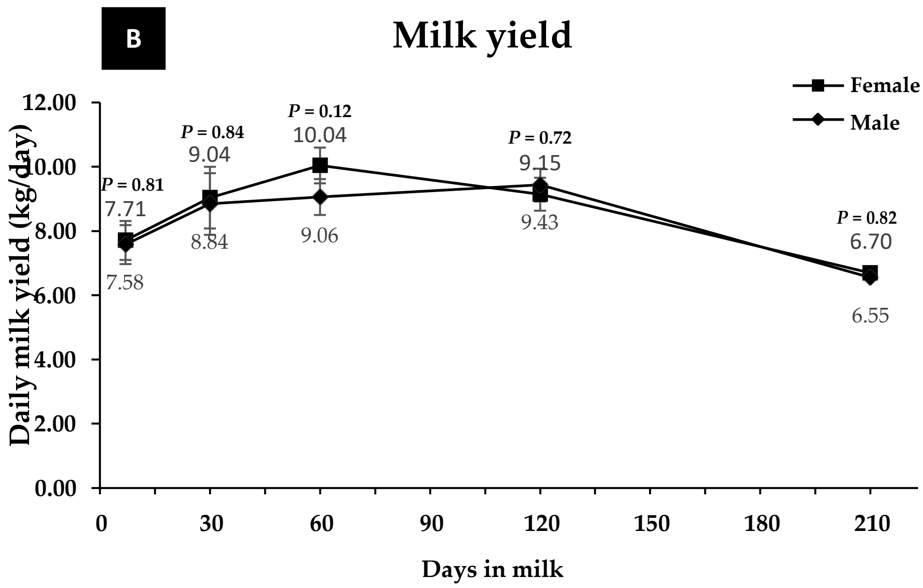


Figure 1: A - Milk production in function of the maternal nutrition; B - Milk production in function of calf sex.

3.3. Milk composition

There was a trend (P = 0.07) toward ~10% additional lactose content for RES cows at 7 DIM. Fat, protein, lactose, total solids, casein, and MUN contents were similar between CON and SUP cows in the remaining points evaluated throughout lactation ($P \geq 0.11$). At 7, 30, 60, and 120 DIM these milk components were similar between dams of males and females ($P \geq 0.17$). Nevertheless, at weaning, cows nursing males presented ~9% and 10% additional milk protein (P = 0.045) and casein contents (P = 0.035) compared to dams nursing females, respectively. MN × CS interactions were detected for fat (P = 0.04) and total solids (P = 0.01) contents. The milk fat content was lower for CON cows suckling females and SUP cows suckling males. The total solids content was reduced in CON cows sucking females, compared to the others groups.

Table 3. Milk composition of Zebu beef cows in response to prenatal feeding regimens and calf sex.

Item	MN		CS		SEM	*P-value*		
	RES	CON	Male	Female		FR	CS	FR × CS
				Fat, %				
7 days	3.41	2.58	3.46	2.53	1.15	0.345	0.309	0.873
30 days	4.10	3.64	3.88	3.85	0.370	0.397	0.951	0.275
60 days	3.94	4.24	4.18	4.00	0.233	0.375	0.588	0.038
120 days	4.14	4.07	4.28	3.92	0.233	0.842	0.260	0.270
210 days	4.74	4.84	5.08	4.50	0.320	0.746	0.160	0.254
				Protein, %				
7 days	3.46	3.74	3.70	3.50	0.140	0.128	0.250	0.151
30 days	3.29	3.18	3.20	3.26	0.097	0.276	0.462	0.069
60 days	3.32	3.40	3.34	3.38	0.095	0.374	0.718	0.311
120 days	3.38	3.42	3.45	3.36	0.066	0.632	0.318	0.602
210 days	3.45	3.49	3.62	3.32	0.108	0.727	0.045	0.377
				Lactose, %				
7 days	4.86	4.42	4.67	4.61	0.189	0.073	0.805	0.131
30 days	4.51	4.50	4.55	4.47	0.229	0.974	0.755	0.201
60 days	4.79	4.79	4.84	4.74	0.067	0.986	0.313	0.684
120 days	4.83	4.75	4.82	4.76	0.072	0.441	0.578	0.854
210 days	4.61	4.63	4.57	4.67	0.123	0.883	0.503	0.883
				Total solids, %				
7 days	12.4	11.3	12.5	11.2	1.37	0.297	0.236	0.972
30 days	12.7	12.2	12.5	12.4	0.495	0.434	0.930	0.245
60 days	13.0	13.4	13.3	13.1	0.228	0.242	0.431	0.010
120 days	13.3	13.2	13.5	12.9	0.285	0.790	0.173	0.310
210 days	13.7	13.9	14.2	13.4	0.375	0.735	0.153	0.274
				Casein, %				
7 days	2.66	2.90	2.82	2.74	0.10	0.110	0.519	0.178
30 days	2.63	2.52	2.53	2.62	0.083	0.327	0.392	0.072
60 days	2.62	2.69	2.65	2.66	0.054	0.357	0.868	0.275

