LUANA RUIZ DOS SANTOS

EFFECTS OF SUPPLEMENTATION OF DEGRADABLE OR UNDEGRADABLE PROTEIN IN THE RUMEN FOR BEEF COWS SUBJECTED TO PROTEIN RESTRICTION DURING MID-GESTATION ON OFFSPRING DEVELOPMENT

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

Prof. Dr. Mateus Pies Gionbelli

Advisor

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Approved on October 4, 2024.

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ABSTRACT

The study investigated how maternal supplementation with a product rich in rumen-protected protein, primarily containing arginine, affects the long-term growth and development of the offspring. Thirty cows were randomly divided into three groups to receive different types of supplementation during the second half of gestation: (1) Control (**CON**, n = 10) – basal diet + mineral supplement containing urea; (2) Rumen-Degradable Protein Supplement (**RDP**, n = 10) – basal diet + protein supplement + ground corn; (3) Rumen-Undegradable Protein Supplement (**RUP**, n = 10) – basal diet + arginine-rich product + mineral supplement containing urea. At birth, the offspring were weighed, vigor was assessed, morphometric measurements were taken, and blood samples were collected. At 48 ± 10 days, biopsies of the muscle were performed on the calves to evaluate muscle fibers and the expression of genes related to metabolism and development of skeletal muscle tissue. The calves were weaned at 210 days and weighed. Out of the 30 cows, only 23 calves remained in the study due to twin births and deaths during the cow-calf phase. At 286 ± 31 days, the offspring (**CON**, n = 9; **RDP**, n = 8; **RUP**, n = 6) were weighed and underwent a backgrounding phase, receiving the same diet for 60 days. At 306 ± 31 days, an evaluation of diet consumption and digestibility was conducted; at 319 ± 31 days, the ingestive behavior of the offspring was assessed; and at 346 ± 31 days, the final weighing of the calves and biopsies of the muscle were performed. Maternal treatment did not influence birth vigor or the blood parameters of the offspring ($P \geq 0.200$). However, the offspring of RUP cows had a greater birth weight ($P = 0.043$) compared to the other treatments; larger morphometric measurements, such as thorax width ($P = 0.015$), distance between ilium bones ($P = 0.006$), ileum-ischium distance ($P = 0.011$), withers height ($P < 0.001$), rump height ($P = 0.019$), and body circumference ($P = 0.006$), compared to the CON group; and greater withers height ($P < 0.001$) compared to the RDP group. Between the RDP and CON offspring, only the RDP group showed greater thorax width ($P = 0.015$) and greater distance between iliac bones ($P = 0.006$). RUP calves exhibited higher expression of the genes *ACACA* ($P = 0.001$), *COL3A1* ($P = 0.050$), *CPT2* ($P = 0.046$), *FASN* ($P = 0.022$), *PPARG* ($P = 0.036$), and *IGFR1* ($P = 0.017$) compared to CON calves. Additionally, the RUP offspring showed greater expression of the genes *ACACA* ($P < 0.001$), *COL3A1* ($P = 0.011$), *IGFR1* ($P = 0.024$), *FABP4* ($P = 0.010$), *PPARG* ($P = 0.044$), *PPARA* ($P = 0.029$), and *SCD1* ($P = 0.042$) compared to the RDP group. Therefore, supplementing diets low in rumen-protected protein for beef cows during the second half of gestation promotes the performance of newborns and may influence the performance and meat quality of the offspring in adulthood.

Keywords: Arginine; bypass protein; fetal programming; ruminants.

RESUMO

O estudo investigou como a suplementação materna com um produto rico em proteína protegida da degradação ruminal, cujo o principal aminoácido presente é a arginina, afeta o crescimento e desenvolvimento a longo prazo da progênie. Trinta vacas foram aleatoriamente divididas em três grupos para receber diferentes suplementações durante a metade da gestação: (1) Controle (**CON**, n = 10) – dieta basal + suplemento mineral contendo ureia; Suplemento proteico degradável no rúmen (**RDP**, n = 10) – dieta basal + suplemento proteico comercial + milho moído; (3) Suplemento proteico não degradável no rúmen (**RUP**, n = 10) – dieta basal + produto rico em arginina + suplemento mineral contendo ureia. Ao nascer, foram avaliados o peso, o vigor, as medidas morfométricas e coletadas amostras de sangue da prole. Aos 48 ± 10 dias, foram feitas biópsias de músculo dos bezerros para avaliar as fibras musculares e a expressão de genes relacionados ao metabolismo e desenvolvimento do tecido muscular esquelético. Os bezerros foram desmamados com 210 dias e pesados. Das 30 vacas, apenas 23 bezerros permaneceram no estudo devido a partos gemelares e mortes durante a fase de cria. Aos 286 ± 31 dias, a prole (**CON**, n = 9; **PDR**, n = 8; **PNDR**, n = 6) foi pesada e passou por um teste de ganho de peso com a mesma dieta por 60 dias. Aos 306 ± 31 dias, realizou-se uma avaliação de consumo e digestibilidade da dieta; aos 319 ± 31 dias, foi avaliado o comportamento ingestivo da prole; e aos 346 ± 31 dias, realizou-se a pesagem final dos bezerros e coletou-se biópsias musculares. O tratamento materno não influenciou o vigor ao nascimento e os parâmetros sanguíneos da progênie ($P \geq 0,200$), porém, bezerros PNDR apresentaram maior peso ao nascer (*P = 0,043*) comparados aos demais tratamentos; maiores medidas morfométricas, como largura do tórax ($P = 0,015$), distância dos ossos do íleo ($P = 0,006$), distância íleo-ísquio ($P = 0,011$), altura na cernelha ($P < 0,001$), altura da garupa ($P = 0,019$) e circunferência corporal ($P = 0,006$), comparados ao CON; e maior altura na cernelha ($P < 0,001$), comparados ao grupo PDR. Entre os descendentes PDR e CON, apenas o grupo PDR apresentou maior largura do tórax ($P = 0,015$) e maior distância entre ílios ($P = 0,006$). Bezerros PNDR apresentaram maiores expressões dos genes *ACACA* ($P = 0,001$), *COL3A1* ($P = 0,050$), *CPT2* ($P = 0,046$), *FASN* ($P = 0,022$), *PPARG* ($P = 0.036$) e *IGFR1* ($P = 0,017$), comparados ao CON. Além disso, bezerros PNDR apresentaram maior expressão dos genes *ACACA* ($P < 0,001$), *COL3A1* ($P = 0,011$), *IGFR1* ($P = 0,024$), *FABP4* ($P = 0,010$), *PPARG* ($P = 0,044$), *PPARA* ($P = 0,029$) e *SCD1* ($P = 0,042$), comparados ao grupo PDR. Portanto, a suplementação de vacas de corte com proteína protegida da degradação ruminal durante a metade da gestação melhora o desempenho dos recém-nascidos e pode influenciar o desempenho e a qualidade da carne na vida adulta da prole

