JAVIER ANDRÉS MORENO MENESES

IMPACTS OF PROTEIN SUPPLEMENTATION DURING MID GESTATION OF BEEF COWS ON MATERNAL PHYSIOLOGY, AND SKELETAL MUSCLE METABOLISM

LAVRAS – MG

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Zootecnia, área de concentração em Produção e Nutrição de Ruminantes, para a obtenção do título de Doutor.

Prof. Dr. Mateus Pies Gionbelli

Orientador

Prof. Dr. Daniel Rume Casagrande

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Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Moreno Meneses, Javier Andrés.
Impacts of protein supplementation during mid gestation of beef cows on maternal physiology, and skeletal muscle metabolism
/ Javier Andrés Moreno Meneses. - 2021.
94 p. .

Orientador(a): Mateus Pies Gionbelli.
Coorientador(a): Daniel Rume Casagrande.
Tese (doutorado) - Universidade Federal de Lavras, 2021.Bibliografia.

1. Maternal Metabolism. 2. Maternal Nutrition. 3. Skeletal Muscle. I. Gionbelli, Mateus Pies. II. Título.

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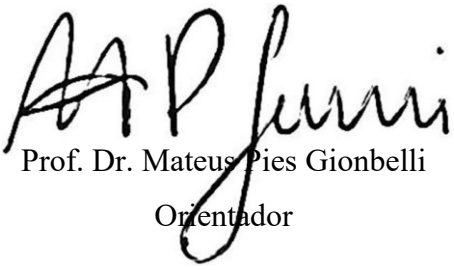
Aprovada em 29/03/2021

Prof. Dr. Marcio Machado Ladeira	UFLA
Prof. Dr. Marcio de Souza Duarte	UFV
Prof. Dr. Daniel Rume Casagrande	UFLA
Prof. Dr. Diego Zanetti	IFSULDEMINAS



Prof. Dr. Mateus Pies Gionbelli

Orientador

LAVRAS – MG

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A minha mãe Flor Maria
A minha esposa Mayra
A toda minha família!

Dedico

AGRADECIMENTOS

A PEC-PG/CAPES, pela concessão da bolsa de doutorado e à FAPEMIG, pelo financiamento do projeto.

À Universidade Federal de Lavras e Universidade de Sucre por me acolherem durante a graduação e pôs graduação.

Ao Programa de Pós-Graduação em Zootecnia da UFLA, pela oportunidade de realização do Mestrado e Doutorado.

A minha mãe Flor Meneses, pelo amor incondicional, apoio e dedicação, fazendo com que eu fosse uma melhor pessoa.

A minha esposa Mayra Alejandra Contreras, pelo apoio incondicional, amor e paciência durante todo este tempo.

Aos meus tios e tias (em especial a minha tia Marlene), que me ajudam e incentivam em todos os momentos.

Ao meu irmão Jorge Eliecer pela amizade, companheirismo e conselhos.

À Universidade Federal de Lavras e ao Programa de Pós-Graduação em Zootecnia por minha formação.

Ao meu orientador, Dr. Mateus Pies Gionbelli, pela oportunidade, confiança e ensinamentos transmitidos. Sempre será um exemplo a seguir como pesquisador e pessoa.

Ao meu coorientador, Dr. Daniel Casagrande, pela confiança, incentivo e apoio em todos os momentos da realização deste trabalho.

Aos membros da branca examinadora, Marcio Machado Ladeira, Marcio de Souza Duarte e Diego Zanneti os quais contribuíram enormemente com a construção deste trabalho.

Aos alunos de iniciação científica orientados do professor Mateus: Pedro, Miguel, Guilherme D, Maria, Helena, Laura, Rafaella, Ariane, Lorena, Matheus Luz, Yanca, os quais foram importantes na coleta de dados e nas atividades laboratoriais.

Aos funcionários do DZO que foram fundamentais para a condução deste trabalho, em especial o Borginho e toda sua equipe.

A todos os funcionários do laboratório de nutrição animal da Universidade Federal de Lavras, em especial aos técnicos Márcio e Flávio os quais me auxiliaram nas análises

laboratoriais. Tambem às pós-doutorandas Tathy, Vânia e Stefânia, pelo suporte com as análises e companheirismo.

O Matheus e Karolina pela amizade, pelo companheirismo durante todo este tempo, pelos conselhos e auxílio na condução do experimento.

Ao NEPEC pelas amizades, especialmente ao Gleidson, Fernando, Gabriel, Luana, Matheus G, Lorena L y Karolina B pela parceria e amizade ao longo da minha estadia em Lavras e aos demais integrantes do grupo muito obrigado pelos ensinamentos e experiências aprendidas nestes seis anos de aprendizado.

Todos vocês contribuíram com minha formação pessoal e acadêmica, sou grato a Deus por tê-los colocado no meu caminho.

RESUMO

Ainda há lacunas no conhecimento que precisam ser preenchidas sobre as formas como as matrizes adaptam-se fisiologicamente para evitar perdas durante a gravidez e como essas adaptações podem comprometer sua eficiência reprodutiva. Atualmente existem cerca de 56 milhões de vacas de corte no Brasil as quais podem ser beneficiadas com os avanços do conhecimento nessa área do conhecimento. Portanto, o objetivo desta pesquisa foi determinar o efeito da suplementação proteica (SP) durante o meio da gestação em vacas alimentadas com forragem de baixa qualidade sobre o desempenho, ingestão de alimentos, fisiologia, e metabolismo proteico no tecido muscular esquelético materno. Cinquenta e duas vacas, multíparas com 490 kg ± 17,8 de peso corporal (PC), 5,63 ± 0,52 de escore de condição corporal (ECC), da raça Tabapuã e portadores de bezerros machos e fêmeas foram distribuídos aleatoriamente em um dos dois tratamentos: Suplementação com 40% de proteína bruta (PB) ou 3,5g / kg de PC (SUP; n = 26) ou não suplementado 0% PB (CON; n = 26). Entre 100 a 200 dias de gestação, as vacas foram alojadas em baias individuais e submetidas a diferentes níveis de alimentação: sendo o CON conformado por uma dieta basal (silagem de milho + bagaço de cana, atingindo 5,5% PB mais mistura mineral) e o SUP conformado pela dieta basal + suplementação proteica. O PC e o ECC foram determinados a cada 30 dias até o pré-parto. O consumo de ração foi monitorado individualmente até o pré-parto, amostras de sangue, biopsias de tecido muscular esquelético e índice de pulsatilidade (IP) e resistência (IR) foram coletadas aos 200 e 270 dias de gestação. Os resultados serão publicados em dois artigos. No primeiro artigo a SP aumentou as reservas corporais representadas em maior PC materno, tecidos gestacionais, área de olho de lombo e garupa ($P \leq 0,01$). Vacas CON perderam quase um ponto de ECC, enquanto SUP ganharam 0,59 pontos de ECC durante 100 dias. Porém, no periparto as vacas SUP mobilizaram maior quantidade de tecido materno ($P \leq 0,01$). A suplementação aumentou a ingestão de forragem, de matéria seca total a digestibilidade total aparente dos nutrientes e a eficiência de síntese microbiana ($P \leq 0,01$). Vacas CON apresentaram maior IR e IP ($P \leq 0,01$) na metade da gestação. No segundo artigo vacas SUP tiveram aumentos concentrações plasmáticas de glicose, insulina e IGF-1, no entanto, os ácidos graxos não esterificados foram maiores ($P \leq 0,01$) nas CON. Vacas SUP apresentaram aumentos de aproximadamente 50% na concentração de amino ácidos circulantes totais ($P = 0,03$), e aumentos entre 30 e 40% na expressão de mRNA de marcadores relacionados à síntese e degradação proteica durante a gestação. Os resultados sugerem que a SP a 3,5 g/kg do PC no meio da gestação pode ser útil para melhorar o estado energético das vacas, além de permitir um maior fluxo de nutrientes para o útero, permitindo um adequado crescimento e desenvolvimento fetal o que ajudará a preservar as reservas de tecido materno quando mantidas em pastagem de baixa qualidade.

Palavras chave: Aminoácidos, Consumo De Matéria Seca, Doppler, Expressão Gênica, Metabolismo Materno, Nutrição Materna, Músculo Esquelético.

ABSTRACT

There are still gaps in the knowledge that need to be filled about how the dams adapt physiologically to avoid losses during pregnancy and how these adaptations can compromise their reproductive efficiency. There are currently about 56 million beef cows in Brazil that can benefit from advances in knowledge in this area of knowledge. Therefore, this research aimed to determine the effect of protein supplementation (SP) during mid-gestation in cows fed low-quality forage on performance, food intake, physiology, and protein metabolism in maternal skeletal muscle tissue. Fifty-two multiparous cows with 490 kg ± 17.8 body weight (BW), 5.63 ± 0.52 body condition score (ECC), Tabapuã breed, and male and female calf carriers were randomly distributed in one of the two treatments: Supplementation with 40% crude protein (CP) or 3.5g / kg BW (SUP; n = 26) or not supplemented 0% CP (CON; n = 26). Between 100 to 200 days of gestation, the cows were housed in individual pens and subjected to different levels of feeding: the CON being conformed to a basal diet (corn silage + sugarcane bagasse, reaching 5.5% CP plus mineral mixture) and the SUP conformed by the basal diet + protein supplementation. CP and ECC were determined every 30 days until pre-delivery. Feed intake was monitored individually until pre-delivery, blood samples, skeletal muscle tissue biopsies, and pulsatility index (PI) and resistance (IR) were collected at 200 and 270 days of gestation. The results will be published in two articles. In the first article, SP increased the body reserves represented in higher maternal CP, gestational tissues, rib eye area, and croup ($P \leq 0.01$). CON cows lost almost one point of ECC, while SUP gained 0.59 points of ECC for 100 days. However, in the peripartum SUP cows mobilized a greater amount of maternal tissue ($P \leq 0.01$). Supplementation increased forage intake, total dry matter, and apparent total digestibility of nutrients, and efficiency of microbial synthesis ($P \leq 0.01$). CON cows had higher RI and PI ($P \leq 0.01$) in mid-gestation. In the second article, SUP cows had increased plasma concentrations of glucose, insulin, and IGF-1, however, non-esterified fatty acids were higher ($P \leq 0.01$) in CON. SUP cows showed approximately 50% increases in the total circulating amino acid concentration ($P = 0.03$), and between 30 and 40% increases in mRNA expression of markers related to protein synthesis and degradation during pregnancy. The results suggest that SP at 3.5 g / kg of BW in the mid-gestation can be useful to improve the energy status of cows, in addition to allowing a greater flow of nutrients to the uterus, allowing for adequate fetal growth and development, which will help preserve maternal tissue reserves when maintained on low quality pasture.

Keywords: Amino Acids, Dry Matter Intake, Doppler, Gene Expression, Maternal Metabolism, Maternal Nutrition, Skeletal Muscle.

Efeito da suplementação proteica durante metade da gestação na fisiologia e metabolismo do músculo esquelético materno de vacas de corte.

Elaborado por **Javier Andrés Moreno Meneses** e orientado por **Mateus Pies Gionbelli**

Geralmente após o diagnóstico de gestação, o pecuarista brasileiro coloca as vacas gestantes em pastos menos cuidados e produtivos da propriedade, provocando que em função da preconização das estações de monta e parição, muitas delas passem restrição nutricional durante o terço médio e parte do terço final da gestação. Tais circunstâncias fazem que vacas de corte sofram adaptações para não produzir bezerros com potencial genotípico inferior para produção. Logo, essa pesquisa foi realizada com objetivo de avaliar os efeitos da suplementação proteica (SP) durante metade da gestação sobre o desempenho, fisiologia e metabolismo de vacas de corte. Foi observado que vacas suplementadas em nível de 0,35% do PV aos 200 dias de gestação apresentaram melhoras no peso corporal (+ 2@), tecidos gestacionais e escore de condição corporal (ECC), apesar de que no peri-parto mobilizaram maior quantidade de tecido corporal materno em função do maior crescimento alcançado pelo feto no terço médio. Contudo, os resultados deste estudo nos permitiram mostrar que houve efeito residual positivo de ganho e ECC em animais suplementados, o que resultou em vacas mais pesadas (+14,9 kg) durante o ciclo produtivo. A SP também aumentou a ingestão de forragem, a digestão total aparente dos nutrientes e a eficiência da síntese microbiana, representado em maiores quantidades de aminoácidos circulantes no plasma e abundância de genes relacionados com síntese proteica no musculo esquelético. Os resultados sugerem que a SP no meio da gestação é útil como corretivo nutricional para melhorar o estado energético das vacas e o crescimento e desenvolvimento fetal quando mantidas em pastagem de baixa qualidade.

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FIRST SECTION

1 INTRODUCTION

Nutritional corrections that seek to optimize livestock production, aiming at efficiency and low input costs are necessary for profitable and sustainable Brazilian production systems. Feeding costs (creep feeding, grazing, or harvested forage) account for approximately 75% of the total operating costs of a cow/calf operation (OAIGEN et al., 2008), indicating that profitability depends largely on the management of these inputs. Besides, there is a relationship between nutrition and reproduction in beef cattle, which has been the subject of study for decades, according to Junqueira *et al*. (2006), it is estimated that reproduction failures represent R$ 77.97 per animal/year, which represents millionaire losses in the meat industry, due to low production rates, delayed reproduction, treatment costs, the low calf birth rate per year (50%). Literature reports report that pre-partum malnutrition results not only in low pregnancy rates (DISKIN and KENNY, 2016; EMERICK et al., 2010; HOUGHTON et al., 1999) and increased interval from birth to first estrus (DEROUEN et al., 1994; DISKIN and KENNY, 2014; Richards et al., 1986) but also in reducing calf birth weight (CATON et al., 2019; FUNSTON et al., 2010), decreased calf survival (BURNS et al., 2010; HOUGHTON et al., 1990) and decreased weaning BW (MARQUES et al., 2016; THONE REINEKE et al., 2006).

Recently, interest studies aimed at assessing the effect of maternal nutrition on performance and long-term physiological and metabolic changes in offspring have increased. It was the English researcher D. J. Barker (1993) who, through epidemiological studies in humans, gave rise to the concept of Metabolic fetal programming, defined as a process through which a stimulus or insult establishes a permanent response in the progeny. Subsequent studies in farm animals have focused on demonstrating how environmental variations and nutritional corrections or restriction affect the dams, the mother's ability to cushion the negative effects on the fetus, and how it affects the long term productivity and profitability in production systems (GREENWOOD et al., 2017; GREENWOOD et al., 2007; UNDERWOOD et al., 2010). This research aims to understand the mechanisms involved in the response of dams under nutritional correction or not in mid-gestation.

This study aimed to understand

a) How beef cows deal with protein restriction during mid-gestation to support fetal growth.

b) Identify possible changes in the maternal feed intake and feed efficiency maternal, weight and body condition score, total apparent digestibility, the plasma level of circulating amino acids, blood flow of the reproductive tract during specific windows during gestation in association with developmental programming,

c) Identify possible changes in abundance of mRNA markers for protein synthesis and degradation in the skeletal muscle and the abundance of mRNA markers for gluconeogenesis in the liver of beef cows during the mid and late gestation as a function of the nutritional treatment during the mid-gestation.

The knowledge of this information will be extremely important in Brazilian beef cattle since the result will allow evaluating the application of a nutritional management strategy that can improve the status of the cow, which will directly impact the efficiency and profitability of the herd.

2. BACKGROUND

Within the systems of production and composition of the herd in cattle, pregnant cows as an animal category still do not have sufficient information on nutritional requirements, metabolism, and physiology when compared with animals in other categories as cow/calf operation, backgrounders, or feedlot (GIONBELLI et al., 2016). Although this information is scarce, it is known that cows within the property represent a significant energy expenditure that depending on the physiological state represents between 50 to 70% of the total energy within the productive system (FERRELL JENKINS, 1985; RITCHIE, 1995). Currently, Brazil has a total herd of more than 210 million heads (INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATISTICA, 2015), and numerically, about 1/3 of the national herd is made up of matrices, which are mostly Zebu. Another reality related to breeding production systems in Brazil is that herds that are kept on pasture are subject to different seasonality in the supply and quality of forage (dry season and rainy season), as they are exposed to these changes, characterized for the scarcity and abundance of forage during the year (DETMANN et al., 2014).

Episode 1.

2.1 Control of the nutrient partition of pregnant cows.

Lavoisier compared the partitioning of nutrients with the concept of "life is a chemical process" (MAYNARD et al., 1979), in which that sustenance involves a series of chemical reactions and physiological processes in which food is transformed into body tissues and activities. In a broad sense, the chemistry of life can be considered a cycle. Food is consumed, and, following digestion in the gut, nutrients are absorbed. These nutrients are utilized by body tissues, as shown in figure 1.

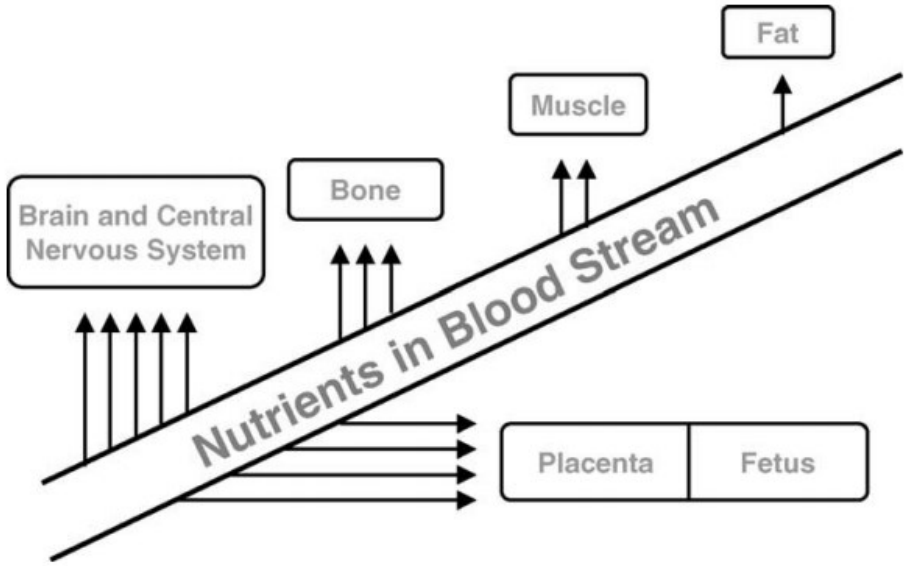

Figure 1. Partitioning of nutrients according to metabolic rates in adults. Arrows indicate relative amounts of nutrients distributed to various tissues. Note the relatively high nutrient flow to the placenta/fetus (gravid uterus), second only to the brain and central nervous system. In the adolescent, there is a higher priority to fat deposition in place of the partitioning of nutrients to the gravid uterus, as compared to the adult. Adapted from Hammond (1994).

In mammals, nutrients are utilized by tissues involved in maintenance and growth and for establishing body reserves including energy stores (lipids), glucose reserves (glycogen), and amino acid reserves (labile protein). Two additional tissues utilizing a substantial portion of maternal nutrients are the developing fetus and the lactating mammary gland (BELL and BAUMAN, 1997). One should not underestimate the importance of partitioning nutrients to support pregnancy and lactation, because these physiological states are the essence of survival of the species and, of course, the foundation of the beef industry.