120 days	2.65	2.69	2.70	2.64	0.059	0.591	0.466	0.763
210 days	2.76	2.78	2.90	2.63	0.079	0.839	0.035	0.361
MUN, *mg/dL*								
7 days	9.65	9.89	9.44	10.0	2.10	0.933	0.799	0.098
30 days	9.53	11.40	9.98	10.90	1.10	0.264	0.470	0.462
60 days	11.9	11.5	11.7	11.7	0.991	0.807	0.955	0.727
120 days	10.2	11.7	11.6	11.3	1.34	0.211	0.539	0.503
210 days	13.7	13.5	14.1	13.1	1.45	0.936	0.594	0.083

Abreviations: MN = Maternal nutrition; CS = calf sex; RES = offspring from unssuplemented cows; CON = offspring from supplemented cows from 102± 5 to 208 ± 6 days of gestation; MUN = Milk urea nitrogen

3.3. Blood parameters.

The BHBA levels were 28,8% greater for SUP dams at 30 days postpartum (*P* = 0.032) compared to CON. By the other hand, NEFA, glucose and MUN levels were similar for maternal nutrition plan (*P* ≥ 0.265). Only BUN levels were affected by calf sex (P = 0.042), being 18.9% greater for dams suckling females compared to those suckling males. No MN × CS interactions were found for blood parameters (*P* ≥ 0.402).

Table 4. Effects of prenatal nutrition and of calf sex on blood parameters of cows at 30 days in milk.

Item	MN		CS		SEM	*P-value*		
	RES	CON	Male	Female		MN	CS	MN × CS
BHBA, *mmol/dL*	0.567	0.719	0.652	0.633	0.049	0.032	0.783	0.404
NEFA, *mmol/dL*	0.523	0.544	0.486	0.582	0.143	0.819	0.290	0.456
Glucose, *mg/dL*	69.8	73.5	69.7	73.6	4.29	0.265	0.236	0.402
BUN, *mg/dL*	19.6	18.6	17.5	20.8	1.16	0.527	0.042	0.790

Abreviations: MN = Maternal nutrition; CS = calf sex; RES = offspring from unssuplemented cows; CON = offspring from supplemented cows from 102± 5 to 208 ± 6 days of gestation; BHBA = β-hydroxybutyrate; NEFA = Non-esterified fatty acids; BUN = Blood urea nitrogen.

3.4. Voluntary intake and total tract digestibility

No difference was observed on digestibility of the other nutrients (P > 0.05) to treatment, calf sex and their interactions. The results of voluntary intake and intake of nutrients are show on Table 5, no difference was observed to intake during 120 and 200 days post-partum. There was a trend (P = 0.061) in NDF digestibility on 200 days post-partum in function of treatment

(Table 6). Cows which received supplementation during the pregnancy trend to have higher NDF digestibility in comparison with no supplemented cows.

Table 5. Effects of prenatal nutrition and of calf sex on voluntary intake and intake of nutrients at 120 and 200 days post-partum.

Item	MN		CS		SEM	P-value		
	RES	CON	Male	Female		MN	CS	MN × CS
				120 days				
DMI, *kg/day*	6.96	7.48	8.17	6.28	1.41	0.626	0.091	0.377
DMI/kg BW	1.43	1.51	1.64	1.30	0.29	0.701	0.131	0.351
OM	6.01	6.22	6.52	5.71	0.75	0.748	0.235	0.539
CP	0.97	1.06	1.15	0.89	0.17	0.532	0.090	0.294
NDFap	3.94	4.27	4.63	3.58	0.69	0.576	0.085	0.371
NFC	1.19	1.25	1.39	1.05	0.46	0.767	0.107	0.236
				200 days				
DMI, *kg/day*	9.26	8.62	9.49	8.39	1.29	0.605	0.376	0.375
DMI/kg BW	1.88	1.69	1.92	1.65	0.30	0.511	0.361	0.268
OM	7.86	7.12	7.79	7.20	1.18	0.517	0.603	0.157
CP	1.32	1.22	1.36	1.18	0.24	0.611	0.325	0.378
NDFap	5.31	4.41	4.97	4.75	0.66	0.142	0.714	0.550
NFC	1.45	1.35	1.50	1.29	0.31	0.621	0.304	0.382

Abreviations: MN = Maternal nutrition; CS = calf sex; CON (control) = offspring from unssuplemented cows; SUP (supplemented) = offspring from supplemented cows from 102± 5 to 208 ± 6 days of gestation;

Table 6. Effects of prenatal nutrition and of calf sex on digestibility at 120 and 200 days post-partum.