Palavras-chave: Arginina; programação fetal; proteína by-pass; ruminantes.

INDICADORES DE IMPACTO

Os sistemas de produção de bezerros no Brasil são fortemente afetados pela sazonalidade da produção de forragens, especialmente devido ao baixo teor de proteína bruta nos pastos durante a estação seca, época esta que coincide com a formação de tecido muscular esquelético da progênie ainda no período pré-natal. Nesse contexto, os estudos de programação fetal em bovinos, envolvendo a suplementação materna com proteína protegida da degradação ruminal durante a metade da gestação, têm impactos significativos nas esferas social, tecnológica, econômica e cultural. Socialmente, ao garantir o crescimento e desenvolvimento saudável da progênie, esses estudos contribuem para a produção de bezerros mais robustos, aumentando a oferta de carne e melhorando a segurança alimentar. Tecnicamente, a pesquisa sobre suplementação nutricional materna impulsiona inovações na alimentação animal, permitindo um melhor aproveitamento de nutrientes e, consequentemente, promovendo maior potencial produtivo dos animais. Economicamente, a suplementação adequada resulta em recém-nascidos mais pesados e com melhores medidas corporais, o que pode encurtar o ciclo de produção e aumentar a lucratividade dos produtores ao melhorar a qualidade da carne. Culturalmente, esses avanços ajudam a transformar a percepção da pecuária, promovendo uma imagem mais científica e ética, e incentivando práticas sustentáveis que se alinham às tendências de produção consciente. Assim, os estudos de programação fetal não apenas melhoram a produção, mas também fortalecem a conexão entre a ciência, a agricultura e a sociedade.

IMPACT INDICATORS

Calf production systems in Brazil are strongly affected by the seasonality of forage production, particularly due to the low crude protein content in pastures during the dry season, which coincides with the formation of skeletal muscle tissue in the offspring during the prenatal period. In this context, studies on fetal programming in cattle, involving maternal supplementation with rumen-protected protein during mid-gestation, have significant impacts in social, technological, economic, and cultural spheres. Socially, by ensuring healthy growth and development of the offspring, these studies contribute to the production of more robust calves, increasing the meat supply and improving food security. Technically, research on maternal nutritional supplementation drives innovations in animal feeding, allowing for better nutrient utilization and consequently promoting greater productive potential in animals. Economically, adequate supplementation results in heavier newborns with better body measurements, which can shorten the production cycle and increase producers' profitability by improving meat quality. Culturally, these advancements help transform the perception of livestock farming, promoting a more scientific and ethical image and encouraging sustainable practices that align with trends in conscious production. Thus, fetal programming studies not only enhance production but also strengthen the connection between science, agriculture, and society.

Inform Graphic

EFFECTS OF SUPPLEMENTATION OF DEGRADABLE OR UNDEGRADABLE PROTEIN IN THE RUMEN FOR BEEF COWS SUBJECTED TO PROTEIN RESTRICTION DURING MID-GESTATION ON OFFSPRING DEVELOPMENT

Brazilian calf production systems are greatly affected by the seasonality of forage production, mainly because pastures have a low crude protein content during the dry season. The aim of this study was to understand the effects of maternal supplementation with rumen-protected protein (containing the amino acid arginine) during mid-gestation on the growth and development of the offspring. In addition, we evaluated whether there are any differences between types of maternal protein supplementation (rumen-degradable or rumen-undegradable protein) on the productive traits of the offspring. In general, the results of this study indicated that the supplementation of rumen-protected protein for beef cows during the second half of gestation results in heavier newborns with greater body measurements, and may also potentially influence the performance and meat quality characteristics in these animals during adulthood.