The partitioning of nutrients to various body tissues involves two types of regulation, homeostasis, and homeorhesis. Homeostatic control involves the maintenance of physiological equilibrium or constant conditions in the internal environment. Homeorhesis is the second type of control in partitioning nutrients, homeorhesis is the orchestrate or coordinated changes in the metabolism of body tissues necessary to support a physiological state (BAUMAN and CURRIE, 1980).

During pregnancy, nutrients are generally partitioned to various tissues of the maternal body in priority order according to their metabolic rate (FOWDEN, 1995; THRIFT et al., 1999). Thus, as nutrients become limiting, tissues with lower metabolic rates and hence reduced priority will receive quantitatively fewer nutrients. Since highly metabolically active tissues receive greater rates of blood flow than less metabolically active tissues, the rate of blood flow becomes the limiting factor for nutrient partitioning to specific tissues. The gravid uterus receives a very large proportion of total cardiac output resulting in a high priority status for the partitioning of nutrients. According to Bell *et al*. (2005), it is expected that during the final third of gestation, 35 to 40% of the fetal energy is supplied as glucose and lactate and about 55% is supplied as amino acids. Most of the remaining 5 to 10% is supplied by acetate, which is very little, about its relative abundance and importance for the maternal system, although they are estimates made with small ruminants, it is expected that the availability of circulating amino acids will increase by the mobilization of protein from maternal carcass tissues (mainly skeletal muscle) by about 10% (BELL et al., 1998), with the residual amount being used as metabolic support, mammary glands and maternal viscera (BELL and EHRHARDT, 2000). However, the hierarchy of nutrient partitioning in young pregnancy shifts from what is seen in the adult to a higher priority.

Age can play an important role in the response of the animal to nutrient deficiency. In general, nutrients are partitioned to maternal tissues according to their metabolic rate, with a greater priority toward maternal tissue growth and fat deposition in adolescent pregnancy compared to adults where the gravid uterus is second only to the brain and central nervous system (REDMER et al., 2004). Heifers bred to calve at two years of age have increased nutrient requirements for growth in addition to those for fetal development, which may lead to a nutrient deficiency when grazing low-quality pastures (CICCIOLI et al., 2003). Increased susceptibility to nutrient deficiencies can result in delayed puberty and extended postpartum periods in young cows (HAWKINS et al., 2000). According to Bell and Bauman (1997), pregnancy induces adaptive responses in maternal hepatic synthesis and peripheral tissue utilization of glucose which are exaggerated by moderate undernutrition. In sheep, much of

the pregnancy-induced increase in maternal gluconeogenesis can be attributed the glucose production is presumably supported by increased peripheral mobilization and hepatic uptake of endogenous substrates such as amino acids from muscle protein breakdown, hepatic lactate uptake derived from metabolism, and increased maternal uterus-placental glucose in peripheral tissues and glycerol from the mobilization of adipose tissue triglycerides (WILSON et al., 1983; IRELAND et al., 2008). Are indications that pregnancy decreases glucose utilization by maternal tissues, progesterone and estrogens could be involved in this diabetogenic effect (PETTERSON et al., 1993).

According to Vonnahme *et al*. (2015), during pregnancy, the maternal physiologic state is associated with significant but reversible modulations to meet metabolic demand as well as alterations to the endocrine and cardiovascular systems. Endocrine secretions from the conceptus allow for communication between the maternal endometrium and fetal membranes of the placenta during mid-to-late-gestation, helping to ensure proper nutrient and waste exchange during the exponential growth of the fetus. Bell *et al.* (2005), showed an increasing concentration in plasma growth hormone and an abrupt decline in IGF-I and insulin, starting two weeks before calving in cows. In contrast to the matrices that presented low body condition score (BSC), those that gave birth to medium and high BSC, the expression of genes involved in the growth hormone / IGF-I signaling axis (GHRIA and IGF-I) was (AKBAR et al., 2015), indicating a probable lower degradation of the muscle signaling pathway, high milk production and greater gluconeogenesis in these animals. Moreover, pregnancy alters the vascularity of the small intestine (SCHEAFFER et al., 2004), allowing greater uptake of nutrients. Maternal cardiovascular capacity also changes dramatically during pregnancy, with decreases in systemic arterial blood pressure and vascular resistance and increases in cardiac output, heart rate, heart stroke volume, and blood volume. The maternal cardiac output has been shown to increase as much as 30%–40% in pregnant *vs.* non-pregnant ruminants (MAGNESS, 1998).

The fetus had acquired approximately 40% of its birth weight during the first 7 mo of gestation (Figure 2). During the last 2 mo of gestation, fetal demands for specific nutrients (glucose and amino acids) are equal to mammary use of nutrients equivalent to about 3 to 6 kg milk/day. Various studies on differential persistency of pregnant versus open cows have indicated differences in the daily production of this order at about 300 days of lactation (OLTENACU et al., 1979). Competition by the fetus for nutrients that otherwise would be used for lactation or maternal replenishment of protein and energy reserves would be increasingly important if drying off were delayed to less than 60 days before expected

calving. The rate of hepatic protein synthesis increased by 45% at the end of gestation in dairy cows, even when intake of dry matter and crude protein was declining (BELL and EHRHARDT, 2000).

This information is consistent with an increase in deposition of liver protein observed by McNeill *et al*. (1997) and a decrease in hepatic deamination of amino acids at the end of ewes' gestation (FREETLY and FERRELL, 1998). Significant nitrogen loss in sheep carcass tissues in the final third of gestation was observed (MCNEILL et al., 1997), possibly due to the mobilization of amino acids from skeletal muscle. However, it has been observed that there are no studies that jointly evaluate nutrient absorption and metabolism of the liver, adipose, and skeletal tissues of cows at the end of pregnancy.

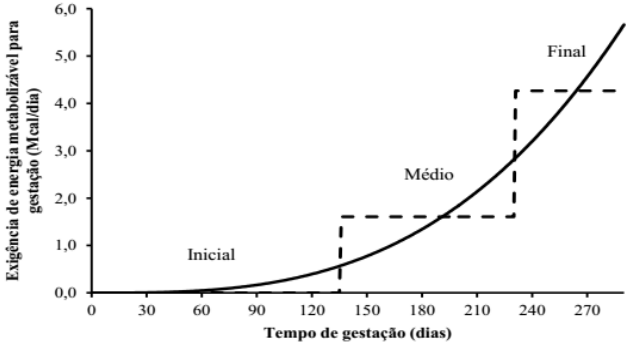

Figure 2. Metabolizable energy requirements for the gestation of an adult Zebu cow (500 kg of PC gestating a calf with an estimated birth weight of 32 kg) divided into three gestation periods (initial, mid, and late) From Gionbelli et al., (2016).

On the other hand, according to Huntington (1990), the maternal gastrointestinal tract is critical for nutrient acquisition but is also a major nutrient sink during pregnancy. In ruminants, the liver and gut consume approximately 40% of maintenance energy demands (FERRELL, 1991). Within the gastrointestinal tract, which consumes approximately 20% of maintenance energy (EISEMANN et al., 1990; WEBSTER, 1989), a majority of these nutrient resources are consumed by ion transport, protein turnover, enzyme secretion, and active transport (CATON et al., 2000; GREGG et al., 1982). The dam needs to maintain a functional capacity of the gastrointestinal tract, even though a decrease in nutrient use by this tissue may be necessary, especially in cases of gestational nutrient restriction.

In the small intestine, most measures have focused on the proximal jejunum, where both digestion and absorption occur (VONNAHME et al., 2015). As with the placenta, these measures of vascularity have been responsive to pregnancy, stage of gestation, and nutritional status during pregnancy. Capillary area density and total vascularity of the jejunum increased

in pregnant multiparous ewes, and total vascularity increased from mid-to-late gestation (SCHEAFFER et al., 2004). According to Caton *et al.* (2009), jejunal capillary surface density and size increased from mid-to-late gestation, while capillary number density decreased from early to late gestation in primiparous ewes, this possibly due to the maternal transition from the anabolic to the catabolic state of pregnancy. Additionally, the capillary area density of the jejunum also increased from mid-to-late pregnancy in beef cattle (MEYER et al., 2010). These studies suggest that vascularity of the small intestine may play more of a role as the nutrient demand of fetal growth increases during late gestation. Overall, small intestinal size and function, including vascularity, are an active component of maternal physiological adaptations during pregnancy. These adaptations occur in response to increased metabolic demands of advancing gestation, altered nutritional plane, and inappropriate levels of specific nutrients.

Episode 2

2.2 Nutrition in the gestating cow.

Pastures are the basis for more practical and economical feeding for beef cattle in Brazil, however, livestock kept on pastures are subject to a different seasonality in the supply and quality of forage, as they are exposed to these changes, characterized by shortage and abundance of forage during the year (DETMANN et al., 2014). These periods of nutrient restriction due to the lack of food availability have been very common in the meat industry. For this reason, we need to know the responses of the animals during the two seasons of the year, to promote good nutritional and reproductive planning of the herd, especially the pregnant dams. In Brazil, the most primitive breeding season used is that in which the bull remains with the herd throughout the year. As a consequence, births are distributed throughout the year, resulting in the occurrence of births at inappropriate times, damaging the development of the calves and the cows; for that, in extensive breeding systems of beef cattle, it is very common the fertility of the herd presents variations due to climatic conditions. The establishment of a limited breeding season is an important decision and of great impact on fertility (MEDEIROS et al., 2015). The breeding season coincides with the season of better quality and greater availability of forage, which provides adequate conditions for the re-establishment of the reproductive activity of females, resulting in higher pregnancy rates. Therefore, fetal programming has important implications for livestock production. It can be used to explain delayed postnatal growth, reduced feeding efficiency, decreased muscle

quality, as well as poor reproductive performance (WU et al., 2006). All of these factors affect livestock profitability.

There is an important association between nutrition and reproduction (KEISLER and LUCY, 1996), and this association may have direct effects under pregnant cows, thus causing variations in the hormonal profile and the ovarian reserve in the progeny. In the dry season, the water deficit associated with the fall in temperature and luminosity leads to a severe limitation to forage growth, at the same time as there is a drastic fall in the nutritive value of the forages, (LAZZARINI et al., 2009). Low-quality forage contains less than 7 % of crude protein and less than 45 % of TDN. Low-quality forages are high in fiber (> 70 percent), but the fiber of low-quality forages are digested/fermented relatively slowly, and only 40 to 50 % of this fiber can be digested (LAZZARINI et al., 2009). That is why the intake and energy content of these forages is so low; beef cows are typically unable to consume adequate energy from forage to meet requirements for maintenance, gestation, or milk production (NATIONAL RESEARCH COUNCIL, 2000). This makes it incompatible with the increase of the nutritional requirements of beef cattle, as the energy and crude protein requirements in the late gestation increase by approximately 20% and 14%, respectively (BELL et al., 2000) compared to the mid-gestation, resulting in a mismatch between the cow's needs and the nutrients available in the forage (ADAMS et al., 1996). This scenario is presented in most productive systems that do not apply nutritional corrections, allowing the inability to meet nutrient needs, which is extremely important for fetal growth and development, in addition to ensuring that the mother has adequate BCS to deliver successfully, produce milk and enter in estrus again within the first 80 days post-partum (NATIONAL RESEARCH COUNCIL, 2000).

Nutrient partitioning occurs according to bodily functions, including maintenance, growth, pregnancy, and lactation, and reserves can be allocated depending on demands as noted in the previous chapter (SHORT and ADAMS, 1988). The relative order of priority for the nutrient partition is: 1) maintenance; 2) activity; 3) growth; 4) energy reserves; 5) pregnancy; 6) lactation; 7) additional energy reserves; 8) estrous cycles and early pregnancy; and 9) excess reserves; although priorities may change depending on the physiological stage (Short et al., 1990).

We could say that there is a greater effect of the time under the reproductive characteristics of the animals that are born in the Brazilian summer (rainy season). This is since females born in the summer spend around 2/3 of gestation in the most critical season of the year, and can lose considerable weight and body condition because there are low

availability and quality of forage. Increased susceptibility to nutritional deficiencies can vary, causing low birth weights due to the delayed growth of the placenta (REDMER et al., 2004), it can accelerate the maturation of the fetal hypothalamic-pituitary-adrenal axis and cause premature birth, produce weak births, greater incidence of dystocic births, increase in perinatal deaths and prolonged postpartum periods in heifers (HAWKINS et al., 2000). Mossa *et al*. (2013); and Evans *et al*. (2012) reported that heifers nursing their first calves needed around 30 days to return to estrus than older cows, which could harm reproductive performance the following year.

Sullivan *et al.* (2009), working with Bos indicus cattle, fed heifers with a low protein diet during the first or second trimester of pregnancy, and their female offspring were monitored until 23 months of age, the largest prepubertal follicle at 5 months of age tended to be smaller in the female offspring of the group that received a low protein diet in the first trimester, conditions that could be related to the nutritional characteristics of dry season forages in Brazil. Although the impacts of nutrient deficiency may be more pronounced in young cows, there is a strong relationship between adequate nutrition and reproductive performance for all cows in the herd, regardless of age (SHORT et al., 1990; RANDEL, 1990). Dunn and Kaltenbach (1980) developed several regression equations that described the relationship between energy status, change in body weight, and reproductive performance. They found that if no weight loss occurred during pregnancy, 91% of multiparous cows and 64% of primiparous cows would return to estrus 60 days after calving. Since cows must conceive within 80 days of calving to maintain a 365-day calving interval (DUNN and KALTENBACH, 1980), reducing the number of days of postpartum anestrus is a critical factor in determining reproductive efficiency and profitability.

In a herd of cows, producing a calf each year is the mother's primary function. Anything below ideal reproductive performance can result in economic loss for the producer. Also, it is well known that cows go through different productive and reproductive cycles throughout the year, causing changes in their nutritional needs represented in weight gain or loss, as well as changes in body condition. Although the assessment of body condition (ratio of fat mass and muscle coverage) is considered a subjective measure, it is still considered a useful tool for estimating the energy reserves and nutritional status of cows (HERD and SPROTT, 1986; KUNKLE et al., 1994; VIZCARRA and WETTEMANN, 1990). It is common for the dams Body Weight (BW) to fluctuate throughout the year, depending on the availability of nutrients and the management strategy; however, there are critical time points in the production cycle that can affect the response to a loss of condition. Body condition

Scoring (BCS) at calving is one of the most important indicators that affect the postpartum interval until estrus and the pregnancy rate in cows (Richards et al., 1986; Selk et al., 1988). Wagner et al. (1988) established the use of body condition scores (BCS), where 1 = emaciated and 9 = obese. On the other hand, several authors (KINCHELOE, 2016; MORRISON et al., 1999; LAKE et al., 2005) demonstrate the ability to successfully manage a herd with a wide range of BCS during mid-pregnancy to give birth to a BCS 5 to 6. Spitzer *et al.* (1995) reported that an increase in BCS of cows calving at 4, 5, and 6 BCS resulted in an increase in birth weight without association with increased dystocia. In a review of the factors that affect reproduction in beef cattle, Dziuk and Bellows (1983) suggested that increasing BCS through better quality grass and supplements is more economical and efficient during pregnancy than after calving, in other words, the initial and mid-gestation are the best times to improve the energy status of cows. Research indicates that BCS in childbirth is a useful indicator of postpartum reproductive performance factors, including reduced postpartum interval (HOUGHTON et al., 1990; RICHARDS et al., 1986; YUSUF et al., 2010) and subsequent pregnancy rate (KAIM et al., 1983; LYIMO et al., 2004). It has been suggested that a BCS of 5 or more at birth is the critical level to ensure acceptable postpartum reproduction (CICCIOLI et al., 2003; CROWE, 2008; DISKIN et al., 2016). We must not forget that mature cows respond to nutrient restriction more favorably than an immature heifer. Heifers that are nutritionally restricted during late pregnancy are more likely to have reduced birth weight than adult cows; according to Wallace *et al.* (2005) older animals can properly distribute nutrients to the fetus, while younger animals do not seem to do.

Episode 3

2.3 Supplementation during pregnancy as a strategy to improve the performance of the dam and offspring.

Gestation is considered a stage characterized by the development and accelerated growth of the fetus, in addition to maternal physiological changes from the moment of conception until birth (BATTAGLIA and MESCHIA., 1988). Generally, After the pregnancy diagnosis, the Brazilian producers place the pregnant cows in less careful and productive pastures on the property, causing that, due to the recommendation of the breeding and calving seasons, many of them go through nutritional restriction during the mid and part of the final gestation, despite lower nutrient requirements due to minimal fetal growth, the mid-gestation is a critical period of development, as tissues of interests in the meat industry (skeletal muscle

and adipose tissue) are in development, however in situations of nutritional restriction the fetus it maintains the growth of organs such as the brain and heart, reducing the growth of less obliged organs and tissues in response to a negative stimulus (HALES and BARKER, 1992).

Supplementation at this stage has been considered crucial to guarantee an optimal outcome in fetal development. It should be noted that fetal growth requires an increase in the net amount of proteins, synthesized entirely from the umbilical supply of Amino acids (AAs). In grazing animals, nutritional corrections are commonly used to meet the greatest nutrient needs of cattle during biologically important times of the year, such as pre-partum, lactation, and reproduction. Protein and energy supplements can result in positive responses to reproduction through greater intake of organic matter, leading to improvements in BCS and energy status and consequently an increase in a reproductive index (DELCURTO et al., 2000). According to Du *et al.* (2010) supplementary during pregnancy is an adequate nutritional strategy to enhance the pool of multipotent cells that will give rise to skeletal muscle, in addition to improving the transfer of nutrients to the fetus, thus promoting adipogenesis in the fetal muscle. On the other hand, according to Bowman and Sowell (1997), some factors contribute to the intake of supplements and their response in the animal we can mention the type and formation of the supplement, the method of administration, the frequency of supplementation, the age of the cow and the nutritional status. The following are the results of different supplementation strategies in pregnant cows.

2.3.1 Protein supplementation

Protein supplementation has become a common strategy for animals grazing in times of low quality and/or amount of forage. Despite the performance responses of cows to supplements with varying concentrations of crude protein, amounts, and frequency of feeding have been well documented, however, the impacts on calves' weight and performance at birth are somewhat variable. Protein supplementation can increase forage intake, which will improve the intake of food and consequently the synthesis of microbial protein in cows, greater flow of microbial protein to the intestine may play important roles related to the fundamental role of amino acids (AAs), according to Manta-Vogli *et al.* (2020), AAs can be used as a source of energy fuels, generators of C1 carbon compounds, and intermediates for gluconeogenesis.

Lopes *et al.* (2020) conducted an experiment that evaluated the effects of protein supplementation during late pregnancy on the maternal metabolism of Nellore cows kept on low-quality pastures. In the experiment, the cows received a protein supplement for 90 days

(2 gr/kg BW with 43.5% CP) before calving. During the supplementation period, supplemented cows were able to maintain greater ADG than cows that did not receive supplementation, in addition to having less mobilization of maternal tissue (P <0.01) when compared to the control treatment, which reflects the possible effect of the supplement. On animal metabolism, supplemented animals can increase body weight, which can guarantee greater accumulation of body reserves at birth. In addition to improvements in animal performance, cows not supplemented by presenting greater mobilization of muscle tissue had higher circulating concentrations of AA and higher expressions of genetic markers for muscle synthesis.