Item	MN		CS		SEM	P-value		
	RES	CON	Male	Female		Treat	Sex	Treat x Sex
				120 days				
DM	587	602	595	593	27	0.496	0.943	0.666
OM	608	630	619	619	26	0.342	0.986	0.495
CP	679	689	688	681	52	0.721	0.820	0.763
NDFap	691	716	702	705	55	0.420	0.906	0.582
NFC	222	257	248	230	241	0.588	0.785	0.997
TDN	1815	2166	2131	1850	1031	0.389	0.487	0.368

			200 days					
DM	600	608	603	605	212	0.626	0.895	0.773
OM	635	618	635	617	15	0.268	0.237	0.533
CP	698	693	688	702	265	0.774	0.384	0.246
NDFap	710	740	736	714	69	0.061	0.162	0.986
NFC	199	149	111	238	229	0.572	0.141	0.166
TDN	2973	2744	2837	2883	255	0.209	0.797	0.382

Abbreviations: MN = Maternal nutrition; CS = calf sex; CON (control) = offspring from unssuplemented cows; SUP (supplemented) = offspring from supplemented cows from 102± 5 to 208 ± 6 days of gestation.

4. Discussion

Due to the seasonality in forage production in countries with a tropical climate and the period of the breeding season for beef cattle in Brazil, it is common that during the middle third of pregnancy, the period in which the calf is going through the process of muscular hyperplasia (DU et al., 2015), there is a lack of food for the cows. This nutritional deficit is detrimental to both the calf being bred and the sows that are pregnant. Meneses et al., (2022) observed that beef cows undergoing nutritional restriction during the middle third of pregnancy showed greater muscle tissue mobilization compared to cows that were receiving adequate nutritional support, and this can also be observed with the increase in blood BHBA concentration.

In the postpartum period, the nutritional deficit that the cows suffered during pregnancy was reflected in the birth weight of the progeny, Nascimento et al., (2022) observed that calves born to mothers who underwent restriction had lower birth weight and consequently this reflected throughout the life of the animals, as there was a reduction in the number of muscle fibers in calves that underwent nutritional restriction (COSTA et al., 2021).

In addition to problems with the progeny, it is known that nutritional restriction impairs milk production in cows. There are some factors that directly influence milk production when the animal undergoes nutritional restriction during pregnancy, according to Meyer et al., 2011, there are three main factors that directly influence the milk production of animals that have or have not undergone nutritional restriction during pregnancy. pregnancy, such as changes in nutrient partitioning during pregnancy, blood flow affects the development of the mammary gland and the endocrine profile during pregnancy and lactation are altered, directly affecting the utilization of nutrients by the mammary gland. The negative effect of pregnancy, especially of cows that have undergone nutritional restriction, occurs mainly at the end of pregnancy, since in this phase the nutritional requirement of the calf increases due to its growth, together with the growth of the fetus, there is the effect of circulating estrogen produced by the fetus-placenta joint (BOUTINAUD et al., 2004).

Estrogen and GH are the main hormones that inhibit prolactin, the main hormone responsible for milk production in mammals. When bromocriptine was administered, a substance that inhibits prolactin production, Shennan et al., 2000 observed a reduction in GLUT1 production in rat mammary tissue, GLUT1 is the main glucose transporter for secretory cells , the glucose absorbed by the cell goes to the Golgi complex and lactose is formed there, which will be secreted into the lumen of the mammary gland. However, the apical membrane of the secretory cell is impermeable to lactose, but permeable to water, so the volume of secreted milk is directly related to the rate of lactose synthesis. The same occurs for GH, Shennan et al., 2000 found that the use of bovine growth hormone releasing factor increased GLUT1 mRNA expression in the bovine mammary gland by 21%. Physiological results were uncertain as there was no increase in GLUT1 concentration in the mammary gland.