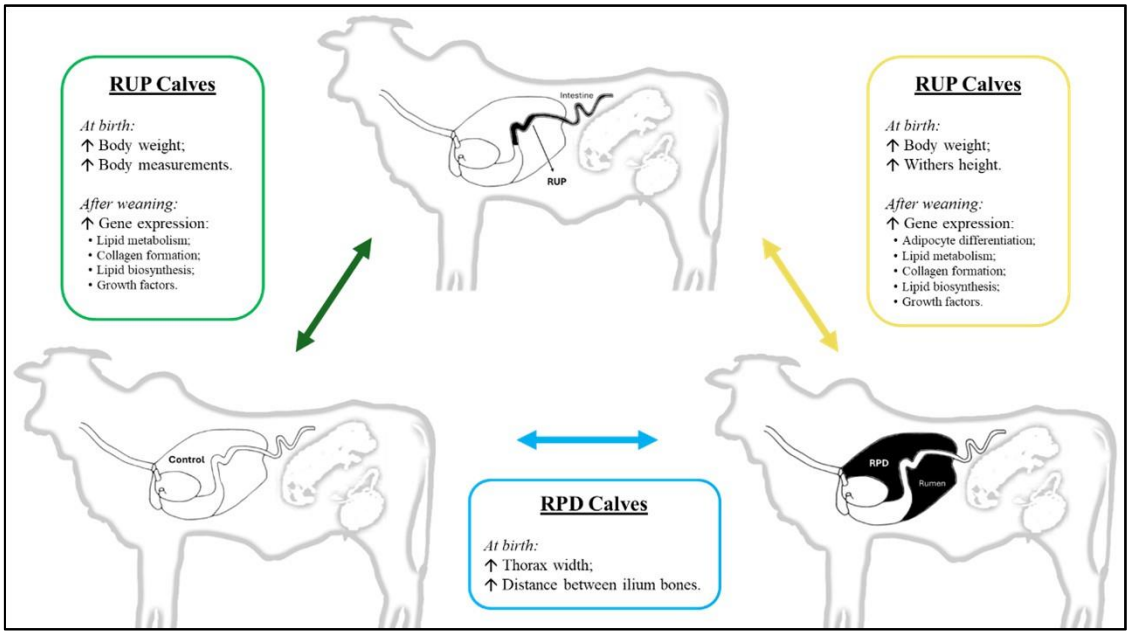

Figure 1. Effects of different sources of protein supplementation for beef cows during mid-gestation under a low crude protein diet (Control = no protein supplementation; RDP = Rumen-degradable protein supplementation; RUP = Rumen-undegradable protein supplementation) on metabolic and developmental characteristics during the postnatal phase of the offspring.

PhD Thesis in Animal Science at UFLA, defended on October 4, 2024.

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Figure 6. Summary of arginine metabolism in mammals. NOS nitric oxide synthase; AT L-arginine:glycine amidinotransferase; ADC arginine decarboxylase; AGMase agmatinase; ODC ornithine decarboxylase; OAT ornithine aminotransferase; P5C pyrroline-5-carboxylate (Morris, 2002). 28

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SUMMARY

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FIRST SECTION

1 INTRODUCTION

Breeding herds in Brazilian production systems experience different scenarios of forage supply and quality throughout the year. This is due to the productive seasonality of forage crops, due to the tropical climate present in a large part of the country, mainly in the Central-West region. Therefore, phases of greater nutritional demands for the dams, such as the final third of gestation and the beginning of lactation, are scheduled for the beginning of spring, so that the animals can benefit from better conditions of supply and quality of forage. Thus, the nutritional requirements of the dams and offspring are better met. However, part of the mid-to-late gestation are compromised, as they occur precisely during the dry season of the year, thus limiting the obtaining of nutrients with better digestibility and in satisfactory quantities, due to the poorer nutritional quality and low supply of forage during this period.

In this scenario, strategies such as storage of excess forage from the rainy season, associated with protein supplementation during the dry season of the year, are essential in order to make more nitrogen available in the ruminal environment for microbial growth and, consequently, improve digestibility and utilization of the forage (Lazzarini et al., 2009). This nutritional management not only benefits the dam, by reducing excessive mobilization of muscle tissue and maintaining adequate body condition. It provides ideal nutritional conditions for the growth and development of the fetus, mainly because it coincides with critical phases for the formation of muscle and adipose tissue in the offspring. Studies have shown that the middle third of gestation is crucial for the growth and development of these tissues in the fetus (Costa et al., 2021a; Du et al., 2010; Long et al., 2009) and that nutritional deficiency from the second half of gestation can impair adipocyte formation and muscle hypertrophy (Du et al., 2010; Underwood et al., 2010). Furthermore, failure to provide nutritional support to the dam, especially in these more advanced stages of gestation, can harm the uteroplacental blood flow and, consequently, the supply of oxygen and nutrients to the fetus (Vonnahme et al., 2007).

In this regard, studies that evaluated the inclusion of the amino acid Arginine (ARG) in the diets of pregnant females, indicated improvements in the supply of nutrients and oxygen to the fetus (Peine et al., 2018; Prezotto et al., 2018; Sun et al., 2017; Zhang et al., 2016). ARG is involved in the synthesis of nitric oxide, a vasodilator that regulates blood flow and increases

vascular permeability (Martin et al., 2001; Roy et al., 2006), processes that improve the efficiency of nutrient absorption between the mother and the fetus, in addition to favoring embryonic and fetal development, subsequent lactation and ovarian function (Meyer et al., 2018). However, it is necessary to better understand the effects of these maternal supplementation strategies on the fetal programming of beef cattle, involving metabolism and maternal/fetal performance, given the challenging nutritional conditions during the dry season of the year.