On the other hands, Marquez *et al.* (2017), worked with Nellore cows supplemented between 1 and 1.5 kg/day, during the middle of gestation indicated that supplementation increased the involvement of mesenchymal stem cells, favoring myogenesis, allowing calves born from supplemented mothers to have a greater formation of secondary muscle fibers. According to Oksbjerg *et al.* (2004) and Micke *et al*. (2011), the greatest proliferative and differentiation activity of myoblasts is associated with IGF-1. Protein supplementation promotes an increase in IGF1R mRNA and, consequently, an increase in IGF2 and IGF2R, which possibly affects fetal and therefore postnatal muscle development. Calves also had higher expression of PPAR-alpha (P = 0.073) and fibroblast growth factor 2 (P = 0.003), which suggests that supplementation stimulated the proliferation of satellite cells, which may contribute to an increase in deposition of skeletal muscle in the postnatal phase.

In another study, Rodrigues *et al.* (2020), used pregnant multiparous cows, receiving or not supplementation (0.2% BW) during the third half of gestation (dry season). Regarding the performance of the mothers, the authors found that supplemented cows gained more weight (79.2 kg vs. 95.3 kg, P = 0.03) and body condition (0.01 vs. 0.36 units, P = 0.05) during pregnancy that cows supplemented tended to have a higher pregnancy rate (62.1% vs 78.6% P = 0.09). After births, the calves' muscle from supplemented mothers showed higher expression of WNT10B (P = 0.01), PPARG (P = 0.03), CD36 (P = 0.04) and TGFβ1 (P = 0.01) and lower expression of C / EBPA (P = 0.01) and FABP4 (P = 0.07 when compared with calves of NS cows. The results indicated that protein supplementation during the middle of gestation besides favoring the performance of the dams favored the myogenic and adipogenic activity of the progeny.

Miguel-Pacheco *et al*. (2016) working with Hereford heifers in two treatments, high (15.7% CP) and low (5.9% CP) of protein during pregnancy, found that high protein intake in the second trimester increased birth weight in females (P = 0.05). At 6 months of age, heifers'

calves fed high nutrition in the second trimester weighed 33 kg heavier than those fed a low diet. The authors pointed out that delayed placental growth may have caused a decrease in the number of muscle fibers during the proliferation of myoblasts as reported in other studies (LONG et al., 2009; PERRY et al., 2002; SULLIVAN et al., 2009) Similar progeny results were found when protein supplements were used during pregnancy (CAFÉ et al., 2006; GREENWOOD et al., 2004; MARTIN et al., 2007; LARSON et al., 2009; STALKER et al., 2007 and UNDERWOOD et al., 2010). The results indicated higher weights at birth, at weaning, higher yields in the carcass, and greater deposition of intramuscular fat. On the other hand, Vanzant and Cochran (1994) provided alfalfa as a supplementary feed at 0.48%, 0.72%, or 0.96% of BW for pregnant cows grazing in high grass prairie for approximately 90 days before calving. Although all cows lost BCS during the supplementation period, cows that received the highest level of alfalfa lost the least amount of BW. The overall conception rate was not affected by the treatment; however, the postpartum 6 intervals were reduced and the live weight of weaning calves increased in mothers who received higher levels of alfalfa.

Stalker *et al.* (2007) found that protein supplementation in the late gestation of grazing cows resulted in improvements in BCS before calving and increased the percentage of live calves at weaning. Although no difference in birth weight was found among calves, calves born from supplemented mothers had 14 kg more weight at weaning compared to calves from non-supplemented mothers. However, supplementation did not affect subsequent pregnancy rates and offspring performance during feedlots. On the other hand, Fike *et al*. (1995) reported no differences in calf birth weight or weaning in cows without supplement or supplemented with 2 kg / d of low protein supplement (12% CP), moderate protein (20.1% CP), and high protein (31, 7% CP). Weight gain and BCS were higher in cows that received the high protein supplement, although reproductive rates were similar between treatments. A similar study conducted by Weder *et al.* (1999) also resulted in greater mother body weight gains and higher calf birth weight in cows fed high (18.8% CP) or low (15.2% CP) alfalfa compared to non-alfalfa cows. Supplemented, but again, there were no effects on reproductive characteristics. The source of nutrients and the place of digestion are a factor that influences the response to protein supplementation. Rusche *et al.* (1993) working with soybean (RDP) or corn gluten/blood meal (rich in RUP) fed 100% or 150% of the NRC recommendations (1984) for CP intake for primiparous cows. Feeding at a higher level of RUP increased the calf's GMD; however, the source and amount of protein did not affect overall conception rates.

Regarding the use of amino acids, there is enough information in the literature in mammals supporting that the use of exogenous AA increases the rate of protein synthesis in skeletal muscle (via mTOR), glucose uptake, and fetal oxygen (BROWN et al., 2019; BROWN et al., 2017; BROWN et al., 2012; DE BOO et al., 2005; ROZANCE et al., 2009). Within the AA studied, arginine has been the target of more research, due to its action as nitric oxide (NO) precursors and polyamines in cells (WU et al., 2014; WU, 1998). NO is essential for its vasodilatory activity and the production of new blood vessels (angiogenesis) in various tissues, but especially in the placental tissue, thus playing a role in regulating the flow of blood from the placenta to the fetus (KWON et al., 2004; REYNOLDS and REDMER, 2001; VONNAHME et al., 2005). Also, polyamines are key regulators of DNA and protein synthesis, which are crucial for the growth, proliferation, and differentiation of mammalian cells (IGARASHI and KASHIWAGi, 2000; ZHAO et al., 2008). Among the most significant results related to the use of arginine being provided exogenously to pregnant animals, are an increase in the size of the placenta and an increase in litter size and weight (GAO et al., 2012; MATEO et al., 2007,), although it should be noted that most results in mammals are in non-ruminant animals, and dietary supplementation does not present the same challenges as for ruminants.

Often in ruminants, the use of arginine or other AA has been provided as infusions directly into the vein or protected in the rumen, among the most frequent results are an increase of between 15 and 20% in the weight of animals at birth (LASSALA et al., 2010; PEINE et al., 2018; SATTERFIELD et al., 2013). therefore, the use of AA plays an important role in maternal metabolism, contributing to body homeostasis, increased consumption of dry matter at the end of pregnancy, increased body protein synthesis (via mTOR), regulation of energy metabolism, stimulate the production of hormones such as insulin, GH, prolactin, glucagon at the end of pregnancy and activation of nutrient signaling pathways in the small intestine (MEIJER and DUBBELHUIS; 2004; MORRIS, 2002; THUREEN et al., 2002; WU et al., 2007; WU et al., 2004 and WU et al., 1999).

2.3.2 Energy supplementation.

Like protein supplements, energy supplementation has been used to supply nutritional deficiencies in periods of shortage or nutritional restriction of animals grazing high-quality pastures. Although the responses to energy supplementation in the literature are diverse, it is clear that the potential negative consequences of energy deficiencies in the gestation of the cow and her fetus, including impacts on cyclicality (CAVESTANY et al., 2009; TYAGI et al.,

2010), reduced maternal body weight, lighter birth weight (RAMIREZ et al 2020) and calf morbidity and mortality (CATON and DHUYVETTE et al., 1997). Energy supplements typically include foods that contain either structural carbohydrates (highly digestible fiber sources, such as soy hulls, wheat bran, or corn) or non-structural carbohydrates (NSC; starchy foods such as corn, wheat, and barley). On the other hand, diets with a higher ME content allow more energy to be distributed between the fetus and the mother, which may explain the increase in fetal growth and maternal performance (GUNN et al., 2012; RADUNZ, 2010; WILSON et al., 2012). In addition to the higher weights recorded at birth in the offspring's who received energy supply during pregnancy, positive results were also found on transcription factors with adipogenesis in the skeletal muscle (PREF-1, CCAAT, C / EBP-β) (Jennings et al., 2016, (DU et al., 2010a).

Tanner *et al.* (2020), evaluated supplementation with dry-rolled corn (0.2% BW) during the middle to the end of gestation in cows fed with low-quality forage and found improvements in the energy status of supplemented cows represented by the changes in body weight and BCS during pregnancy, although there was no difference in the performance of calves in the postnatal phase. The authors concluded that supplementing corn at low levels during pregnancy can be useful as a substitute for forage to improve the cow's energy status while decreasing the need for forage.

Supplementation with non-structural carbohydrates (NSC) generally has an impact on the intake and digestibility of OM, usually can result in decreased intake and digestibility of forage, which can influence the response to supplementation. Radunz *et al*. (2012) investigated the effects of supplementing three types of energy sources consisting of grass hay, corn, or dry corn distiller's grains for beef cows from 160 days of breeding to calving. The results found showed higher weights at birth and weaning from calves born to mothers supplemented with corn or distillery grains. As stated by Radunz *et al.* (2012), diets with high concentration content may allow more energy to be distributed to the fetus, which could help explain the increased fetal growth in calves of cows fed corn or distillery grains.

Few studies have compared the direct effects of supplementing with energy and protein at the same time. Huston *et al.* (1993) tested for 4 years in grazing cows to provide equal amounts of CP and 10%, 20%, or 40% of the requirements of energy digestibility (ED). The results found showed loss of BW and BCS for cows and increased weight at weaning of calves compared to the progeny of mothers not supplemented. On the other hand, Marston *et al.* (1995) were fed with protein or supplemental energy for approximately 120 days before partum. Cows fed with energy supplementation had slightly higher body weight gains and

pregnancy rates 11% higher than cows with protein supplementation. BW at weaning calves was not affected by supplementation, according to the authors that influences on reproduction may occur even without major changes in other performance measures.

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SECOND SECTION

ARTICLE 1- Impacts of protein supplementation during mid-gestation, in Zebu beef cows on maternal voluntary feed intake, performance, digestibility and uterine flow.

Article formatted according to Livestock Science guides

Short title: Protein supplementation improves performance in pregnant beef cows

Highlights

- Mid gestation is the best time to improve the energy reserves of beef cows.
- Protein supplementation in mid-pregnancy improves the average daily gain of maternal tissues.
- Beef cows supplemented in mid-gestation mobilize more body reserves.
- Protein restriction in mid-gestation reduces placental growth and development

Abstract

To determine the effect of protein supplementation (**PS**) during mid-gestation in cows fed low-quality forage on feed intake, body weight pregnant (BWp), empy bodyweight not pregnant (EBWnp), body condition score (BCS), plasma metabolites, dry matter intake, digestibility, the efficiency of microbial synthesis and pulsatility (IP) and resistance index (IR) in beef cows, 52 multiparous Zebu cows (490.5 ±17.8 kg BWp, 5.63 ± 0.50 BCS, 6.29 ± 0.6 yr of age) of Tabapuã breeding and carrying male and females calves were assigned randomly to (**SUP**) or unsupplemented (**CON)**) treatments. In the mid-gestation (100 to 200 days of gestation), cows were housed in a feedlot with individual pens and submitted to one of two treatments. **CON:** supply of basal diet (corn silage + sugarcane bagasse, achieving 5.5% CP plus a mineral mixture) and **SUP** basal diet plus 3.5 g/kg of BWp of protein supplementation (400 g/kg). Body Weight pregnant and BCS were determined every 30 days from day 100 of gestation until weaning. Feed intake was monitored individually from d 100 of gestation until pre-partum. Jugular blood samples and IP and IR also were collected at 200 and 270 d of pregnancy. Data were analyzed using the MIXED procedure of SAS. Protein supplementation increased (P = <0.01) daily gain in maternal tissues (0.359 kg/day), increase

in 34.7% the daily gain of the PREG component and 15% the eye muscle area, and P8 rump area in SUP cows. CON cows lost almost 1 point of BCS, while SUP gained 0.59 points of BCS during 100 days. However, in the peripartum SUP cows (-0,306 kg) mobilized a greater amount of maternal tissue ($P \leq 0.01$). Protein supplementations increased in 15% the forage intake, increase total DMI, and improves apparent total digestibility of nutrients, microbial CP, and efficiency synthesis ($P \leq 0.01$). CON cows showed a higher IR and IP ($P \leq 0.01$) when compared to SUP in the mid-gestation. PS influenced total maternal gain from 100 days of gestation until weaning in the SUP group (+15 kg). Protein supplementation at 3.5 g/kg of BW during mid-gestation can be useful as a nutritional correction to improve the dam's performance throughout pregnancy and postpartum, in addition to allowing a greater flow of nutrients to the uterus, which will allow adequate fetal growth and development.

Keywords: Doppler, gestational nutrition, hormones, maternal tissue, metabolism, offspring sex.

1. Introduction

The seasonality of production has become a barrier in the production systems of beef cattle managed in grazing systems raised in tropical regions (de Medeiros et al., 2015). Beef cows usually birth in the rainy season, the season that coincides with higher quality and availability from pastures, as a consequence these cows spend around 2/3 of gestation in the most critical season of the year, the season where forage levels with crude protein levels between 4 to 6% (DM basis), less than 45 percent TDN, low concentrations of nitrogenous compounds and high concentrations of (>68%) insoluble fiber and lignin (Detmann et al., 2014; Lazzarini, et al., 2009). Once forages represent the main source of energy for grazing animals and although energy is the main attribute of the diet for the development of gestating cows, protein is traditionally considered the main limiting nutrient (Paulino et al., 2006; Detmann et al., 2014).

Thus, the supply of nutrients via protein supplementation during pregnancy aims to provide moderate gains to the dams between 200-400 g / day per animal, searching for the addition of the recovery of body score and greater supply of nutrients for fetal growth and development. In this way, it is possible to use protein supplements at a level of 3.5 g/kg BW, which could provide the expected weight gain (Reis et al., 2014). On the other hand, In pregnant cows, most research focused on the effects of protein supplementation at the end of pregnancy, with a positive response to the dry matter intake, blood metabolites, digestibility, increase in maternal body weight, and BCS at birth (Bohnert et al., 2002; Stalker et al., 2007; Wilson et al., 2016; Tanner et al., 2020), However, there is few information on how this

nutritional correction could be addressed to improve the cow's performance during mid-gestation and your persistence during pregnancy. Recently, it has been demonstrated that the mid gestation is a critical window for the growth and development of muscle and fat tissue in the bovine fetus (Du et al., 2010; Long et al., 2012); therefore, maternal nutritional deficiencies, especially protein during the mid-gestation can cause loss of BW, BCS, changes in the resistance and pulsatility indices resulting from inadequate uterus-placental blood flow, affecting the delivery of oxygen and nutrients to the fetus (Vonnahme and Lemley.,2012), inducing cows to mobilize skeletal muscle tissue as the main source of energy reserve to meet the needs of fetal development, this situation negatively impacts the nutritional status of mothers, compromising future reproductive efficiency and the development of their offspring (Meteer et al., 2015).

Therefore, we hypothesized that protein supplementation with 40% CP at the level of 3,5g/kg of BW (DM basis) to cows fed low-quality forage during mid-gestation will improve the performance of the cows throughout pregnancy and the flow of nutrients to the gestational tissues, prioritizing fetal growth. This study seeks to understand how beef cows deal with protein restriction during mid-gestation to support fetal growth. We aim to identify possible changes in the maternal feed intake, body weight pregnant (BWp), empy bodyweight non-pregnant (EBWnp), ADG to PREG compounds, body conditions score (BCS), total apparent digestibility, and blood flow of the reproductive tract during specific windows during gestation.

2. Material and methods

Location and weather conditions

This experiment was carried out at the Beef Cattle Facilities of the Department of Animal Science of the Universidade Federal de Lavras (UFLA) in Lavras-MG, Brazil. This study was approved by the Brazilian Ethics Committee on Animal Use (CEUA/UFLA – a process no. 015/17), according to ethical principles of animal experimentation established by the National Council of Animal Experimentation Control (CONCEA). Weather conditions during the experiment were: 140.9 mm of rain and an average temperature of 23.1°C (initial gestation); 28.56 mm of rain and average temperature 18.4°C (mid-gestation); and 128.4 mm of rain and average temperature 20.8°C (late gestation).

Animals and diet

Fifty-two multiparous Tabapuã cows (*Bos taurus indicus*), with initial body weight 490.5 ± 17.8 kg BWp, 5.63 ± 0.50 BCS, 6.29 ± 0.6 years of age were used in this study. Cows were inseminated and at 60 days of gestation, fetal sexing was performed to have homogeneous control over treatments. To 0 to 100 days of gestation, all cows were kept in *Brachiaria brizantha* cv. Marandu pasture with mineral supplementation. In the mid-gestation (100 to 200 days of gestation), cows were housed in a feedlot with individual pens and submitted to different feeding levels: **Control (CON) -** supply of basal diet [75% of corn silage + 25% of sugarcane bagasse, achieving 5.5% crude protein (CP), thus representing levels of PC similar to those found in pastures under dry season conditions in the central west and southeastern in Brazil, and a mineral mixture] or **Supplement (SUP) -** basal diet plus protein supplementation [40% CP at the level of 3.5 g / kg body weight (BW). The composition of supplements and basal diet are shown in Table 1. The protein supplement consisted of a 50: 50 mixture of soybean meal and commercial supplement (40% CP). For made adjustments in the supplement supply, once a month the cow's body weight was recorded after 16 hours of fasting. From 200 days of gestation until the parturition, all cows received a diet containing only corn silage and mineral supplement. Cows had free access to water and were individually fed *ad libitum* twice daily (07:00 a.m. and 01:00 p.m.). The amount of diet provided was adjusted for DM content weekly, based on DM content of corn silage and sugarcane bagasse. Dry matter intake was obtained by the difference between the individual diet offered and the refusals observed daily. For each animal, samples of the offered feeds and refusals were collected every week for dry matter analysis.

Measurements

2.1 Weight gain and maternal body condition

The bodyweight of the cows to estimate the daily weight gain was obtained after a period of 16 h without feeding, Every 30 days, cows were weighed in the morning (0700 h, before feeding) from 100 to 270 days of gestation. The adjustment of the pregnant body weight **(BWp)** and the daily gain of the conceptus **(ADGcon)** were considered according to Gionbelli et al. (2015). To establish a better relationship between the gain in maternal body tissue and pregnant tissue, Gionbelli et al. (2015), determined the pregnancy component (PREG), mathematically estimated as an extra component of the cow (cow BW + PREG). The PREG component considers the increase in maternal tissues in pregnancy (GUdp: gravid

uterus + increase in Udder). The GUdp represents the weight of the pregnant uterus minus the weight of the uterus of the non-pregnant cows.

Discrimination of each of the components was necessary to calculate ADG of maternal tissues, considering the bodyweight of the cows minus the PREG component at 100, 200, and 270 days of gestation. The gain of the pregnancy component (with ADG) was calculated as the estimated weight of the PREG in each of the evaluated phases. The maternal adBWP was then considered as the calving weight of the cow minus the estimated PREG at calving.

Body condition score evaluations were performed every 30 days from 100 to 270 days of gestation together with the weighing activities of the animals. The scores evaluated were represented on a scale from 1 to 9, with 1 being considered very emaciated and 9 very obese (Nicholson and Butterworth, 1986 and Richards et al 1986). A score of 0.5 was considered a partial evaluation. The evaluation was carried out by three trained people who carried out observations and palpations on the animals. The final score was calculated as the average of each of the anatomical points evaluated between the evaluators.