In our study, lower milk production was observed for animals that underwent nutritional restriction at 7 and 30 days postpartum, and this difference was not observed after 60 days postpartum, indicating that the nutritional restriction suffered by cows during the middle third of gestation directly influenced the milk production of cows right after delivery, thus indicating problems in the development of the mammary gland. Meyer et al., 2011 reported that 98% of mammary gland development in sheep occurs during pregnancy and only 2% occurs during lactation.

Another factor that influences milk production in cows is the sex of the progeny. According to the theory proposed by Trivers-Willard, 1973, the sex of the progeny during pregnancy influences the milk production of cows, based on the authors, cows pregnant with females tend to produce more milk in the postpartum period, maternal investment is greater for the sex that will provide the perpetuation of the species (BARCELOS et al., 2022). Another explanation is regarding the nutritional requirement of cows pregnant with females, according to BR-Corte system 2016, the nutritional requirement of cows pregnant with females is smaller than pregnant cows with male calves and this is explained by the growth rate of the calves males compared to females (BARCELOS et al., 2022). According to Ithurralde et al., 2019, the deleterious effects are greater in male calves, according to these authors, cows pregnant with males tend to prioritize themselves nutritionally over the calf.

In their work evaluating uterine hemodynamics, Meneses et al., 2022 observed that cows pregnant with females had a higher pulsativity index, resistance, and a higher systolic/diastolic ratio, indicating that there is a difference between blood flow and, consequently, nutritional intake is different depending on the sex of the progeny. However, little is known about the mechanism by which the cow "understands" the sex of the progeny being sired and how cows "know" the sex of the progeny to make the necessary physiological adjustments for good fetal development (BARCELOS et al., 2022). This effect occurs during the gestational phase, however, in the postpartum period, milk production, in general, is higher for male mother cows. Galvão, 2018 working with Zebu beef cows observed that mother cows of male calves produced more milk compared to mother cows of female calves, this is due to the higher nutritional requirement of males compared to females.

Another point observed in this work was that the frequency of feeding of males was higher than that of females, according to Svennersten-Sjaunja et al., 2005, the frequency of milking stimulates the milk production of cows due to the increase in the rate of cellular apoptosis. In their study with beef calves, Nascimento et al., 2022 observed that male calves spent 26% more-time suckling compared to female calves. However, in our study, a difference was observed in the milk production of cows according to the sex of the progeny throughout the entire breeding phase. However, more studies need to be carried out to verify how nutritional restriction and the sex of the progeny, during pregnancy, can influence the milk production of cows.

Nutritional restriction is one, if not the main factor that influences the performance of animals. According to the BR-Corte 2016 system, regnant animals have greater nutritional requirements than non-pregnant animals this is because the pregnant animal needs nutrients for fetal growth, development of the mammary gland and maintenance. Meneses et al., 2022, working with Zebu beef cows that underwent protein restriction during the middle third of gestation, observed that the animals that underwent restriction lost 16 kg and approximately 1 point in the BCS, in addition, a reduction in the AOL of the cows was observed. restricted.

This occurs because cows need to mobilize tissues to meet the growth requirements of calves, in this same work it was observed that the concentration of circulating amino acids was 33% higher for cows that were on restriction when compared to cows without restriction. Both the fetus and the placenta need amino acids for their development, according to Battaglia et al., 2002, the metabolic demand of the placenta is practically the same as that of the fetus, the placenta represents only 10% of the placenta-fetus set , and the rate of use and production of each amino acid is determined by the rate of uptake by the fetus, and these amino acids can be used for fetal growth itself, substrate for the synthesis of other metabolites or as a source of energy for the fetus. Due to the possible mobilization of muscle tissue, the cows that did not receive supplementation were lighter at parturition, and this was directly reflected in the weight of the cows at 7 and 30 days of lactation and this was directly reflected in the weight of the cows at 7 and 30 days of lactation, where they were 11% and 10% lighter, respectively.

5. Conclusions

Protein supplementation at the level of 3.5 g/kg of body weight during the middle third of pregnancy was favorable in terms of milk production in cows, however, this better milk production was observed only in the 7 days postpartum, and this difference was not maintained throughout the lactation period. Cows that were supplemented during pregnancy had a better body condition score and were heavier at 7 and 30 days of lactation, showing that there was tissue mobilization during pregnancy and that 30 days were necessary for these cows to recover. Intake of dry matter and nutrients and their respective digestibilities are not affected by nutritional restriction in the postpartum period.

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Institutional Review Board Statement: The study was conducted in accordance with the ethical principles of animal experimentation established by the National Council of Animal Experimentation Control (CONCEA) of the UFLA Ethics Committee on Animal Use (CEUA/UFLA – protocol 015/17).

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