Therefore, our hypothesis is that supplementation with a product with protein protected from ruminal degradation, rich in Arginine, can promote a greater flow of nutrients between dams and fetuses, even in low-quality diets. The objective of this research was to identify the effects of maternal supplementation of a commercial product rich in protected protein from ruminal degradation, whose main amino acid is arginine, on the characteristics of growth and development of offspring in the long term.

2 BACKGROUND

2.1 Offspring developmental programming

Maternal nutrition, environmental heat stress, hypoxic stress and multiple fetuses are the main factors that can affect fetal growth and development (Reynolds et al., 2006; Caton and Hess, 2010). Among them, maternal nutrition stands out, because it has been extensively studied in terms of fetal development programming (Reynolds et al., 2017; Reynolds et al., 2019). Both the lack and excess of energy/nutrients can influence the metabolism and physiology of the new individual that is being generated.

During the first half of gestation in cattle, energy demand is relatively low when compared to more advanced stages, since total energy needs increase as gestation progresses (Bell et al., 2005). However, it is necessary to pay attention to this phase, due to the critical events that begin in it, such as, for example, establishment of functional fetal and uteroplacental circulation, organogenesis, myogenesis and adipogenesis (Du et al., 2010), to guarantee survival and development. embryonic growth.

Up to 50 days of gestation in beef cattle, the conceptus grows from a single cell to a fully formed embryo, with fully recognizable organ systems and a functional placenta (Caton

et al., 2019). In addition, dams who experience some type of stress (nutritional or environmental, for example) only during early pregnancy can give rise to offspring with normal birth weight, but with great potential for slow growth and metabolic problems due to stress suffered in early pregnancy (Ford et al., 2007; Vonnahme et al., 2007; Reynolds e Caton, 2012).

According to Caton et al. (2020), some nutrients, metabolites and metabolic processes may be critical for maintaining pregnancy, including: (1) hexoses, such as fructose and glucose, which provide energy for the developing conceptus and activate nutrient detection pathways , as the target of rapamycin (*mTOR*) in mammals (Kim et al., 2012), whose gene is involved in the signaling of several processes, including protein synthesis and prevention of protein degradation (Latres et al., 2005). Furthermore, these sugars play a critical role in the production of NADPH and nucleotides through the pentose phosphate pathway, by donating electrons to scavenge oxygen free radicals (OFR), and in the synthesis of nucleotides, through the production of ribose-5 phosphate; (2) some specific amino acids necessary for rapid cell growth and proliferation; (3) metabolism of arginine via nitric oxide synthase, to produce nitric oxide and reduce OFR through glutathione peroxidase and NADPH, which plays a key role in OFR metabolism, stimulates cell proliferation and prevents cell death due to oxidative stress; (4) metabolism of arginine to polyamines, with S-adenosyl methionine donor carbon skeletons (Clare et al., 2019) to further enhance cell proliferation that is required for successful elongation of trophectoderm to maintain pregnancy; and (5) methyl donors such as methionine (metabolized to S-adenosyl methionine), which may be crucial for early embryonic development, not only for regulating OFR and polyamine synthesis, but also for regulating transcription and modifications post-translational DNA and histones (Crouse et al., 2020).

Not only the initial third of pregnancy, but also the middle and final thirds of pregnancy are considered critical, especially for the development of skeletal muscle and for determining the potential for muscle growth in the postnatal phase (Greenwood et al., 2000; Du et al., 2010; Du et al., 2013).

2.1.1 Growth and development of muscle tissue

The formation of muscle fibers is called myogenesis, being divided into three stages, determination, proliferation and differentiation. Mesenchymal stem cells (pluripotent cells) multiply and are mobilized to the process of myogenesis, transforming into myoblasts. The

regulation of the myogenic process is carried out by a set of transcription factors, including *Wingless and Int* (Wnt), *β-catenin*, *PAX3*, *PAX7*, which induce the expression and activity of myogenic regulatory factors called *MRF's* (Chargé and Rudnicki, 2004; Du and Zhu, 2009). These are proteins that act as activators of the transcription process, binding to DNA through specific sites in the promoter region of several muscle-specific genes, promoting their expression and controlling myogenesis events (Lassar et al., 1991).

In the embryonic phase, the process called primary myogenesis occurs, in which the commitment of pluripotent cells begins through the *PAX3* / *PAX7* pathway (Kassar-Duchossoy et al., 2005; Grifone et al., 2007), which is responsible for keeping these cells undifferentiated, but in proliferation, to that there is expansion of the cells to the myogenic lineage (Amthor et al., 1999). Next, there is expression of factors *MYF5* and *MYOD* (Tajbakhsh et al., 1997; Du et al., 2010), which control the differentiation of myogenic cells, that is, the transformation of myogenic progenitor cells into myoblasts. Subsequently, these myoblasts proliferate, migrate and merge to form myotubes, a process regulated by the expression of the *MYOG* factor (Megeney e Rudnicki, 1995), also known as *Myogenin*. The myotubes will finally give rise to primary muscle fibers, from the expression of the *MRF4* factor, which regulates the differentiation of these cells into muscle fibers (Megeney e Rudnicki, 1995). Although the amount of primary muscle fibers formed is very limited, these in turn serve as “templates” for the formation of secondary muscle fibers during the fetal phase (Du et al., 2010). In addition, there is a regulatory factor in bovine skeletal muscle, *Myostatin* (a member of the *TGF-β* superfamily), which negatively regulates muscle hyperplasia by controlling the proliferation and differentiation of myoblasts during the early stages of myogenesis (Kambadur et al., 2004). In addition, *Myostatin* acts in the regulation of satellite cell activation during postnatal life (Kambadur et al., 2004).