2.2 Total nutrient digestibility.

To evaluate the nutritional characteristics of diet, total digestibility of DM and dietary constituents were assessed by determining the average intake of DM, and diet components during fecal collection. A digestibility trial was performed for 4 consecutive days at 150, 200, and 270 days of gestation as suggested by Barbosa (2005), Paixão et al (2007), and Ferreira et al (2009). Total feces were sampled from droppings on a concrete floor. At the end of 24 h of sampling, the buckets containing the samples were weighed, fecal samples were homogenized, and a subsample per day was collected, Samples were dried in a forced-air oven at 55°C for 72 h. After drying, samples were ground to pass through a 1-mm screen. Subsequently, a composite sample of feces for each bull was prepared, taking into account the number of feces excreted relative to the amount of the oven-dried sample.

2.3 Microbial protein synthesis.

During the same period of feces collection to evaluate digestibility, urine samples were collected from each cow, which after thawing became a compound for the evaluation of creatinine, allantoin and subsequent calculation of microbial synthesis. The concentration of creatinine in the urine was analyzed using a commercial kit (Creatinine K016, Bioclin, Belo Horizonte, Brazil). The concentration of allantoin was quantified according to the colorimetric method (Chen and Gomes, 1992). Uric acid was estimated by the concentration of allantoin (Santos et al., 2016), as follows: uric acid (mmol / d) = 0.1104 × allantoin (mmol / d). The microbial synthesis of CP was estimated by the technique of purine derivatives in urine (Chen

and Gomes, 1992). Urine volume was estimated using the creatinine concentration as a marker and assuming a daily creatinine excretion (mg / d) of 37.88 × PAS 0.9316, where PAS is the bodyweight reduced in kg (Santos et al., 2016). The excretion of purine derivatives in the urine was calculated by adding the excretions of allantoin and uric acid, obtained by the product between its concentrations in the urine and the daily urine volume. The absorbed purines were calculated from the excretion of purine derivatives (Prates et al., 2012), as follows: absorbed purines (mmol / d) = excretion of purine derivatives (mmol / d) - (0.389 × BW 0.75) / 0.99; where 0.99 = recovered absorbed purines. The value of 0.389 × BW 0.75 = endogenous excretion of purine derivatives (mmol / d). The ruminal synthesis of MCP was calculated as a function of the absorbed purines (Prates et al., 2012), as follows: MCP = 70 × absorbed purines (mmol / d) / (0.93 × 0.11 × 1000); where 70 = purine N content (mg N / mol), 0.93 = purine digestibility and 0.11 = purine N ratio: total N of microorganisms. The efficiency of MCP synthesis was calculated by dividing the amount of MCP by the intake of digestible OM.

2.4 Resistance indices.

To assess the hemodynamic behavior of the uterine artery, measurements were taken at the end of the supplementation period (200 ± 5 days of gestation) and in the pre-partum period (270 ± 5 days of gestation) using an ultrasound device with B and Doppler modes (Color and Spectral) and 7.0 MHz transrectal linear probe (distributed by Corometrics Medical Systems, Wallingford, CT; the veterinary, transrectal, linear, UST-5813-5 model, was used in this research). After inserting the probe through the rectum and activating digital B mode, the pregnant uterine body and the 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 (IR), Pulsatility (IP), Systolic/diastolic ratio (S/D), and speed were displayed after activation of the spectral Doppler function in the uterine artery. The uterus was not palpated before evaluations to avoid influencing blood flow through transrectal manipulation. The analysis of the Doppler indices was performed using the equipment's analysis software.

2.5 Ultrasound measurements.

The ultrasonic measurements of the thickness of the subcutaneous fat were measured between the 12th and 13th rib in the longissimus muscle area and P8 rump fat depth on the hip bone at d 200 and 270 of gestation on the right side of each animal. In both locations, the transducer was positioned directly on the skin, perpendicular to the vertebral column. Insonification was performed with an Aloka 210DX real-time ultrasound unit (Corometrics

Medical Systems, Wallingford, CT) equipped with a 3.5-MHz linear array probe with 17.2 cm of length. The coupler was mineral oil.

2.6 Laboratory Procedures and Analyses

The forage provided, supplements, refusals, feces, were processed through a 1-mm sieve for further determination of DM (method 934.01; AOAC, 1990); organic matter (OM) determined by ash (method 924.05; AOAC, 1990); crude protein (CP) obtained by multiplying total nitrogen, determined using the micro Kjeldahl technique (method 920.87; AOAC, 1990), by a fixed conversion factor (6.25); Non-fibrous carbohydrate (NFC) levels were calculated following proposed by Detmann and Valadares Filho (2010), with NFC = 100 - ((% CP -% CP derived from urea +% urea) +% apNDF +% EE +% ASH), NDFap, according to Mertens (2002), using thermostable α-amylase, without using sodium sulfite; insoluble NDF containing neutral detergent insoluble nitrogen (NDIN) was quantified by following the recommendations of Van Soest and Robertson (1985) and ether extract (EE), determined gravimetrically after extraction using petroleum ether in a Soxhlet apparatus (method 920.85; AOAC, 1990). The energy intake of the animals was evaluated according to Detmann et al. (2010): TDN (%) = DCP + 2.25 x DEE + DNFC + apDNDF, where DCP, DEE, DNFC, and apDNDF mean, respectively, digestible crude protein, digestible ether extract, digestible and digestible non-fibrous carbohydrates free of ash and neutral detergent fiber proteins, which were calculated from the digestibility tests of the present study.

2.7 Statistical analyses

A completely randomized 2 × 2 factorial design was used, referring to maternal nutrition and progeny's sex (treatment as fixed effects) and year 1 and 2 (random effect). A full fixed-effect model was used:

$$Y_{ijk} = \mu + D_i + S_j + F_k + (DS)_{ij} + (BW)_{ijk} + e_{ijk}$$

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 dietary treatment (2 levels); S_j is the fixed effect of the j^{th} level of calf sex (2 levels); F_k is the random effect or the F^{th} level of the year (2 years); DS_{ij} is the interaction between D and S; BW_{ijk} is covariate of initial empy body weight, initial BCS, gestation time and parity of the dam (when pertinent); and e_{ijk} is the random error associated with Y_{ijk}, with $e_{ijk} \sim N(0,\sigma_e^2)$.

Before the final analyses, studentized residuals were removed when not within ± 3 standard desviation, and normality (P-value > 0.05) was assessed using Shapiro-Wilk's test. When pertinent, repeated measurement procedures were used. Least-squares means were separated using Fisher's least significant difference test. Results were deemed significant

when *P*-value ≤ 0.05 and trending when 0.05 < *P*-value ≤ 0.10. All analyses were performed using SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA).

3. Results

3.1. Average daily gain and body condition score during the gestation period

During measurements at 100, 200, and 270 days of gestation, there was no interaction between maternal nutrition and calf sex, for maternal BWp, SBWp, EBWnp, and BCS (Table 2). As expected, there was no effect of maternal nutrition on variable BW and BSC at 100 days of gestation, demonstrating the effectiveness of the distribution of animals applied between treatments to measure the effects of protein supplementation on performance variables. However, at 200 and 270 gestation days, there was a treatment effect (P <0.01), where SUP was heavier than CON cows during the protein restriction period (from 100 to 200 days of gestation) and at parturition. Concerning BCS, CON treatment cows lost almost 1 point of BCS, while cows in SUP treatment gained 0.59 points of BCS during 100 days (protein restriction period; P <0.01; Table 2). After a period of protein supplementation, the results showed a residual effect of the BCS gain in the animals that received protein supplementation in the mid-gestation, SUP animals showed 20% more BCS gain compared to the animals in the CON group.

At the end of the supplementation period, ADG, ADG adjusted for EBW, AGD of maternal tissue and the pregnancy component were higher for animals in SUP treatment (P <0.01; Figure 1). Cows in the SUP group showed increases on average five times more for ADG (0.599 vs -0.155) and ADG adjusted for EBW (0.572 vs -0.127). Supplemented cows also had a greater daily gain in maternal tissues (P <0.01; 0.359 vs -0.288) and showed a 34.7% increase in the daily gain of the PREG component. After a period of protein supplementation, cows in the CON group showed a 53.5% increase in total daily gain (Figure 2). When the weight was adjusted to EBW, cows SUP showed lower losses in daily weight gain (P = 0.03; -0.321 vs -0.173). On the other hand, SUP cows were those that mobilized the greatest amount of maternal tissue until partition compared to the cows of the CON group, SUP animals showed losses on average of 300 gr/day (P <0.01), which represented losses five times greater of maternal tissue. Although animals in the SUP group showed greater mobilization of maternal tissue at the end of gestation, maintained a 24% greater gain in the PREG component and total gain (P <0.01; Figure 3), when compared to the CON cows. The ribeye area (Longissimus muscle area) and the ribeye area expressed per 100 kg of a carcass in SUP cows (P = 0.03; and P = 0.02; Table 3), had a 15% increase during mid-gestation,

which represents carcass with a greater degree of muscularity. Change in the percentage of Intramuscular fat at 200 and 270 days of gestation did not differ between supplemental treatments, the ribeye area expressed per 100 kg of carcass did not show significant differences when compared between the cows at 270 days of gestation. The rump area at 200 (CON= 99.06 cm^2; SUP=109.70 cm^2) and 270 (CON= 96.98 cm^2; SUP=105.30 cm^2) days differed between treatments, being higher in supplemented than in unsupplemented cows (P = 0.03). There was no change in intramuscular fat in the rump between treatments.

3.2 DMI and digestibility.

Forage, total dry matter, and nutrient intake data are shown in Table 4. The forage intake in absolute values, increased by 15.1% (P = 0.03; 6.27 vs 6.92 ± 0.30), while the total DMI (Forage + Supplement) was also higher in SUP cows (P <0.01; 6.01 vs 8.16). When intake is expressed as g / kg BWp DMI was greater (P <0.01) in SUP cows than CON (12.77 v. 15.91 ± 0.95 g / kg BW), and when expressed as EBWnp supplements cows showed higher DMI also (13.47 v. 16.85 ± 1.04 g / kg EBWnp). At 270 days of gestation, SUP animals also showed higher values in DMI when compared to the CON group, but when expressed as g / kg BWp and EBWnp was no difference between treatments (P = 0.10 and 0.27) respectively.

There was no interaction between maternal nutrition and calf sex for nutrients intake. During the supplementation period (100 to 200 days of gestation) the protein intake of cows in the SUP treatment was 325 % higher than that of cows in the CON, which represented 98% in the amount of protein ingested concerning the animal's requirement. CON cows had about 70 % protein deficit according to the BR-Corte (2016). By the end of gestation, both treatments had protein restriction at similar levels, according to the DMI, both treatments ingested about 40% of their protein requirements, which resulted in the mobilization of maternal tissue in both groups, being higher in the SUP group due to the increased requirement about the weight of the PREG component. On the other hand, the Intake of OM, apNDF, EE, NFC, and TDN were higher (P <0.01) in SUP cows only during the mid-gestation.

Total apparent digestibilities data are shown in Table 5. There was no interaction between maternal nutrition and calf sex ($P \geq 0.05$) for DM and OM at mid-gestation, cows in SUP treatment showed 12 and 17 % higher total apparent digestibility compared with CON cows. Cows in SUP treatment showed greater (P ≤ 0.01) protein (240.39 v 694.52 ± 42.6), apNDF (516.18 v 605.97 ± 22.8), and EE (697.0 v 801.1 ± 20.4) digestibility respectively, as affected microbial protein and synthesis efficiency, being higher (P <0.01) in animal SUP at

mid-gestation. There were no differences for nutrients ($P \geq 0.05$) digestibility's between groups at 270 days of gestation.

3.3. Resistance index

The resistance index is presented in Table 6. There was no interaction between maternal nutrition and calf sex for indices of resistance (IR), pulsatility (IP), and Systolic/diastolic ratio (S/D). Maternal treatment influenced both indices. Protein restriction in the mid-gestation produces an increase ($P < 0.01$) in IP and IR in CON cows. During the prepartum period, both groups showed a decrease in the indices values in the uterine artery but were no differences between treatments ($P \geq 0.10$). When considering the effect of sex, cows pregnant with females showed increases of 20 %, 15%, and 24% in the indices of pulsatility, resistance, and Systolic/diastolic ratio respectively in the mid-gestation, although no differences were found in the values of indices between treatments at the late gestation, as the gestation time advanced, the differences between females and males increased, being 30% and 20% for pulsatility and resistance, respectively.

4. Discussion

In Brazil, beef cows are normally exposed to low-quality forage during almost 2/3 of the gestation period, which promotes an inadequate intake of nutrients, which allows applying nutritional corrections via supplementation to avoid limiting maternal protein requirements**.** Supplement protein (DM basis) around 0.35% of BW program used in this study resulted in increases in average daily gain, BCS, ribeye area, and the rump area during mid-gestation, although BCS and precocity index is subjective, it can be a good indication of the degree of finish and muscularity of the carcass, which suggested that SUP cows had a better energy and protein status compared to CON. On the other hand, the results of this study allowed us to show that there was a positive residual effect of gain and BCS in SUP animals during birth, presenting 20% more in average daily gain, which represented 14.9 kg more during the production cycle. In contrast to the results found during the supplementation phase, the SUP cows, post supplementation period had greater losses of maternal tissues. As pregnancy progresses, the uteroplacental and fetal metabolism increases, with the apex the last 90 days before Partum (Battaglia and Meschia, 1988; Bell et al., 2005). As noted in our study, the animals in the peripartum had crude protein intake lower than the protein requirements for pregnancy, which caused the greater mobilization of the maternal skeletal muscle tissue to supply the fetal demand, this mobilization was greater in SUP cows, which had to withstand greater demand for nutrients by the fetus (greater PREG component), that suggests there are

adjustments in maternal metabolism in response to the increased nutritional requirements in the fetal environment.

The effect of protein supplementation for cows grazing on low quality forages on the increase of dry matter intake, organic matter, available energy, and apparent total digestibility has been well documented by several authors (Batista et al., 2017; Rufino et al., 2016; Poppi et al., 1995; Mccollum et al., 1990; Poppi et al., 1987). The level of protein supplementation (0.35% of the BW) was chosen to potential positive associative effects on the intake of forages, SUP animals had an increase to 15.1 % in forage intake and 58 % in the OM intake, which caused an increase in energy intake, which is likely due to the significant increase in BWp, EBWnp, BCS and ADG to PREG component in animals that received supplementation during mid-gestation. Similar results in the intake variable in pregnant cows supplemented were found for de Lana Ferreira et al., (2020); Sotelo et al., (2019); Moura et al., (2018); Schauer et al., (2005), and Sletmoen -Olson et al., (2000).

Protein supplementation of grazing animals consuming low-quality forage leads to changes in the rumen fermentation pattern, SUP cows had a higher intake of CP at 200 days of gestation, which led to greater apparent digestion of OM, CP, apNDF, and other potentially digestible nutrients (Detmann et al 2014; Almora et al., 2012; Köster et al., 1996). Likewise, SUP animals showed greater microbial and efficiency of microbial protein during mid-gestation. Microbial protein has become an essential element of the PM system used by food systems and therefore represents the main source of amino acids for the animal (Galyean and Tedeschi, 2014; NRC 2000). Greater production of microbial protein and better efficiencies found in SUP animals during the supplementation period was due to greater intake of MO since there is a high correlation between DMI and microbial protein synthesis (Gomes et al., 1994; Pathak ., 2008; Galyean and Tedeschi, 2014). On the other hand, although there was a residual effect of supplementation in mid-gestation in SUP animals on DMI, there was no difference at the end of gestation for microbial synthesis and efficiency between the groups, this is probably since at the end of gestation there is greater digesta passage rate (kp) (Hanks et al., 1993; Linden et al., 2014)

Resistance indices and PI measured via Doppler have been considered as an important tool to assess fetal growth and development and to predict results of fetuses with restricted growth (Maršál., 2009; Vonnahme et al., 2015; Lekatz et al., 2015; Burton et al., 2012; Owens et al., 1986). The increases in the RI and PI indexes found in this study for CON cows in the mid-gestation are due to the reduction in placental vascularization or reduced uterine or

umbilical blood flow caused by dietary insults, since there is a negative correlation between the values of the indices and the blood flow parameters (Panarace et al.,2006), in other words, higher indices can be explained how a placental compensation strategy to overcome the nutrient loss. On the other hand, lower rates found in SUP animals may be related to the development of new blood vessels in response to the growing demand for nutrients.

In cows, placental growth increases throughout gestation, with the change in capillary area density being the main variable related to blood flow and the developing placenta (Vonnahme et al., 2007; Reynolds et al., 1990; Funston et al., 2010). There have been few studies that have measured the flow of nutrients during periods of restriction and realimentation. In this study, vascular resistance and pulsatility in late pregnancy were similar between the CON and SUP treatments, although both treatments received ingested about 40% of their protein needs. The values were numerically lower than the values found during mid-gestation due to final placental development at late gestation, which reflects a greater supply or volume of uterine blood. We believe that the placenta acts as a sensor for maternal stressors and undergoes modifications, which some have called placental programming, to ensure the healthy development of the concept for that in the refeeding period the placental arterial viscosity returned to the CON animals after the restriction period. These results are in agreement with the findings of Ekskine and Ritchie, (1985) and Serin et al., (2010), who reported reductions in those indices during late gestations, as vascularity increases to allow for the supply of sufficient nutrients. Thus, further studies are needed to elucidate the effect of restriction and realimentation in pregnant beef cows under tropical conditions.

Regarding sex effect, studies in mammals have shown sex-specific differences in fetal growth and development concerning sexual dimorphism on gene expression and signaling of genes that generate these differences between the sexes when they are compared in intrauterine environments (Di Renzo et al., 2007). Higher levels of resistance, pulsatility, and systolic/diastolic ratio found in this study for female fetuses regardless of experimental treatment, may lead to a decrease in fetal development, but at the same time can be considered as a mechanism for reducing situations adverse or, in other words, "adaptive allocation of maternal resources", since in various species of mammals when there are genetic or environmental factors that can have adverse effects on fetuses, maternal resources are allocated differently to one of the two sex. According to the Trivers-Willard theory (Trivers and Willard, 1973), it is suggested that females, depending on their physiological state, evolve within the species to allocate their resources preferentially to sex, aiming at the perpetuation of the species, while male fetuses prioritize growth when compared to female fetuses, and

have continued to grow despite the unfavorable intrauterine environment, which can put them at risk for lack of nutrients (Widnes et al., 2018; Panarace et al.,2006; Morrow et al., 1993). The results found in this study are different from those found by Hernandez-Medrano et al, (2015), where cows pregnant with male fetuses that passed nutritional restriction during pregnancy showed increases in PI and RI.

5. Conclusion

Protein supplementation at 3.5 g/kg of BW (DM basis) during mid-gestation improved the energy status of the cows, when forage quality was limited, being represented by the increase in maternal weight and body condition score with positive residual effect until pre-partum. The application of a nutritional correction in mid-pregnancy increased the daily gain of the fetus, inducing greater muscle growth and development in the progeny and promoting the greater maternal mobilization of the skeletal muscle tissue at late gestation as a mechanism to ensure adequate nutrient flow; it was an important discovery of this work. With the use of these nutritional strategies, producers will be able to take advantage of the best time to increase the energy status of the cow and enhance the development of progeny muscular tissue and reduce the production costs of the animal in the entire cycle within the farm.

Declaration of conflicts of interest.