During the middle third of pregnancy, the mesenchymal precursor cells around the primary muscle fibers continue to proliferate and, when they merge with the primary myofibrias, give rise to secondary muscle fibers (Oldham et al., 2001), a process called secondary myogenesis, which is responsible for the formation of most of skeletal muscle fibers (Zhou et al., 2021). This muscle growth characterized by an increase in the number of muscle fibers and their grouping is called hyperplasia, a process that occurs only during the prenatal phase of many species, including ruminants (Zhu et al., 2004). Therefore, it is a determining factor in animal's growth potential, since the increase in muscle mass in the postnatal period

occurs exclusively from the increase in the size of previously formed cells (Stickland, 1978; Zhu et al., 2004; Du et al., 2010).

Muscle fibers are specialized according to their contractile and metabolic properties, which depend on the proportion of myosin heavy chain isoforms (*MyHC*) and the development of enzymatic pathways of energy metabolism, respectively (Bonnet et al., 2010; Picard and Gagaoua, 2020). They are classified as: (1) Slow-twitch oxidative fibers (type I); (2) Fast-twitch glycolytic fibers (type IIx and IIb); and (3) Fibers IIa that exhibit mixed characteristics, that is, they have fast contraction and oxidative metabolism (Picard and Gagaoua, 2020).

Given the importance of the processes that occur during the fetal stage, it is imperative to provide adequate nutrition at this stage to allow the proliferation of myogenic precursor cells and, consequently, increase the growth of lean tissue in animals destined for meat production. As nutrients directed to the fetus are derived from maternal circulation, maternal nutrition has strong impacts on fetal muscle development. Furthermore, skeletal muscle has a lower priority in nutrient partitioning compared to vital organs including the brain, heart, and liver (Zhu et al., 2006). Thus, muscle development is particularly vulnerable to changes in nutrient intake, as muscle mass is the component with the highest body proportion (Zhu et al., 2006). In cattle, whose gestation period is long, and the rearing system is basically characterized by livestock on pasture, important stages of pregnancy may coincide with the dry season of the year, when the nutritional value of forage is impaired, affecting maternal nutrition and fetal.

Primary muscle fibers in bovine fetuses form within the first 2 months after conception (Figure 1; Russell e Oteruelo, 1981; Du et al., 2010). Secondary myogenesis, the formation phase of more muscle fibers, occurs between 2 and 7 months of gestation in cattle (Figure 1; Du et al., 2010). In the final third of pregnancy, there is a very limited formation of new muscle fibers and muscle growth is due to the increase in size and length.

Therefore, nutritional restrictions in these phases of pregnancy can lead to a reduction in the proliferation of myogenic precursor cells, which decreases the number of muscle fibers formed, with long-lasting and irreversible negative effects on the muscle growth of the offspring (Stannard e Johnson, 2004; Zambrano et al., 2005; Zhu et al., 2006).

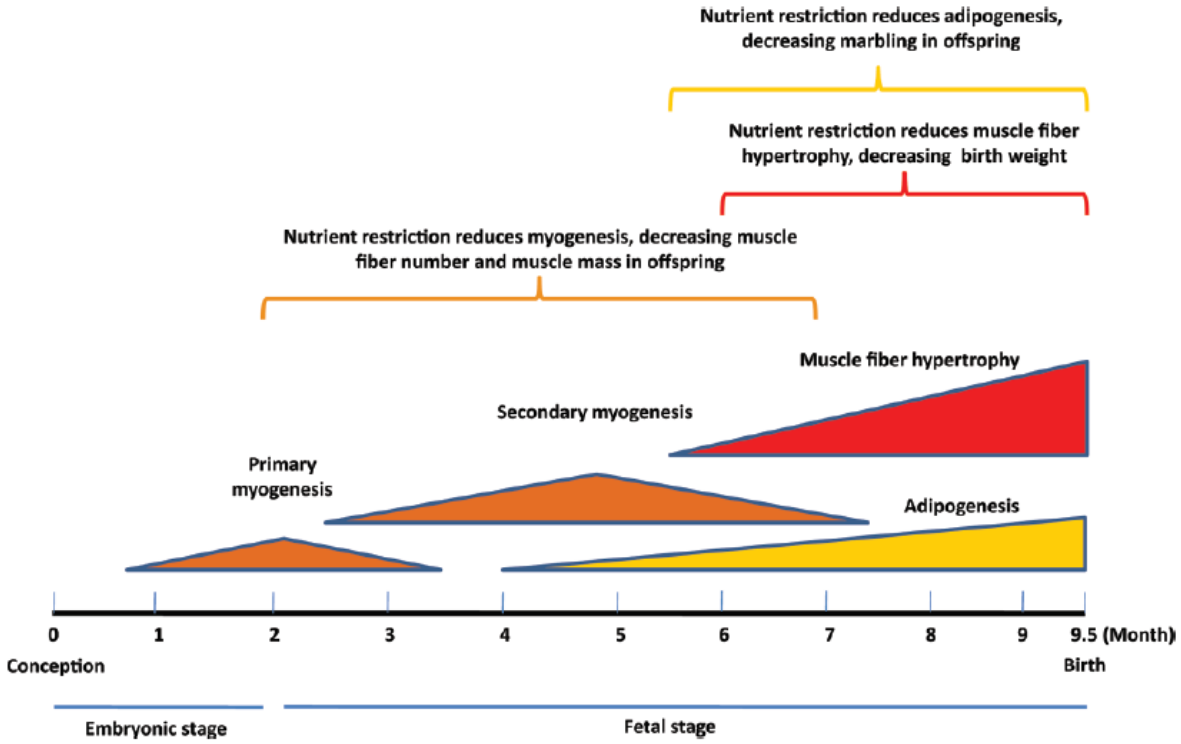

Figure 2. Development process of bovine fetal skeletal muscle tissue. Adapted from Du et al. (2010).

In a study with ewes, receiving 50% of the nutritional requirements from 28 to 78 days of gestation (equivalent to approximately 2 to 5 months of gestation in cattle), there was a reduction in the total formation of secondary muscle fibers (Zhu et al., 2004). In addition, poor maternal nutrition decreased the concentration of growth factors (*IGF-1* and *IGF-2*) in the fetal circulation, which reduced the proliferation of myogenic cells and the formation of muscle fibers (Gonzalez et al., 2013). Beef cows receiving diets with 60% of the nutritional needs according to NRC, up to 85 or 140 days of gestation, reduced the expression of *IGF-1* in the fetuses and the density of myogenic progenitor cells, resulting in a smaller number of muscle fibers at 254 days of gestation (Gonzalez et al., 2013). These data clearly indicate that nutritional restriction from early to mid-pregnancy reduces the number of muscle fibers and muscle mass, affecting the growth and development of the offspring.

At the end of secondary myogenesis, that is, when all the muscle fibers are already formed, the myogenic cells continue to proliferate and merge with the existing muscle fibers, increasing their diameter, that is, increasing cell hypertrophy (**Figure 2**). In addition, muscle fibers also increase in length through the synthesis of new myofibrillar proteins. There is a group of myogenic cells that do not form muscle fibers, but which are responsible for postnatal

regeneration and growth of skeletal muscle (Chargé e Rudnicki, 2004). These are called satellite cells (myogenic stem cells), located beneath a thin layer of connective tissue around mature muscle fibers. These are mononuclear and mitotically active cells, which, when incorporated into existing muscle fibers, increase both their nuclear density, promoting the synthesis of myofibrillar proteins, and the size of muscle fibers (Kuang et al., 2007).

In cases of inadequate nutrient intake during the gestational period, the proliferation of fetal myogenic cells may be impaired. Maternal nutritional deficiency during late pregnancy reduced fetal muscle fiber size, although fiber number was unaffected (Greenwood et al., 1999; Zhu et al., 2004). In addition, the muscle growth of the animal in the postnatal phase can be affected by the reduction in the density of satellite cells. Nutritional restriction until mid-pregnancy in ewes decreased the number of muscle fibers and, as compensation, there was an increase in fiber diameter in the offspring at 8 months of age (Zhu et al., 2006). Increased muscle fiber size has also been reported in other studies with cattle and sheep when nutrient intake by dams was restricted during early pregnancy (Du et al., 2005; Du et al., 2010). This can affect the tenderness of the meat depending on the fiber diameter. Maltin et al. (1997) verified that as the average diameter of the muscle fibers increases, the shear force also increases, indicating a more resistant muscle. Zhu et al. (2004) found that nutritional restriction until mid-pregnancy reduced the number of fetal skeletal muscle fibers, which may be related to a negative regulation of rapamycin target protein signaling in mammals (*mTOR*). In contrast, protein supplementation strategies for cows during the middle third of pregnancy provided an increase in the number of muscle fibers and, consequently, improved the postnatal performance of calves (Marquez et al., 2017).

2.1.2 Growth and development of adipose and connective tissue

Adipogenesis (formation of adipocytes) is characterized by two main steps, determination and differentiation (Du et al., 2013), and adipocytes also originate from undifferentiated progenitor cells, which become pre-adipocytes when they are committed to the adipogenic lineage. Adipogenesis in ruminants begins shortly after primary myogenesis (Fève, 2005; Gnanalingham et al., 2005; Muhlhausler et al., 2007), that is, the initial cellular adipogenic impairment occurs simultaneously with secondary myogenesis, from the second mid-gestation (Figure 2; Du and Stephen, 2018). However, much of adipocyte formation occurs

during the final third of gestation until weaning of the animal (Figure 2; Mersmann e Smith, 2005; Du e Stephen, 2018). Thus, maternal nutrition during the fetal phase can impact adipocyte hyperplasia, altering the development of adipose tissue in the offspring as a whole, as well as the deposition of intramuscular fat (Du et al., 2013).