The authors declare no conflicts of interest.

Acknowledgments.

This work was supported by the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), Minas Gerais State Agency for Research, and Brazilian National Council for Scientific and Technological Development (CNPq) and Minas Gerais State Agency for Research and Development (FAPEMIG). The authors would like to thank Dr. Pedro Veiga R. Paulino (Cargill Animal Nutrition) for his support and insights on this research, the Beef Cattle Research Complex, the beef cattle study group (NEPEC), and those responsible for the installations of the animal science laboratory.

Author contributions:

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Table 1. Nutrient content of dietary supplements and forage offered to cows during gestation

Chemical composition	Supplements[3]	Forage		
		Early	Mid[2]	Late[3]
DM (g/kg DM)	881.1	284.9	418.26	330.3
OM (g/kg DM)	957.8	916.7	951.05	941.4
CP (g/kg DM)	400.53	154.33	53.31	72.2
NDFap (g/kg DM)	213.03	568.0	631.48	549.2
NFC (g/kg DM)	342.1	112.57	242.13	290.7
EE (g/kg DM)	41.2	25.5	24.12	29.2
TDN [1](g/kg DM)	788.83	585.33	544.15	596.20

1 calculated according to Detmann et al. (2010)
2(corn silage + sugarcane bagasse)
3(corn silage)
[4]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).

Table 2. Influence of protein supplementation during mid-gestation on body weight (BW), empy body weight pregnant (EBWp), empy body weight not pregnant (EBWnp) pregnant components and body condition score (BCS) in beef cows.

Item	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
Measurements at 100 days of gestation (initial weights)								
Cow BWp, kg	486.34	495.70	467.54	514.49	17.8	0.67	0.04	0.34
Cow EBWp, kg	431.17	439.64	413.21	457.60	16.4	0.68	0.04	0.34
Cow EBWnp, kg	427.89	436.75	410.73	454.92	16.2	0.70	0.04	0.34
Cow BCS	5.64	5.62	5.59	5.67	0.502	0.90	0.68	0.97
PREG[1], kg	3.27	3.37	3.23	3.41	0.250	0.70	0.54	0.98
Measurements at 200 days of gestation (end of supplementation period)								
Cow BWp, kg	469.80	547.60	508.56	508.54	5.54	<0.01	0.97	0.735
Cow EBWp, kg	396.86	463.91	430.66	430.11	4.83	<0.01	0.93	0.778
Cow EBWnp, kg	417.28	489.75	453.37	456.66	5.18	<0.01	0.96	0.723
Cow BCS	4.84	6.21	5.40	5.65	0.247	<0.01	0.19	0.507
PREG, kg	20.23	25.99	22.23	23.99	1.31	<0.01	0.31	0.985
Measurements at 270 days of gestation (prepartum)								
Cow BWp, kg	490.14	556.94	522.33	524.75	6.27	<0.01	0.78	0.46
Cow EBWp, kg	392.42	443.19	417.91	417.70	5.15	<0.01	0.97	0.40
Cow EBWnp, kg	438.12	500.94	468.35	470.72	5.15	<0.01	0.77	0.46
Cow BCS	4.89	5.83	5.20	5.45	0.11	<0.01	0.11	0.47
PREG, kg	45.43	57.78	49.63	53.58	2.65	<0.01	0.27	0.80
Measurements postpartum								
Cow EBW, kg (30 d)	384.9	433.5	413.5	404.9	7.74	<0.01	0.43	0.69
Cow EBW,kg (120 d)	422.4	444.7	443.5	421.5	7.60	0.05	0.04	0.38
Cow EBW,kg (210 d)	441.4	456.3	461.8	435.9	6.54	0.08	<0.01	0.31
Total Maternal Gain, kg [2]	20.72	35.63	41.13	15.22	6.54	0.08	<0.01	0.31

CON = control; unsupplemented

SUP= supplemented

[2]Maternal gain in the whole cycle (100 days of gestation until weaning)

BWnp = maternal BW (discounting gravid uterus weight and udder accretion); EBWnp = maternal EBW (discounting gravid uterus weight and udder accretion); PREG = pregnancy component 1 Gravid uterus minus the non-pregnant uterus plus the accretion in udder related to pregnancy

Table 3. Influence of protein Supplementation during mid-gestation on carcass characteristics in beef cows.

	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
Measurements at 200 days of gestation (end of supplementation period)								
Ribeye area, cm^2	105.54	121.10	110.75	116.00	5.39	0.03	0.45	0.54
Fat thickness in Ribeye área, cm	5.53	5.67	5.90	5.29	0.59	0.72	0.14	0.07
Ribeye area, cm/100kg	24.89	28.45	26.33	27.01	1.33	0.02	0.66	0.73
Rump area ,cm^2	99.06	109.70	106.17	102.61	3.51	<0.01	0.28	0.38
Rump fat thickness,cm^2	4.32	4.65	4.85	4.11	0.47	0.44	0.08	0.49
Rump area ,cm^2 /100kg	23.38	25.73	24.97	24.13	0.58	<0.01	0.27	0.31
Measurements at 270 days of gestation (prepartum)								
Ribeye area, cm^2	87.82	91.20	86.64	92.38	4.20	0.55	0.33	0.23
Fat thickness in Ribeye área, cm	3.30	3.02	3.07	3.25	0.39	0.60	0.75	0.07
Ribeye area, cm/100kg	22.75	20.99	20.91	22.84	1.03	0.21	0.18	0.13
Rump area ,cm^2	96.98	105.30	100.61	101.67	3.08	0.05	0.80	0.41
Rump fat thickness,cm^2	3.42	2.97	3.38	2.02	0.48	0.49	0.59	0.57
Rump area ,cm^2 /100 kg	25.33	24.38	24.60	25.11	0.80	0.38	0.64	0.31

CON = control; unsupplemented

SUP= supplemented

Table 4. Influence of protein Supplementation during mid-gestation on Dry matter and nutrient intake in beef cows.

	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
Measures from 100 to 200 days of gestation (end of supplementation period)								
Intake. kg/day								
Forage	6.01	6.92	6.78	6.42	0.30	0.03	0.29	0.88
Total	6.01	8.16	7.14	6.82	0.38	<0.01	0.44	0.81
OM	4.51	7.17	6.10	5.58	0.34	<0.01	0.27	0.31
CP	0.27	0.88	0.60	0.55	0.05	<0.01	0.34	0.80
apNDF	2.89	4.01	3.64	3.26	0.20	<0.01	0.18	0.32
EE	0.13	0.23	0.19	0.17	0.01	<0.01	0.28	0.57
NFC	1.24	1.98	1.70	1.53	0.25	<0.01	0.24	0.32
TDN	2.75	4.97	4.03	3.69	0.41	<0.01	0.32	0.44
Intake. g/kg BW								
Forage	13.47	13.87	13.92	13.48	0.60	0.12	0.22	0.91
DM	13.47	15.91	14.79	13.90	0.95	<0.01	0.26	0.98
DM *g/kg EBWnp*	13.53	16.85	15.62	14.70	1.04	<0.01	0.27	0.96
OM	9.99	13.15	11.99	11.08	0.63	<0.01	0.30	0.22
apNDF	6.36	7.40	7.17	6.59	0.39	0.06	0.29	0.30
TND	6.04	9.13	7.84	7.32	0.65	<0.01	0.42	0.35
Measurements from 200 to 270 days of gestation (prepartum)								
DM	6.59	7.84	7.35	7.08	0.63	0.01	0.57	0.59
OM	4.80	5.43	4.93	5.29	0.26	0.08	0.31	0.16
CP	0.37	0.40	0.37	0.40	0.04	0.34	0.20	0.10
apNDF	2.75	3.10	2.80	3.05	0.15	0.09	0.22	0.12
EE	0.15	0.17	0.163	0.167	0.022	0.20	0.78	0.13
NFC	1.52	1.74	1.30	1.66	0.097	0.09	0.66	0.36
TDN	2.97	3.29	2.91	3.34	0.26	0.36	0.22	0.21
Intake. g/kg BW								
DM	12.71	13.68	13.27	13.11	0.84	0.10	0.79	0.48
DM *g/kg EBWnp*	15.40	16.72	16.19	15.83	1.73	0.27	0.74	0.92
OM	10.08	9.86	9.87	10.07	0.52	0.76	0.78	0.11
apNDF	5.76	5.63	5.62	5.76	0.29	0.74	0.72	0.07
TND	6.29	5.92	8.82	6.39	0.84	0.59	0.43	0.19

CON = control; unsupplemented

SUP= supplemented

Organic matter (OM, kg), crude protein (CP, kg), neutral detergent fiber corrected for ash and protein (apNDF, kg), Ether Extract (EE, kg), Non-fibrous carbohydrates (NFC, kg), Total Digestible Nutrients (TDN, kg)

Table 5. Influence of protein Supplementation during mid-gestation on Total apparent digestibility in Beef cows.

	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
Measurements at 200 days of gestation (end of supplementation period)								
Total apparent digestibility, g/kg or DM								
DM	540.9	598.4	582.8	556.4	59.9	0.02	0.29	0.95
OM	575.5	668.3	615.3	628.5	42.9	<0.01	0.61	0.38
CP	240.3	694.5	495.7	439.2	42.6	<0.01	0.33	0.11
apNDF	516.8	605.9	548.8	574.01	22.8	<0.01	0.41	0.20
EE	697.0	801.1	744.4	753.7	20.48	<0.01	0.73	0.06
NFC	751.05	774.5	757.03	768.56	68.54	0.47	0.72	0.84
TDN	572.8	675.4	614.9	633.4	37.3	<0.01	0.47	0.37
Nmic[1]	253.2	588.6	443.1	398.7	33.1	<0.01	0.21	0.70
Emic[2]	96.3	123.7	110.4	109.7	1.66	<0.01	0.74	0.58
Measurements at 270 days of gestation (prepartum)								
DM	532.1	513.5	496.8	548.8	43.34	0.65	0.21	0.54
OM	574.4	560.7	545.2	589.9	47.1	0.72	0.26	0.48
CP	338.9	241.4	259.1	321.2	102.9	0.16	0.36	0.22
apNDF	519.01	497.1	496.2	519.8	73.3	0.64	0.61	0.35
EE	729.6	702.5	714.4	717.74	24.6	0.41	0.92	0.90
NFC	711.2	714.6	668.02	757.8	27.5	0.92	0.01	0.96
TDN	568.8	559.1	543.1	584.8	40.2	0.80	0.28	0.49
Nmic[1]	299.7	333.8	297.9	335.5	24.8	0.31	0.27	0.19
Emic[2]	107.7	107.4	107.8	107.3	2.43	0.93	0.87	0.92

CON = control; unsupplemented

SUP= supplemented

Organic matter (OM, g/kg), crude protein (CP, g/kg), neutral detergent fiber corrected for ash and protein (apNDF, g/kg), Ether Extract (EE, g/kg), Non-fibrous carbohydrates (NFC, g/kg), Total Digestible Nutrients (TDN, g/kg),

1 Ruminal synthesis of microbial nitrogen (NMic, g/d),

2 Efficiency for the synthesis of microbial protein (Emic, g microbial CP synthesis kg dOM intake)

Table 6. Influence of protein Supplementation during mid-gestation on pulsatility, resistance indices and Systolic/diastolic ratio in beef cows.

Item	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
Measurements at 200 days of gestation (end of supplementation period)								
PI[1]	0.905	0.717	0.900	0.721	0.04	<0.01	0.01	0.83
RI[2]	0.628	0.498	0.611	0.516	0.31	<0.01	0.03	0.56
(S/D)[3]	2.20	2.17	2.35	2.03	0.14	0.87	0.11	0.89
Measurements at 270 days of gestation (prepartum)								
PI[1]	0.788	0.765	0.906	0.647	0.04	0.69	<0.01	0.41
RI[2]	0.508	0.522	0.568	0.462	0.01	0.55	<0.01	0.67
(S/D)[3]	1.99	2.02	2.22	1.79	0.09	0.78	<0.01	0.58
Calf birth weight	27.29	31.08	29.22	29.16	3.96	0.05	0.98	0.56

CON = control; unsupplemented

SUP= supplemented

[1] pulsatility index

[2] resistance index

[3] Systolic/diastolic ratio

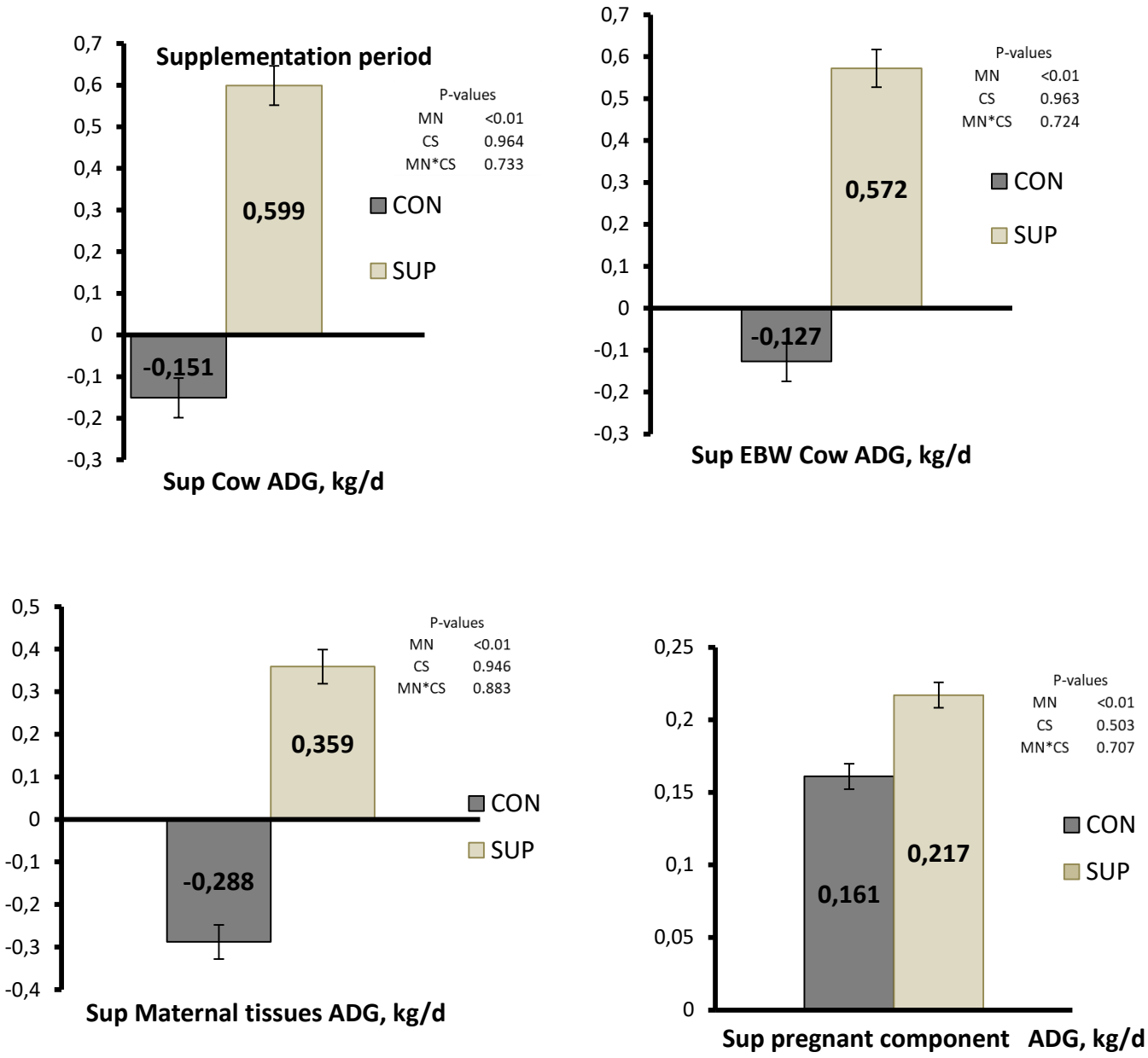

Figure1. Influence of protein Supplementation during mid-gestation on ADG (BW) ADG (EBW), maternal tissues, and ADG of pregnant components during 100 to 200 d of gestation in beef cows.

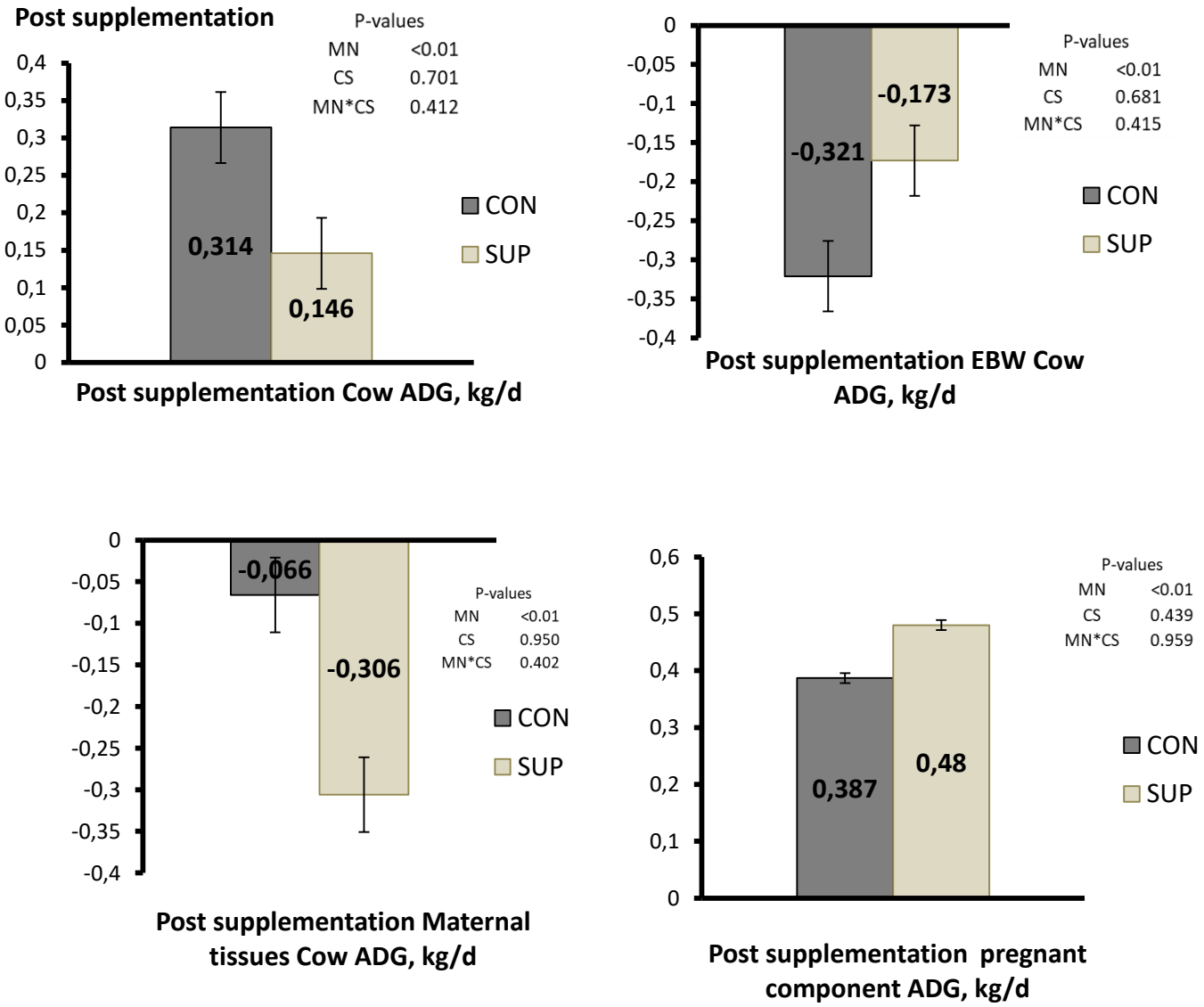

Figure 2. Influence of protein Supplementation during mid-gestation on ADG (BW) ADG (EBW), maternal tissues, and ADG of pregnant components during 200 to 270 of gestation in beef cows.