Fat deposition in cattle follows a developmental order, in which adipocytes are first detected in visceral fat, followed by subcutaneous, intermuscular, and intramuscular fat, respectively. The increase in the number of visceral fat adipocytes extends to the neonatal stage, while the hyperplasia of subcutaneous and intermuscular fat extends to weaning (Du et al., 2013). The formation of intramuscular adipocytes occurs later, mainly between the end of pregnancy and close to 250 days of age in cattle (Du et al., 2013; Du and Stephen, 2018). Therefore, this chronological difference in the formation of adipocytes allows an increase in marbling formation, without increasing adipogenesis in the other deposits, which is called the "marbling window" (Corah e McCully, 2007; Du et al., 2013). Therefore, when providing nutritional or energy supplementation to calves, from the beginning of weaning until 250 days of age, more intramuscular fat will be deposited, further increasing the degree of marbling of the final product (Wertz et al., 2001; Wertz et al., 2002; Pyatt et al., 2005a; Pyatt et al., 2005b; Du et al., 2013).

The amount of fat mass of an individual is determined by the number and size of adipocytes. The number of adipocytes is mainly defined during the last two thirds of pregnancy, extending to the initial postnatal phase, making the total number of adipocytes fixed right after puberty (Figure 2; Spalding et al., 2008). After 250 days of age, nutritional supplementation becomes less effective in increasing the number of adipocytes due to the depletion of multipotent cells (Figure 2; Du et al., 2013). Therefore, adipose tissue growth is primarily driven by hypertrophy of already existing adipocytes (Figure 2; Mersmann e Smith, 2005).

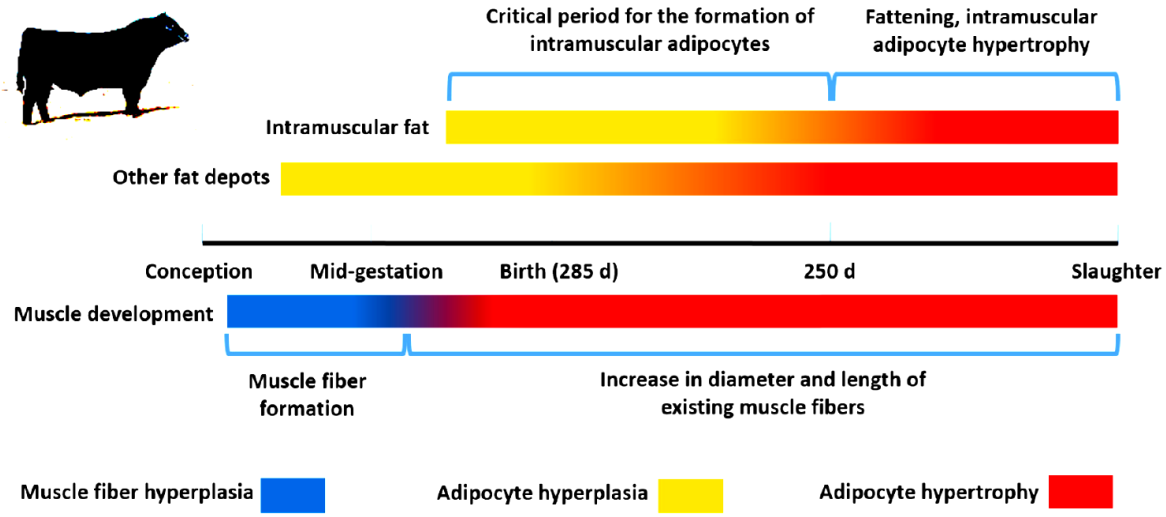

Figure 3. Development process of skeletal muscle and adipose tissue in beef cattle. Adapted from Du and Stephen (2018).

Adipocytes and fibroblasts share a common source of progenitor cells (Du and Stephen, 2018). These cells are called fibro/adipogenic progenitor cells (FAPs), due to their ability to differentiate both into adipocytes and fibrogenic cells (Joe et al., 2010; Uezumi et al., 2010). Adipogenesis and fibrogenesis (connective tissue formation) usually occur simultaneously, with adipocytes embedded in a connective tissue network, together forming the basic structure of adipose tissue. In skeletal muscle tissue, connective tissue is divided into layers: (1) endomysium, which covers the muscle fiber, (2) perimysium, which covers the muscle fiber bundles, and (3) epimysium, which covers the entire muscle (Astruc, 2014a; Astruc, 2014b).

The regulation of adipogenic and fibrogenic processes are involved with *Wnt* (*Wingless and Int*) signaling, a family of signaling proteins that, in addition to recruiting pluripotent cells for the formation of muscle, adipose and connective tissues, are related to embryonic development and differentiation cell (Liu et al., 2013; Nakamura et al., 2013). *Wnt* proteins are responsible for positively modulating the expression of the *MYF5* factor (Kuhn et al., 2012), previously mentioned, being a transcriptional factor that, when expressed, directs mesenchymal stem cells to the myogenic lineage. Basically, *Wnt* signaling phosphorylates and inactivates the enzyme glycogen synthase kinase-3β (*GSK-3β*), stabilizing and allowing the entry of *β-catenin* into the cell nucleus (Kassar-Duchossoy et al., 2005). This, in turn, acts on a complex of members of the T cell factor/lymphoid enhancing factor (*TCF*/*LEF*) family to activate factors *MYF5* and *MYOD*, via *PAX3* and *PAX7* (Kassar-Duchossoy et al., 2005). When the *Wnt*

pathway is inhibited, the *MYF5* factor is not expressed and the undifferentiated cells are mobilized to the fibro-adipogenic lineage, originating FAPs or pre-adipocytes (Du et al., 2010).