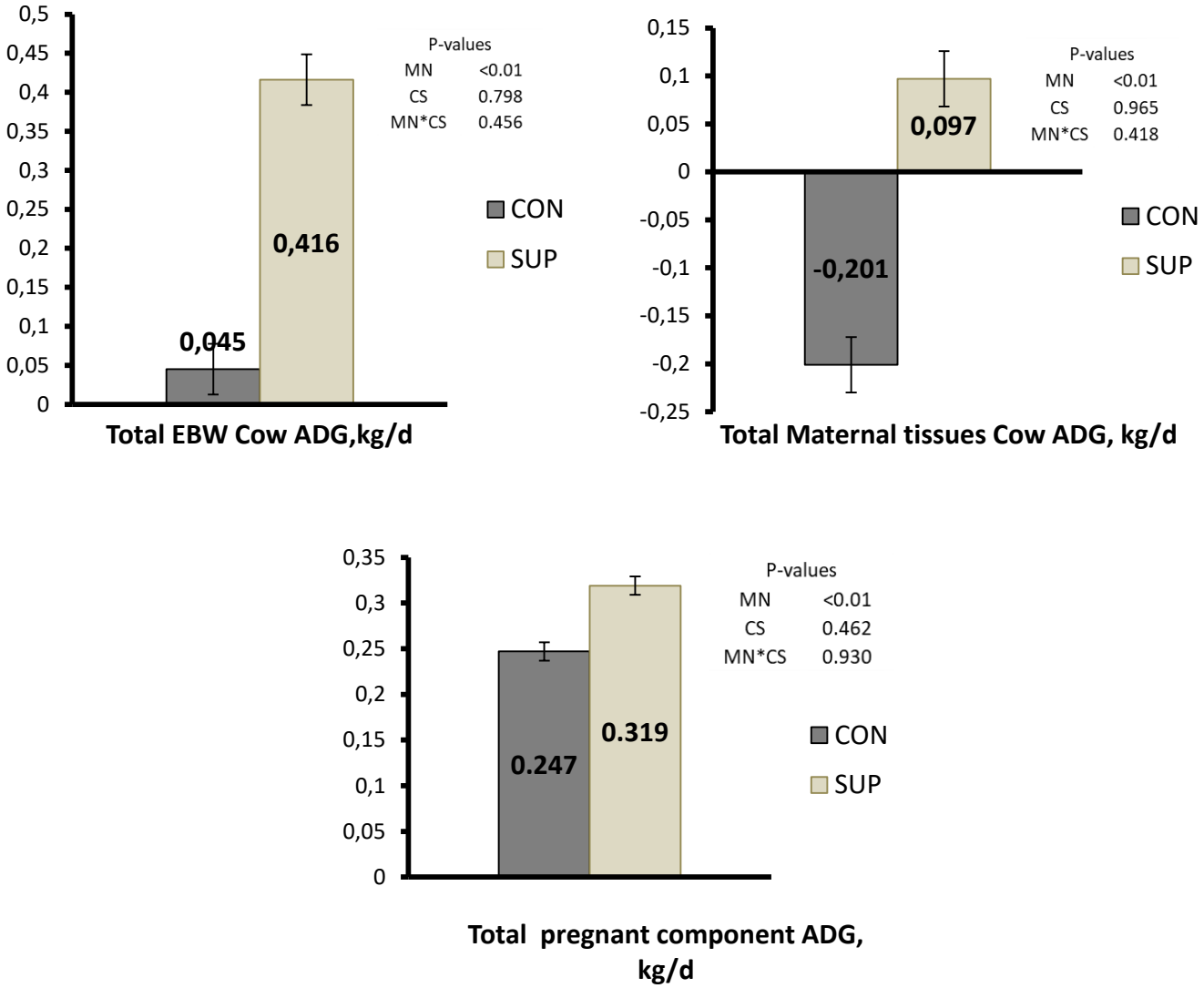

Figure 3. Influence of protein Supplementation during mid-gestation on total ADG (EBW), maternal tissues, and ADG of pregnant components during 100 to 270 d of gestation in beef cows

ARTICLE 2- Protein supplementation during mid-pregnancy alters the amino acid patterns and regulates maternal muscle protein synthesis and degradation in skeletal muscle in beef cows

Article formatted according to Livestock Science guides

Short title: Protein supplementation induces adaptations in maternal tissues in pregnant beef

Highlights

- Nutritional corrections via supplementation alter the metabolism in pregnant cows
- Supplement protein at mid-gestation compromises the pool of plasma amino acids.
- Gestational nutrition influence adaptive changes in maternal muscle metabolism.
- Protein supplementation stimulates the growth of maternal muscle tissue

Abstract

To determine the effect of protein supplementation (**PS**) during mid-gestation in cows fed low-quality forage on circulating plasma amino acids (AAs), hepatic and maternal skeletal muscle gene expression in beef cows. Fifty-two multiparous Zebu cows (490.5 ±17.8 kg BWp, 5.63 ± 0.50 BCS, 6.29 ± 0.6 yr of age) predominately of Tabapuã breeding and carrying male and females calves were assigned randomly to supplement treatments (**SUP)**: 40%CP or 3.5g/kg of body weight (*n* = 26) or unsupplemented (**CON) :**0% CP (*n* = 26) from d 100 to 200 of gestation. In the mid-gestation (100 to 200 days of gestation), cows were housed in a confinement with individual pens and submitted to different feeding levels: **(CON) -** supply of basal diet (corn silage + sugarcane bagasse, achieving 5.5% crude protein (CP) and a mineral mixture) and **SUP** basal diet plus + protein supplementation. Blood samples to assess plasma levels of hormones, metabolites and AAs and skeletal muscle biopsies to assess mRNA expression were performed at 200 and 270 days of gestation. Data were analyzed using the MIXED procedure of SAS. Protein supplementation positively influenced ($P \leq 0.01$) the concentration of glucose (54.72 mg/dL), IGF-1 (61.9 Ng/mL) and insulin (10.4 µIU/mL) in plasma during pregnancy, represented by increases of around 30% in SUP cows. CON cows showed a 25% increase (0.105 mmol/L) in plasma NEFA concentration. Supplemented cows had an approximately 50% increase in the concentration of total circulating AAs ($P = 0.03$). Supplement cows showed an increase of approximately 26% in plasma concentration of glucogenic and ketogenic AAs to late gestations ($P \leq 0.01$). The

mRNA expression of genes related to protein synthesis (EIF4E and GSK3BETA) and degradation (MUFR1) in maternal skeletal muscle were up-regulated, showing an increased between 30 and 40% in SUP cows. Protein supplementation at 3.5 g/kg of BW during mid-pregnancy can be useful as a nutritional strategy to support fetal development and stimulate greater hepatic glucose production from a greater flow of plasma amino acids, which will help preserve the reserves of maternal tissue when maintained on low quality pasture.

Keywords: Amino acids, gene expression, gluconeogenesis, maternal nutrition, skeletal muscle.

1. Introduction

Pastures are the basis for a more practical and economical feeding of beef cattle in Brazil, but livestock kept on pasture is subject to different seasonality in the supply and quality of forage between April and October (dry season) and November to March (rainy season), as it is exposed to these changes, characterized by quality and abundance of forage during the year (Detmann et al., 2014). Knowing the responses of animals during the two seasons of the year is important to promote good nutritional and reproductive planning of the herd, especially the dam. In more technical production systems, the breeding season coincides with the season of better quality and greater availability of forage, which provides adequate conditions for the recovery of body reserves and, consequently, the reestablishment of the reproductive activity of females, resulting in higher pregnancy rates. Unfortunately, primitive reproductive systems still prevail where the bull remains with the herd throughout the year. As a consequence, births are distributed throughout the year, resulting in the occurrence of births at the inappropriate season, impairing the development of calves and cows (de Medeiros et al., 2015).

In general for pregnant cows in Brazil, the low nutritional value of the pasture consumed results in low intake of dry matter (DMI) and, consequently, the supply of metabolizable energy (EM) and metabolizable protein (PM) is often below the nutritional needs of grazing ruminants. During pregnancy, nutrients are generally partitioned to various tissues of the maternal body in priority order according to their metabolic rate (Fowden, 1995; Thrift et al., 1999). During the late gestation, fetal growth dramatically increases and results in increased nutrient demand (Ferrell, 1991; NRC, 2000), thus, dams may be able to reduce maintenance energy costs to support the energetic demands of the fetus (Freetly et al., 2008), for this reason, as nutrients become limiting, tissues with lower metabolic rates and hence

reduced priority, will receive quantitatively fewer nutrients, and are the first to be degraded. It has been shown that pregnant cows fed below their maintenance requirements may be able to conserve energy through metabolic changes and adaptations of liver tissue and skeletal muscle tissue, since glucose production required by gestational tissues is supported by increased peripheral mobilization and hepatic uptake of endogenous substrates, such as amino acids from muscle protein breakdown, becoming the main substrate to stimulate fetal growth and development (Lopes et al., 2020; Caton et al., 2020; Micke et al., 2011; Bell, 1997; Wilson et al., 1986). Little is known about the underlying cellular mechanisms involved in these processes concerning the pregnancy of beef cows fed low-quality pastures in tropical conditions.

There are still gaps in knowledge that need to be filled about the ways that the dams seek to adapt physiologically to avoid losses during pregnancy and how these adaptations cause damage to the maternal organism and to achieve later pregnancies. When researching proteins related to protein turn over skeletal muscle, namely: Ribosomal protein S6 kinase **(p70S6k)**, glycogen synthase kinase 3β **(GSK3B)**, eukaryotic translation initiation factor 4E **(eIf4E)**, muscle ring finger 1 **(MuRF1),** and muscle atrophy F-box protein **(atrogin-1)**, we believe that these molecular markers may play a fundamental role in the energy and protein partitioning of cows during pregnancy. We hypothesize that dams' protein supplementation during mid-gestation will bring greater amino acids to support the development of the fetus, it will also stimulate greater hepatic glucose production from higher levels of gluconeogenic marker mRNA and greater gains in maternal muscle tissue from expression skeletal muscle protein synthesis markers, and thus reduce mothers' need for tissue mobilization while preserving maternal tissue reserves when maintained on low quality pasture. The objective of this experiment was to investigate the effect of supplementing pregnant beef cows with 40% CP at the level of 3.5 g / kg body weight during mid-gestation on the plasma concentration of hormones, metabolites and pool of total AAs and the cellular mechanisms related to the energy and protein metabolism in beef cows.

2. Material and methods

2.1 Location and weather conditions

This experiment was carried out at the Beef Cattle Facilities of the Department of Animal Science of the Universidade Federal de Lavras (UFLA) in Lavras-MG, Brazil. This study was approved by the Brazilian Ethics Committee on Animal Use (CEUA/UFLA – a

process no. 015/17), according to ethical principles of animal experimentation established by the National Council of Animal Experimentation Control (CONCEA). Weather conditions during the experiment were: 140.9 mm of rain and an average temperature of 23.1°C (initial gestation); 28.56 mm of rain and average temperature 18.4°C (mid-gestation); and 128.4 mm of rain and average temperature 20.8°C (late gestation).

2.2 Animals and diet

Animal handling procedures have been previously reported (Meneses et al., 2021). 52 multiparous Tabapuã cows (*Bos taurus indicus*), with initial body weight 490.5 ± 17.8 kg BWp, 5.63 ± 0.50 BCS, 6.29 ± 0.6 years of age were used in this study. Cows were inseminated and at 60 days of gestation, fetal sexing was performed to obtain homogeneous treatment. In the first third of gestation (0 to 100 days) all cows were kept in *Brachiaria brizantha* cv. Marandu pasture (DM basis: DM = 284.9 g/kg CP = 154.33 g/kg and NDF = 213.03 g/kg), In the mid-gestation (100 to 200 days of gestation), cows were housed in a confinement with individual pens and submitted to different feeding levels: **Control (CON) -** supply of basal diet [75% of corn silage + 25% of sugarcane bagasse, achieving 5.5% crude protein (CP), thus representing levels of PC similar to those found in pastures under dry season conditions in the central west and southeastern in Brazil, plus a mineral mixture] or **Supplement (SUP) -** basal diet plus protein supplementation 40% CP at the level of 3.5 g / kg body weight (BW). The composition of supplements and basal diet are shown in Table 1. The composition of supplements consisted of a 50:50 mixture of soybean meal and commercial supplement. For made adjustments in the supplement supply, once a month the cow's body weight was recorded after 12 hours of fasting. From 200 days of gestation until the parturition, all cows received a diet containing only corn silage and mineral supplement. Cows had free access to water and were individually fed *ad libitum* twice daily (07:00 a.m. and 01:00 p.m.). The amount of diet provided was adjusted for DM content weekly, based on DM content of corn silage and sugarcane bagasse.

3. Measurements

3.1 Insulin, glucose, and plasma amino acids.

Blood samples were taken from all cows on d 200 ±5 and d 270 ±5 of gestation. The samples were taken at 0700 h, before feeding, from the jugular vein using tubes with coagulation accelerator and vacutainer tubes containing sodium heparin, EDTA K3. Samples (10 mL), the blood samples were placed on ice immediately and plasma was separated and

stored at -20◦C until analyzed. One blood sample from each animal was carried to a commercial laboratory (VIÇOSA LAB laboratory, Viçosa, MG, Brazil) and analyzed high-performance-liquid chromatography according to Visser et al., (2011). AAs were evaluated individually, being classified as essential (Arginine, Phenylalanine, Histidine, Isoleucine, Methionine, Leucine, Tryptophan) and non-essential (Aspartate, Glutamate, Asparagine, Serine, Glutamine, Tyrosine, Alanine), and grouped according to their functions (branched-chain AA, glucogenic and ketogenic).

Blood samples were collected at 200 d (final supplementation) and 270 d of gestation via caudal vein puncture using tubes with coagulation accelerator and vacutainer tubes containing sodium heparin, EDTA K3. Samples (10 mL), were placed on ice and immediately centrifuged at 2700 G for 20 minutes, and then stored at - 20 °C. Samples from each cow were analyzed in a duplicated assay.

Blood glucose analyses were performed by the colorimetric method (Glucose PAP Liquiform, Labtest®, Lagoa Santa, Brazil, limit of photometric detection 0,41mg/dL). Concentrations of glucose were quantified with Oxidase / Peroxidase Liquid Stable Reagent using God-Trinder methodology. In triplicate, plasma at a concentration of 0.01ml was maintained at 4 °C in a water bath before the addition of reagent until incubation. Immediately following vortexing of samples and reagent, samples were incubated in a water bath at 37 °C for 10 min; after incubation, the equipment was adjusted to a wave compression of 500 nm for reading. Plasma urea-N was determined using the urease Berthelot procedure by the enzymatic method according to the modified diacetyl method (Urea PAP Liquiform, Labtest®, Lagoa Santa Brazil).

To analyze the energy profile, serum concentrations of b-hydroxybutyrate (BHBA) in plasma samples were determined by the enzymatic kinetic method (Randox Laboratories Ltd), non-stereized fatty acids (NEFA) by the colorimetric method (Randox Laboratories Ltd). Bovine plasma IGF-1 (*Cloud-Clone* Corp) and Insulin (*Cloud Clone* Corp) concentrations were quantified by the Elisa test.

3.2 Skeletal muscle tissue sampling

Skeletal muscle samples were biopsied from each animal at 200 and 270 days of gestation. 3 mL subcutaneous and 3 mL intramuscular lidocaine were applied to the biopsy site. Samples of about 1 g muscle tissue were obtained from right *Longissimus dorsi* muscle through percutaneous biopsies with Bergström-type needles according to Van Thienen et al.,

(2014). All muscle samples were immediately placed in 2 mL cryovial, snap-frozen in liquid nitrogen, and stored at –80°C until analysis.

Total RNA extraction was performed from 50 mg of muscle samples using QIAzol (QIAGEN, Valencia, CA) and treated with DNA-free DNase (Ambion, Austin, TX) according to the manufacturer's instructions. To evaluated RNA integrity, was analyzed the 28S and 18S rRNA bands, the total RNA was electrophoresed in a 1.0% (m/v) agarose gel, stained with GelRed nucleic acid gel stain (Biotium, Hayward, CA), and visualized with a UVItec FireReader XS D-77Ls-20M (UVItec, Cambridge, UK). cDNA synthesis was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and samples were stored at −20°C. RIN/RQI or Cq of 3 and 5 transcripts Inhibition testing (Cq dilutions, spike, or other).

Reverse-transcription quantitative PCR (RT-qPCR) was performed on the Eppendorf Realplex system (Eppendorf, Hamburg, Germany) using the SYBR Green detection system (Applied Biosystems, Foster City, CA, USA). For each reaction, 1.0 µL of cDNA (100 ng/µL), 0.3 µL of each primer (1.5 µM; forward and reverse) and 5.0 µL of SYBR Green Master Mix were combined in a 10.0-µL final volume per sample in a 96-well MicroAmp Optical plate (Applied Biosystems). The results were normalized using the threshold cycle (CT) Primers of each gene are presented in **Table 1**. Method for the expression of the reference genes 18S and D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers for target gene amplification and endogenous amplification were designed using the PrimerQuest program (https://www.idtdna.com/site/account/login?returnurl=%2FPrimerquest%2FHome%2FInd x) with sequences obtained from the Gen-Bank database. The PCR primers were commercially synthesized (Life Technologies, São Paulo, BR) and reconstituted to a final concentration of 10 µmol.

3.4 Statistical analyses

A completely randomized 2 × 2 factorial design was used, referring to maternal nutrition and progeny's sex (treatment as fixed effects) and year 1 and 2 (random effect). A full fixed-effect model was used:

$$Y_{ijk} = \mu + D_i + S_j + F_k + (DS)_{ij} + (BW)_{ijk} + e_{ijk}$$

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 dietary treatment (2 levels); S_j is the fixed effect of the j^{th} level of calf sex (2 levels); F_k is the random effect or the F^{th} level of the year (2 years); DS_{ij} is the interaction between D and S; BW_{ijk} is covariate of initial empy body weight, initial BCS,

gestation time and parity of the dam (when pertinent); and e_{ijk} is the random error associated with Y_{ijk}, with $e_{ijk} \sim N(0,\sigma_e^2)$.

Before the final analyses, Studentized residuals were removed when not within ± 3 standard deviations, and normality (*P*-value > 0.05) was assessed using Shapiro-Wilk's test. When data were not normally distributed, they were transformed using PROC RANK of SAS. When pertinent, repeated measurement procedures were used. Least-squares means were separated using Fisher's least significant difference test. Results were deemed significant when *P*-value ≤ 0.05 and trending when 0.05 < *P*-value ≤ 0.10. All analyses were performed using SAS 9.4 (Statistical Analysis System Institute, Inc., Cary, NC, USA).

4. Results

Results of Average daily gain, body condition score, and DMI during the gestation period have been previously reported (Meneses et al., 2021a unpublished data). There was no interaction between maternal nutrition and calf sex at 100, 200, and 270 days of gestation for maternal body weight pregnant, empy bodyweight not pregnant, and body condition score. The protein supplementation between 100 and 200 days of gestation increased by 15.1% (P = 0.03; 6.27 vs 6.92 ± 0.30) the forage intake in absolute values, while the total DMI (Forage + Supplement) was also higher in SUP cows (P ≤ 0.01; 6.01 ± vs 8.16 ±). Protein supplemented (SUP) cows had a greater average daily gain (ADG) than CON cows (P ≤ 0.01; 0.359 vs -0.288) as well as the daily gain of the PREG component, who showed a 34.7% increase in the SUP group.

4.1 Insulin, glucose, and plasma amino acids.

There was no interaction between maternal nutrition and calf sex for plasma concentration of hormones and metabolites (Table 3). Concentrations of insulin, glucose and IGF-1 in plasma of SUP cows were greater (*P* ≤ 0.01) during 200 and 270 d of gestation compared with CON cows. NEFA and urea plasmatic concentration at 200 were no different between treatments (P ≥ 0.10), but during the last gestation CON cows showed higher (*P* ≤ 0.01) plasma concentrations of NEFA (0.110 mmol/L v. 0.078 mmol/L ± 8.07) and Urea (35.44 mg/dL v. 30.64 mg/dL ± 3.15) respectively. BHBA plasmatic concentration tended (*P* = 0.08) to be greater in supplemented than in unsupplemented cows at mid-gestation.

There was no interaction between maternal nutrition and calf sex in most of the AAs evaluated in the plasma (P ≥ 0.10). However, at 200 d of gestation, maternal treatment and progeny sex interaction (P = 0.08) was observed for non-essential and Glycogenic AAs, as for

the 270 days of gestation, there was interaction for Branched-chain AAs (P = 0.06). AAs were grouped according to their metabolic functions in table 3 to improve understanding of the metabolic processes in which they participate. Supplement cows had an approximately 50% increase in the concentration of total circulating AAs (P = 0.03) during mid and late gestation when compared to CON cows. The level of essential AAs tended to be higher in SUP cows (P = 0.07) in both evaluated periods. On the other hand, in the mid-gestation, there was an interaction between maternal treatment and sex for AAs, with pregnant CON cows with female calves showing on average 30% and 40% less in the plasma concentration of non-essential and Glycogenic AAs respectively (Figure 1). However, at 270 days of gestation, the same experimental group showed a 60% increase in the plasma concentration of Branched-chain AAs (Figure 2). No differences were found for the remaining AA groups (ketogenic, glycogenic, and ketogenic) in the two gestation times evaluated except for glycogenic and ketogenic AAs to late gestations ($P \leq 0.01$), where SUP cows showed an increase of approximately 26% in their plasma concentration.

The AAs presented individually are shown in Table 4. There were no differences and interactions between maternal nutrition and calf sex for the majority of AAs considered essential in mid-gestation except for Phenylalanine, whose concentration was higher in CON cows pregnant with female calves. In the case of AAs considered non-essential, glutamine presented plasma concentration 3 times lower in CON cows pregnant with female calves (P = 0.02) (Figure 3), while tyrosine and alanine showed increases (P = 0.03) of approximately 25% in SUP cows. In late gestation within the group of essential AAs, there were increases (P = 0.02) in the plasma concentration of phenylalanine, leucine, and tryptophan in cows that received protein supplementation in mid-gestation, however, there was an interaction between maternal treatment and calf sex for AAs involved in the metabolism of one-carbon (methionine and isoleucine, Figure 4), with concentrations of 50% higher in CON cows pregnant with female calves. While for the group of non-essential AAs, only differences between groups were found for the AAs tyrosine and alanine, with greater concentration on SUP cows.

4.3 Skeletal muscle tissue gene expression

There was no interaction between maternal nutrition and calf sex for mRNA expression of markers in skeletal muscle tissue in mid and late gestation ($P \geq 0.10$). In mid-gestation, SUP cows increased between 30 and 40% in mRNA expression of markers related to muscle protein synthesis and degradation EIF4E (P = 0.04) and GSK3β (P = 0.03), MUFR1

(P = 0.09) when compared with CON cows. On the other hand, no differences were found for the expression of ATROGIN and P7056K mRNA (Table 5). In late gestation, the behavior about the increase in the relative mRNA expression of markers related to the synthesis and degradation of muscle protein EIF4E (P = 0.06) and GSK3β (P = 0.10), MUFR1 (P = 0.04) was maintained for SUP cows, while no differences were found for the expression of ATROGIN and P7056K mRNA.

5. Discussion

This study provides clear evidence that nutritional corrections via supplementation during the mid-gestation (DM basis) around 3.5 g/kg of BW alter the performance and metabolism of maternal tissues. Besides, this study also shows that protein supplementation during mid-gestation will bring higher amino acids (Thr, Ala) since it improves the intake of CP what is needed to give a live weight gain response in the dams and help to support the development and increase the genotypic growth potential of the fetus and, thus, despite increasing the need for mobilization of the dams tissue at the end of gestation (increase in the growth potential of the calf, increase in the nutritional requirement and decrease in the dry matter intake) the protein supplementation nonetheless helped to preserve greater reserves of maternal tissue in cows during throughout pregnancy (+15 kg) when kept on low quality pasture, also help to improve the efficiency of intestinal protein utilization (Poppi and McLennan., 1995). These discoveries are of significant interest to the cattle industry in Brazil, as protein is the most deficient nutrient in pastures during the dry season, a time that coincides with around 2/3 of the gestation period for beef cows in most regions of the country (Ruas et al., 2000; Lazzarini et al., 2009).

Previous studies have investigated the effects of protein supplementation for cows grazing on low-quality forages on the increase of dry matter intake, (Summer et a., 2015; Köster et al.,1996; Poppi et al., 1995 Bandyk et al., 2001). The level of protein supplementation (3.5 g/kg of the BW) was chosen to potential positive associative effects on the intake of forages, SUP animals had an increase to 15.1 % in forage intake and total dry matter intake, which caused an increase in energy intake. On the other hand, elevations in plasma AAs concentration in SUP cows after 100 days of supplementation indicate that the nutritional correction applied was successful, due to the higher proportion of metabolizable protein absorbed in the small intestine. When the intake of MO is adequate, the absorption and subsequent deposition of protein are linearly related to the weight gain and body condition of the animal (Summer et al., 2015; Stalker et al., 2007; Titgemeyer and Loest

2001).

On the other hand, hormonal and metabolic results have resulted in a good model for determining the possible effects of nutritional correction during mid-gestation. According to Lents et al., (2005) and Ciccioli et al., (2003), increases in plasma concentrations of insulin, glucose, and IGF-1 in SUP cows may indicate an increase in the availability of circulating nutrients, which allowed the animals to improve BCS and greater body weight gains at the end of the supplementation period. Results similar to those found in this study were presented by McLean et al., (2018). Even, lower concentrations of IGF-1 in the CON cows was probably due to the lower number of GH receptors in the liver, which caused this response due to the state of catabolism in which the animals were found (lower concentrations of glucose and plasma insulin) (Pires et al., 2013). According to Yambayamba et al., (1996) and Breier et al., (1999), IGF-1 is the main regulator of protein synthesis in several tissues, including skeletal muscle tissue. Regarding plasma urea concentration, although there was no difference between the experimental treatments, at 200 days, both groups presented high plasma concentrations, according to Chapa et al., (2001), high plasmatic concentrations reflect an energy imbalance in the diet or high concentrations of dietary protein, situations that could happen in this study with the CON and SUP treatments.

Regarding, the plasma concentrations of NEFA and BHBA at 200 days of gestation suggests that there was a little mobilization of maternal adipose tissue. These differences in maternal metabolism indicate that the cows in the SUP group provided the fetus with a greater amount of nutrients during the phase of greater muscle fiber formation, which was represented in the increase in the PREG component and calves with higher birth weights (P = 0.04, 27.09 kg v 31.08 kg ±3.68; Nascimento et al., unpublished data). In the late gestation, the plasma concentration of glucose and insulin decreased in both treatments but remained higher in the cows of the SUP group, which can be explained by the greater demand for fetal nutrients. According to Kolnes et al., (2015) and Bell and Bauman (1997), lower plasma concentrations of glucose and low sensitivity to insulin responsiveness obey the direct influence of homeorectic hormones, which prioritize the distribution of maternal glucose for favoring of the conceptus. Data in the literature report a decrease in dry matter intake at the end of pregnancy due to factors that are either physical or physiological (Gionbelli et al., 2013). Decreased intake results in weight loss and BCS, in addition to high plasma concentrations of NEFA and BHBA, which causes the cow to enter a negative energy balance. In this study, the plasma concentrations of NEFA were affected by the treatment applied in mid-gestation, SUP cows had lower concentrations of NEFA in the prepartum,

which indicates a lower lipolytic rate of adipose tissue when compared with the CON (Tanner et al., 2020; Lopes et al., 2016)

In the study, SUP cows significantly increased the majority of the concentration of free amino acids in plasma evaluated in this study, indicating greater availability of AAs for muscle protein synthesis and accretion, which may eventually lead to better maternal performance and benefit the flow of AAs to the GEST component, which showed a 30% increase in weight at the end of the supplementation period and late gestation, when compared to the CON group. Several authors have reported that the addition of body tissue reflects the physiological conditions and the energy balance of the animals since the reduction in the body protein synthesis process are suggested as a potential reason for weight loss in animals that are fed below their protein requirements, as happened in this study with CON cows (Monirujjaman et al., 2014; Bohnert et al., 2013; Cynober et al., 2002)

On the other hand, the concentration of intracellular AAs has been suggested as the main reason that induces the process of protein synthesis in the skeletal muscle, and the metabolism of glucose and lipids (Nie et al., 2018; Wu et al., 2014). Protein supplementation in mid-gestation increased the use of almost all AAs in SUP cows, the results indicated differences and trends for Tryptophan (P= 0.02), Phenylalanine (P= 0.03), leucine (P <0.01), isoleucine (P= 0.03), methionine (P= 0.05), total amino acids (P= 0.03) non-essential amino acids (P= 0.02), glycogenic and ketogenic AAs (P <0.01) and glycogenic amino acids (P= 0.10) in both periods of gestation. Higher plasma glucose concentrations in SUP animals suggest that gluconeogenesis would be increased for the greater availability of AAs. Some authors indicate that the net consumption of glutamate, serine, and leucine by uteroplacental tissues indicates catabolism or transamination of these essential AAs for fetal growth (Roos et al., 2007; Wu et al., 2010).

On the other hand, higher concentrations of methionine and isoleucine in pregnant cows with female calves can be seen as a mechanism for compensating the placenta to increase the growth of placentomas, thus improving their ability to absorb nutrients and transport O^2, according to Cetin et al., (1996) and Garnerd et al., (2002), an increased amount of amino acids would be directed towards the synthesis of placental protein, being important not to impede fetal growth and development, since the placenta in response to changes in the supply of nutrients regulate the delivery of nutrients to the fetus (Mordhorst et al., 2017).

Another evidence that shows that SUP animals used the essential AAs better, was the higher plasma concentrations of Leucine, glutamine, aromatic amino acid (tryptophan, Tyrosine, histidine), and alanine (important glycogenic AA), which in addition to probably

explaining the greater growth of the GEST component and may explain the greater gains in the Ribeye area (P = 0.03; **SUP**: 121.10 vs **CON**: 105.54) and Rump area, cm2 (P = <0.01; **SUP**: 109.70 vs **CON**: 99.06; Meneses et al., 2021a unpublished data) respectively since an increase in the circulating level of this AAs in the blood has the potential to activate cell signaling pathways via MTOR, that stimulate protein synthesis in mammary tissues, signaling protein synthesis in skeletal muscle, starting from protein phosphorylation (p70S6 kinase and 4E-BP1) and the subsequent synthesis of polypeptides (Lei et al., 2012; Murgas et al., 2010). Wu and Thompson, (1990) and Meijer and Dubbelhuis, (2004), demonstrated that glutamine and leucine also inhibit protein breakdown in skeletal muscle. Lower concentrations of these AAs during pregnancy may contribute to an increase in protein degradation and a decrease in protein synthesis in maternal and fetal skeletal muscle. Greater muscle growth shown by SUP cows in mid-gestation can also be explained too by the higher concentrations of aromatic amino acids. According to Dukes et al., (2015) and Glass (2003), tryptophan is a precursor to serotonin, which has the function of modulating the production of growth hormone (GH) in the hypophysis, which in turn induces the production of the insulin-like growth factor (IGF-I), derived from the liver. IGF-1 signaling is known to play a key role in regulating muscle mass.

In the case of alanine (high plasma concentrations), which receives an amino group from BCAAs, has the role of regulating the processes of gluconeogenesis and glycolysis inhibiting pyruvate kinase, to guarantee the production of glucose for hepatic cells during periods of food restriction or during the period of greatest fetal demand (Pre-partum), higher concentrations of this AA in SUP cows represent the greater mobilization of skeletal muscle tissue to support the growth and development of heavier fetuses through glucose uptake (main fuel source for the fetus and placenta, since alanine is quantitatively considered an important nitrogen transporter from skeletal muscle to the liver) since according to bell (1995) skeletal muscle tissue is the largest body reserve of AAs to support changes in hepatic metabolism at the end of pregnancy and the start of lactation (Bell et al., 2000; Drackley et al., 2001). In this study, differences were found between the plasma concentrations of glucose (P = 0.05; **SUP**: 53.3 Mg / dL vs C**ON**: 48.4 Mg / dL) and insulin (P <0.01; **SUP**: 9.05 µIU / mL vs **CON**: 6.58 µIU / mL) between treatments in the late gestation. About insulin, there was a decrease in plasma concentration of approximately 10%, between mid-gestation and late gestation, which can be explained by the regulation of gluconeogenesis via endocrine control between experimental treatments, since insulin inhibits uptake hepatic from subtracts such as alanine, glycerol, lactate, and glutamine for glucose production (Donkin, 1999).

In this study, mRNA levels of skeletal protein synthesis markers (EIF4E, GSK3β) and

degradation (MUFR1) changed based on CP dietary differences between experimental treatments ($P \leq 0.05$). These markers are involved in the dynamic relationship of protein synthesis and degradation and energy metabolism of muscle tissue (Jones et al., 1990; Lobley, 2003). We observed in the present study that SUP cows during the protein supplementation period showed an increase in the abundance of protein synthesis markers in the *longissimus dorsi* muscle of eIF4E and GSK3β. Both transcription factors are involved in the regulation of protein anabolism in skeletal muscle tissue in response to food, since by the hormonal pathway by increasing the plasma concentration of insulin and IGF-1 or by the plasma concentration of AAs (Balage et al., 2001), both factors increased in this study for the application of maternal treatment in mid-gestation. EIF4E is part of a group of specific proteins called eukaryotic initiation factors and is in charge of regulating the initiation of translation (Kelly and Jefferson, 1985; Kimball et al., 1998). Insulin and AAs regulate the start of translation involving phosphorylation/dephosphorylation of the group of eukaryotic initiation proteins, especially the translation regulator eIF4E binding protein-1 (4E-BP1) which has the role of inhibiting translation, that is, it downregulates protein synthesis. According to Tomek et al. (2002), insulin and amino acids increase the phosphorylation state of 4E-BP1, which results in the dissociation of the 4E-BP1 complex, releasing eIF4E to participate in the assembly of the eIF4E complex and subsequent protein synthesis. ·

In the case of glycogen synthase kinase, Shin et al., (2014), showed evidence that (GSK) -3β promotes cell proliferation through the positive regulation of protein synthesis. The authors found that GSK-3β phosphorylates and inactivates 4E-BP1, thereby increasing eIF4E-dependent protein synthesis. On the other hand, MuFR1 P (P=0.09), tended to be higher in animals supplemented in mid-gestation, suggesting that skeletal muscle synthesis and rupture was occurring (indicating that the breakdown of the muscle was the result of increased protein synthesis), which confirms the greater growth of muscle tissue during mid-gestation (higher body weight, better BCS, greater area of the rib eye and greater area of the rump). These results are similar to those found by Lopes et al., (2020), who found greater expression of markers (GSK3β and p7056k) for protein synthesis in supplemented animals during gestation.

In late gestation, both experimental groups had protein restriction at the same levels, according to the DMI, both treatments ingested about 40% of their protein needs, this caused a decrease in the abundance of protein synthesis markers when compared to what occurred in the mid-gestation, therefore, there was less weight gain, less BCS, smaller eye areas of rib and rump, also the results of this study indicate that there was a breakdown of the skeletal muscle

tissue (MuFR1 P = 0.04).

MuFR1 was evaluated as one of the main genes in the atrophy process due to its predominance of expression in skeletal muscle. In this study, at the end of gestation, the abundance of MuFR1 was higher in SUP cows, which ended up losing more maternal body tissue when compared with CON cows, since skeletal muscle is considered the main repository of proteins in the body, a source that is used to provide a pool of amino acids for the fetus, tissue repair and gluconeogenesis in conditions of malnutrition and other metabolic stresses. According to Bodine et al., (2001), the expression of MuRF1 is positively regulated by the family of transcription factors known as FOXO, which have been cataloged as the main regulators of muscular atrophy (Schiaffino and Mammucari, 2011). The family of FOXO acts inhibit the IGF 1-Akt pathway and induce decreased IGF-1 production and consequently protein synthesis (Schiaffino and Mammucari, 2011; Waddell et al., 2008; Glass, 2005). The MuRF1 is stimulated when the nerve that innervates a muscle is cut, resulting in paralysis and severe atrophy; or when they are positively regulated by simple immobilization of skeletal muscle or by an increase in glucocorticoids (cortisol and corticosterone), stress and nutritional status, causing muscle atrophy. In this study, CON cows showed proteolysis of the maternal carcass throughout pregnancy, which was consistent with the performance results observed, while SUP cows showed greater expression of EIF4E (0.06) and GSK3β (0.10) which meant slight protein synthesis despite the degradation of maternal skeletal muscle tissue.

6. Conclusion

This study showed that supplementing in mid-gestation is the best strategy to improve the energy status of cows, which was supported by the increase of glucose, IGF-1, circulating amino acids in the plasma and the abundance of protein synthesis markers in maternal skeletal muscle tissue. Furthermore, this study suggests that the maternal skeletal muscle plays an important role at the end of gestation since the supplemented cows also went through catabolic processes of muscle degradation attributed to the production of calves probably with greater gain potential (greater gestational component).

Declaration of conflicts of interest.

The authors declare no conflicts of interest.

Acknowledgments.

This work was supported by the Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), Minas Gerais State Agency for Research, and Brazilian National Council for Scientific and Technological Development (CNPq) and Minas Gerais State Agency for Research and Development (FAPEMIG). The authors would like to thank Dr. Pedro Veiga R. Paulino (Cargill Animal Nutrition) for his support and insights on this research, the Beef Cattle Research Complex, the beef cattle study group (NEPEC), and those responsible for the installations of the animal science laboratory.

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Table 1. Nutrient content of dietary supplements and forage offered to cows during gestation

Chemical composition	Supplements[2]	Forage		
		Early	Mid	Late
DM (g/kg DM)	881.1	284.9	418.26	330.3
OM (g/kg DM)	957.8	916.7	951.05	941.4
CP (g/kg DM)	400.53	154.33	53.31	72.2
NDFap (g/kg DM)	213.03	568.0	631.48	549.2
NFC (g/kg DM)	342.1	112.57	242.13	290.7
EE (g/kg DM)	41.2	25.5	24.12	29.2
TDN [1](g/kg DM)	788.83	585.33	544.15	596.20

[1] calculated according to Detmann et al. (2010)
[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 Flavomy

Table 2. Sequence, NCBI[1] accession number, amplicon size, and efficiency of the primers used for quantitative PCR.

Gene abbreviation	Access number	Primer	Tissue	Function
p70S6k	AY396564.1	F TTGAACCAAAAATCCGATCC R AGCACCTCTTCCCCAGAAA	Muscle	Protein synthesis
GSK3B	NM_001101310.1	F GCCCAGAACCACCTCCTTT R TGCTGCCATCTTTGTCTCTG	Muscle	Protein synthesis
eIf4E	NM_174310	F AAACCACCCCTACTCCGAAT R TGCCCATCTGTTCTGTAAAGG	Muscle	Protein synthesis
MuRF1	NM_001046155.1	F GGGACAGATGAGGAAGAGGA R CCTCATCATCGCCTTACTGG	Muscle	Protein degradation
Atrogin-1	NM_001046155.1	F CCTTGAAGACCAGCAAAACA R AGACTTGCCGACTCTTTGGA R GAGTCATGCCGTAGTGGTTG	Muscle	Protein degradation
18S	NM_001033614	F CCTGCGGCTTAATTTGACTC R AACTAAGAACGGCCATGCAC	Muscle	Endogenous control
GAPDH	NM_001034034.1	F CGACTTCAACAGCGACACTC R TTGTCGTACACAAGGAAATGAGC	Muscle	Endogenous control

p70S6k = ribosomal protein S6 kinase, GSK3B = glycogen synthase kinase 3β; eIf4E = eukaryotic translation initiation factor 4 E, MuRF1 = muscle ring finger 1, ACACA = acetyl-CoA carboxylase alpha; Atrogin-1= muscle atrophy F-box protein; GHR1A = growth hormone receptor 1A; PC = pyruvate carboxylase; PCK1 = phosphoenolpyruvate carboxykinase 1;18S = 18S ribosomal; GAPDH = glyceraldehyde-3-phosphate dehydrogenase.

1 NCBI, National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov)

Table 3. Influence of protein Supplementation during mid-gestation on plasma hormones and metabolites in beef cows.

	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
Measurements at 200 days of gestation (end of supplementation period)								
IGF-1 (Ng/mL)	61.9	66.9	62.6	66.2	2.00	0.06	0.18	0.64
Insulin (µIU/mL)	6.70	10.4	8.77	8.37	0.45	<0.01	0.51	0.43
Glucose (mg/dL)	46.9	54.72	51.1	50.5	1.31	<0.01	0.71	0.62
Urea (mg/dL)	36.09	36.43	36.23	36.20	2.16	0.85	0.94	0.78
NEFA (mmol/L)	0.108	0.100	0.1086	0.099	0.01	0.56	0.50	0.63
BHBA (mmol/L)	0.34	0.26	0.29	0.31	0.04	0.08	0.59	0.93
Measurements at 270 days of gestation (prepartum)								
IGF-1 (Ng/mL)	58.80	62.09	59.57	61.33	3.20	0.20	0.48	0.33
Insulin (µIU/mL)	6.58	9.05	7.78	7.85	0.59	<0.01	0.87	0.14
Glucose (mg/dL)	48.4	53.3	52.5	49.1	1.90	0.05	0.18	0.79
Urea (mg/dL)	35.44	30.64	33.43	32.65	3.15	0.09	0.70	0.61
NEFA (mmol/L)	0.105	0.078	0.089	0.099	0.90	0.01	0.42	0.07
BHBA (mmol/L)	0.40	0.48	0.45	0.42	0.05	0.10	0.57	0.12

CON = control; unsupplemented

SUP= supplemented

Table 4. Influence of protein Supplementation during mid-gestation on the plasma concentration of total amino acids, and Glycogenic, Ketogenic and Glycetogenic amino acids in beef cows.

	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
			(nmol/mL)					
Measurements at 200 days of gestation (end of supplementation period)								
Total AA	1018.66	1532.94	1106.70	1444.90	128.06	0.03	0.34	0.10
Essential AA	611.72	661.67	646.91	626.47	66.1	0.11	0.66	0.31
Nonessential AA	579.23	833.66	621.04	791.04	66.7	0.02	0.16	<0.01
Glucogenic AA	787.87	965.78	842.49	911.16	71.9	0.10	0.44	0.08
Ketogenic AA	140.79	131.98	152.33	120.44	17.6	0.69	0.15	0.13
Glucogenic and ketogenic AA	400.27	449.85	426.46	423.66	37.9	0.49	0.48	0.65
Branched-chain AA	354.67	351.52	382.75	323.44	47.1	0.95	0.30	0.26
Measurements at 270 days of gestation (pre-partum)								
Total AA	1120.91	1698.86	1226.23	1583.23	128.9	0.01	0.47	0.17
Essential AA	689.00	802.23	761.01	730.22	53.5	0.07	0.50	0.81
Nonessential AA	677.88	886.63	711.50	853.01	71.2	0.09	0.21	0.19
Glucogenic AA	980.81	1003.79	1027.37	957.23	62.7	0.76	0.36	0.69
Ketogenic AA	201.57	156.24	212.18	135.63	54.79	0.17	0.03	0.15
Glucogenic and ketogenic AA	416.73	528.83	455.18	490.38	31.06	<0.01	0.35	0.51
Branched-chain AA	618.76	372.33	660.78	330.31	86.30	0.36	0.05	0.06

CON = control; unsupplemented; SUP= supplemented.

Table 5. Influence of protein Supplementation during mid-gestation on the plasma concentration of amino acids in beef cows.

	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
			(nmol/mL)					
Measurements at 200 days of gestation (end of supplementation period)								
			Essential					
Arginine	70.79	93.74	79.79	84.74	13.05	0.16	0.75	0.59
Fhenylalanine	78.05	74.40	88.51	63.95	11.7	0.80	0.10	0.08
Histidine	97.38	89.07	102.13	84.33	11.54	0.55	0.22	0.68
Isoleucine	124.62	121.84	138.16	108.30	18.29	0.90	0.19	0.31
Methionine	27.98	28.37	30.41	25.94	4.06	0.94	0.37	0.47
Leucine	121.84	117.31	129.08	110.07	12.16	0.76	0.21	0.19
Tryptophan	25.60	39.33	32.63	31.31	4.47	0.02	0.95	0.94
			Nonessential					
Aspartate	15.47	21.55	18.84	18.18	4.39	0.26	0.90	0.73
Glutamate	141.69	122.21	131.75	131.15	19.4	0.41	0.98	0.81
Asparagine	6.87	8.24	8.16	6.95	1.23	0.39	0.42	0.90
Serine	82.21	88.04	76.08	94.17	12.4	0.71	0.24	0.10
Glutamine	176.66	257.35	191.37	232.74	45.04	0.11	0.45	0.09
Tyrosine	43.40	59.88	48.27	55.01	4.37	<0.01	0.21	0.25
Alanine	229.48	282.05	256.64	254.89	19.3	0.03	0.94	0.15
Measurements at 270 days of gestation (prepartum)								
			Essential					
Arginine	65.33	85.29	69.48	81.14	10.4	0.12	0.36	0.23
Fhenylalanine	47.71	63.24	57.26	53.70	2.88	0.03	0.58	0.84
Histidine	94.94	101.30	96.71	99.52	9.66	0.59	0.81	0.48
Isoleucine	191.49	121.48	196.51	116.05	22.53	0.69	0.20	0.03
Methionine	44.18	27.95	47.48	26.64	5.18	0.36	0.02	0.05

Leucine	122.05	156.24	142.63	135.63	5.97	<0.01	0.52	0.67
Tryptophan	21.63	31.32	27.66	25.30	3.52	0.02	0.55	0.69
			Nonessential					
Aspartate	9.22	9.31	8.54	8.90	4.62	0.89	0.64	0.96
Glutamate	286.27	317.08	299.18	304.16	26.02	0.33	0.87	0.12
Asparagine	25.92	27.33	25.42	26.55	17.4	0.70	0.71	0.72
Serine	107.20	103.81	100.03	107.97	13.8	0.84	0.51	0.24
Glutamine	98.35	87.74	87.92	98.17	12.2	0.47	0.49	0.89
Tyrosine	37.81	55.90	42.50	51.21	2.85	<0.01	0.02	0.26
Alanine	210.63	286.57	244.13	253.08	25.2	0.02	0.77	0.97

CON = control; unsupplemented
SUP= supplemented

Table 6. Influence of protein Supplementation during mid-gestation on changes in gene expression in the longissimus dorsi and liver tissue in beef cows.

	Maternal nutrition		Calf sex		SEM	P-value		
	CON (n = 26)	SUP (n = 26)	Female (n = 22)	Male (n = 30)		MN	CS	MN×CS
Measures from 100 to 200 days of gestation (end of supplementation period)								
ATROGIN	0.974	0.784	0.707	1.050	0.155	0.55	0.15	0.17
MUFR1	0.986	1.379	1.073	1.291	0.173	0.09	0.49	0.91
EIF4E	0.986	1.456	1.029	1.414	0.210	0.04	0.11	0.16
GSK3BETA	1.004	1.332	1.184	1.152	0.150	0.03	0.61	0.43
P70S6K	0.989	0.954	0.898	1.041	0.119	0.91	0.39	0.90
Measurements from 200 to 270 days of gestation (prepartum)								
ATROGIN	1.104	0.979	1.063	1.020	0.33	0.75	0.31	0.93
MUFR1	0.923	1.395	1.176	1.143	0.196	0.04	0.40	0.95
EIF4E	0.504	1.235	0.840	0.898	0.320	0.06	0.49	0.74
GSK3BETA	1.023	1.256	1.186	1.094	0.099	0.10	0.51	0.28
P70S6K	1.018	1.056	1.179	0.895	0.105	0.79	0.05	0.91

CON = control; unsupplemented
SUP= supplemented

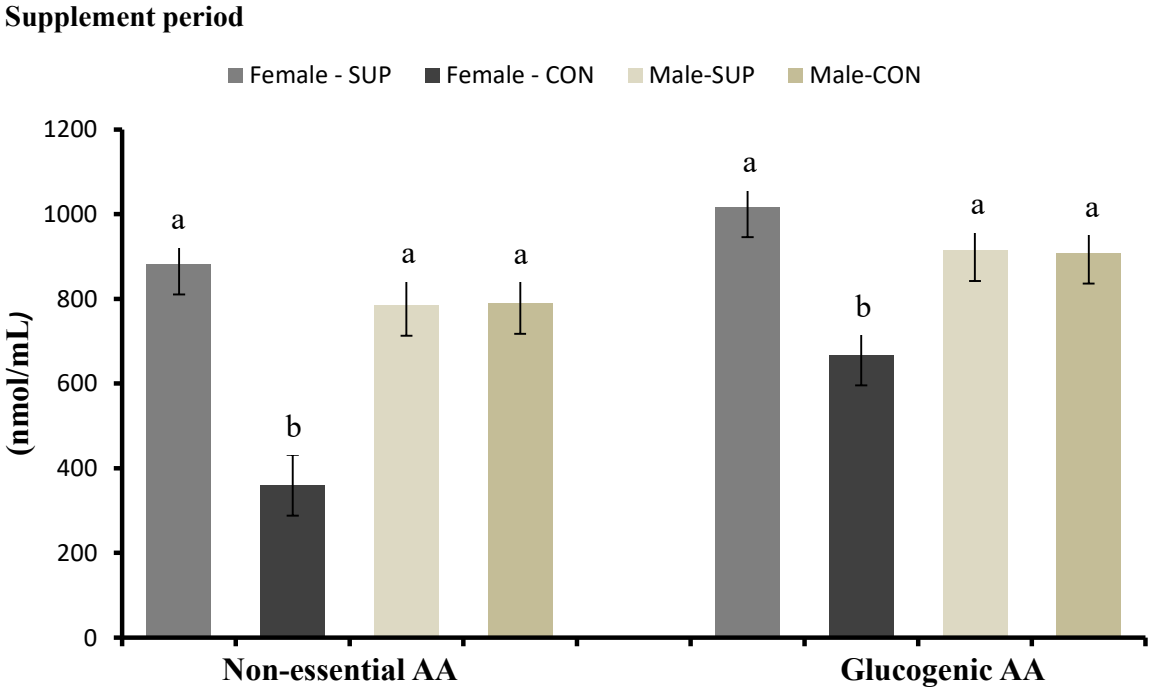

Figure 1. Interaction between maternal treatment and progeny sex at 200 d of gestation for non-essential and Glycogenic amino acids in beef cows. Means followed by a different superscript are different (P<0.05) by Tukey test.

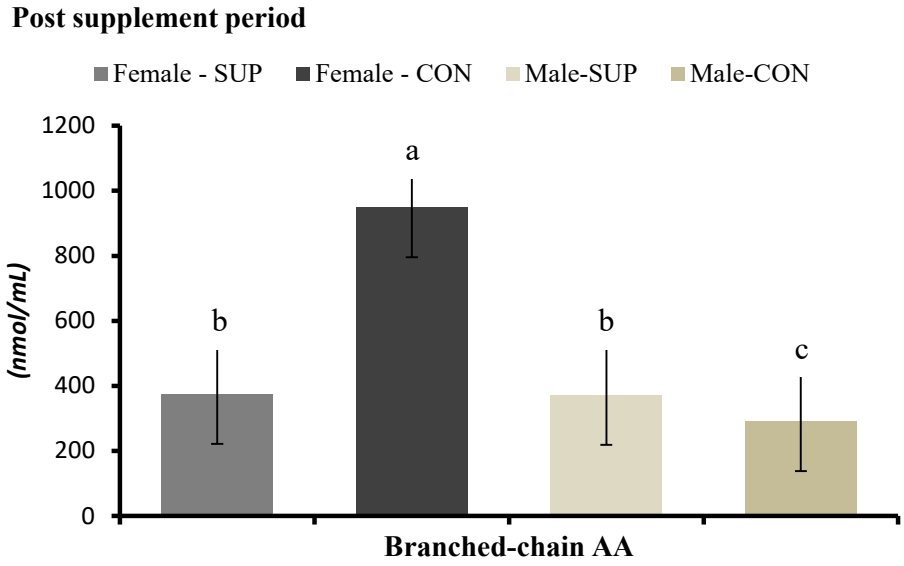

Figure 2. Interaction between maternal treatment and progeny sex at 270 d of gestation for branched-chain amino acids in beef cows. Means followed by a different superscript are different (P<0.05) by Tukey test-

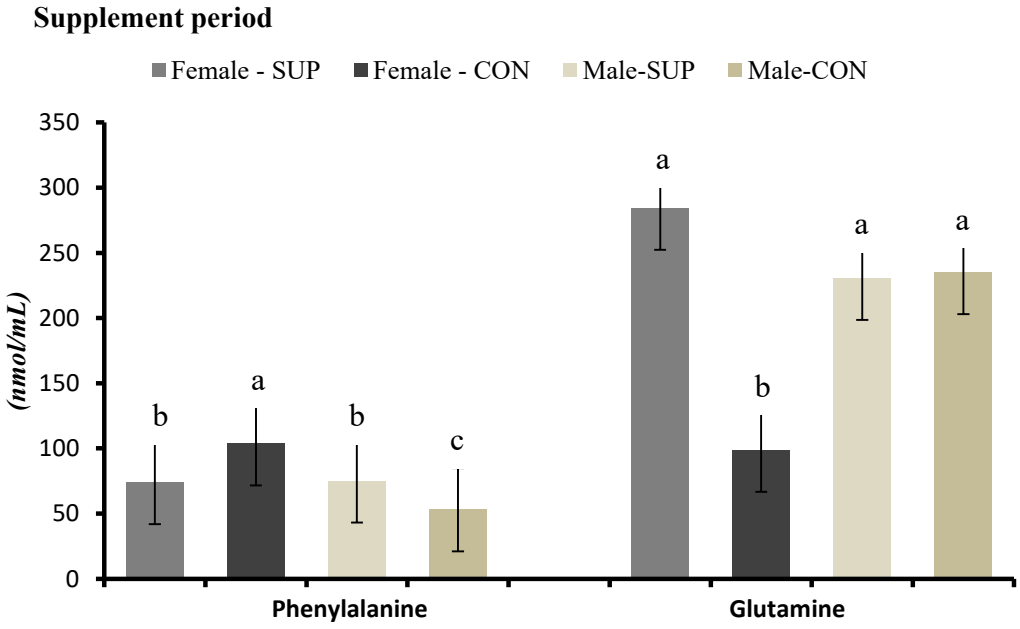

Figure 3. Interaction between maternal treatment and progeny sex at 200 d of gestation for Phenylalanine and Glutamine amino acids in beef cows. Means followed by a different superscript are different (P<0.05) by Tukey test-

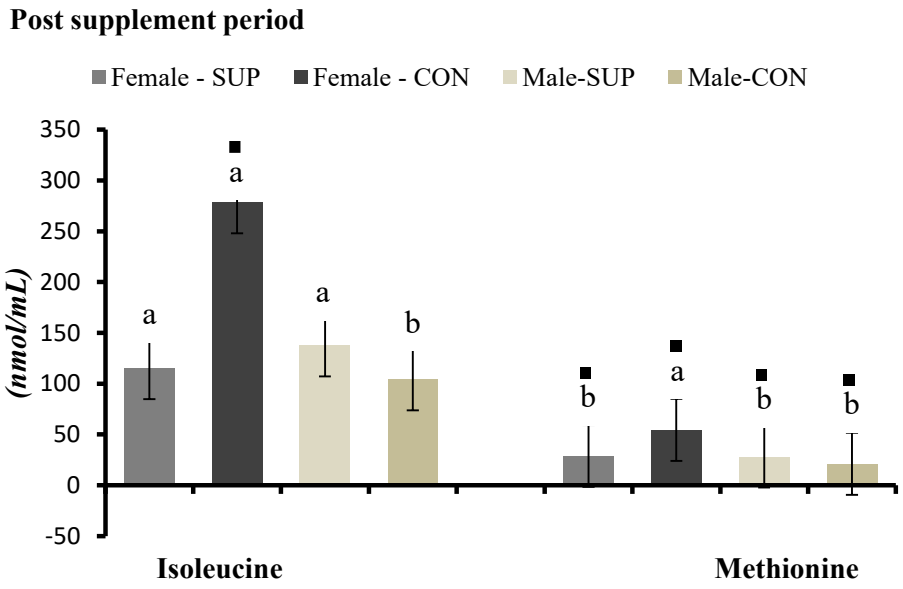

Figure 4. Interaction between maternal treatment and progeny sex at 270 d of gestation for isoleucine and Methionine amino acids in beef cows. Means followed by a different superscript are different ($P<0.05$) by Tukey test.