During the determination phase, the adipogenic marker, *ZFP423*, acts in the recruitment of progenitor cells to pre-adipocytes (Ghaben e Scherer, 2019), being of perivascular origin (Gupta et al., 2012). The next step is the differentiation phase, which is controlled by the expression of two major adipogenic factors, *CCAAT*/enhancer binding protein alpha (*C/EBPA*) and peroxisome proliferator-activated receptor gamma (*PPARG*) (Ladeira et al., 2016; Moseti et al., 2016). *ZFP423* acts on *PPARG*, stimulating the increase of *C/EBPA* within preadipocytes, further activating *PPARG*, which results in preadipocyte differentiation (Duarte et al., 2013). Thus, the maturation of pre-adipocytes into mature adipocytes occurs, initially characterized by the accumulation of lipid droplets (Duarte et al., 2013). For this, a synchronized action between *PPARG* and *C/EBPA* is important (Du et al., 2013). There are three isoforms (alpha, beta and gamma) that participate in the PPARs family, and that are differentiated by their physiological action and the target tissue in which they are expressed (Desvergne and Wahli, 1999). *PPARG* is more expressed in adipose tissue compared to muscle tissue (Kersten, 2014), and has an important role in lipid metabolism, as it regulates the expression of genes associated with fatty acid storage, including *FABP*, *ACSL1* and *LPL* (Bionaz et al., 2012). Another important isoform is *PPARA*, expressed in adipocytes, as well as in the heart, liver and small intestine (Bunger et al., 2007). *PPARA* acts on the expression of long-chain fatty acid transporters and enzymes involved in the β-oxidation of fatty acids (Ladeira et al., 2016).

There is another family that is involved in the metabolism of adipose tissue cells, which is the SREBP family. It is composed of three isoforms (*1a*, *1c* and *2*), and the sterol regulatory element binding protein 1c (*SREBP-1c*), encoded by the *SREBF1* gene, is the most prominent isoform. *SREBF1* is an important transcriptional factor, as it positively regulates the expression of other genes (*ACACA*, *FASN* and *SCD1*) involved in the de novo synthesis of fatty acids (**Figure 4**; Shimano, 2001). In addition, it induces transcriptional activation of genes encoding important lipogenic enzymes (Tang and Lane, 2012) such as Fatty Acid Binding Protein 4 (*FABP4*), adiponectin and leptin (Ladeira et al., 2016). It is also during this phase that adipocytes begin to acquire insulin sensitivity, a fact related to the increase in the number of insulin receptors and glucose transporters (Ladeira et al., 2016). *FABP4* expression occurs mainly in mature adipocytes, encoding proteins related to the absorption and transport of fatty

acids, in addition to being involved in fat deposition, and its accumulation is indicative of the number of adipocytes in muscle tissue (Pino et al., 2017).

The hypertrophy resulting from the deposition of triglycerides in adipocytes is intensified after puberty and animal maturity, while there is a decrease in muscle growth (Owens et al., 1995). Lipogenesis and subsequent cell hypertrophy depend on the relative rates of synthesis, degradation and oxidation of fatty acids (Bonnet et al., 2010). The first step for the *de novo* synthesis of fatty acids is marked by the presence of acetyl-CoA in the cytosol, originating from the metabolism of acetate and/or glucose. Acetyl-CoA undergoes the action of an enzyme called acetyl-CoA carboxylase, which is encoded by the *ACACA* gene, and which carboxyl the malonyl-CoA molecule. Subsequently, malonyl-CoA receives acetyl-CoA molecules through multiple reactions of the fatty acid synthase multienzyme complex (encoded by the *FASN* gene), resulting in the formation of a long chain of fatty acids (Ladeira et al., 2016). Palmitic acid is one of them (C16:0), and can undergo the process of elongation or implantation after its formation, through the action of the enzyme stearoyl-CoA desaturase, which is encoded by the *SCD1* gene (Duckett et al., 2009; Ladeira et al., 2014).

According to Ward et al. (2010), when there is greater deposition of intramuscular fat, greater is the action of acetyl-CoA carboxylase and the multienzyme fatty acid synthase complex. Fat deposition in adipose tissue occurs through fatty acid uptake, through the participation of membrane proteins and intracellular transporters that transport fatty acids into cells (**Figure 4**; Gerbens, 2004; Glatz et al., 2010). Initially, triglycerides, originating from blood transport carried out by lipoproteins, are hydrolyzed by the enzyme lipoprotein lipase (*LPL*), located in the endothelial cells of capillaries (**Figure 4**; Gerbens, 2004). Then, the fatty acids are transported into the adipocyte by the action of transporters, depending on the affinity of the fatty acid to the receptor, or by association with FABP and acyl CoA synthase (Miyamoto et al., 2016; Ladeira et al., 2018). The binding of fatty acids linked to FABP inside adipocytes allows their transport from the membrane to the site of esterification or directed to β-oxidation in mitochondria (Gerbens, 2004). For β-oxidation to occur, there must be lipolysis of fatty acids, and the binding of acyl CoA groups to a carnitine molecule by the action of the enzyme carnitine palmitoyltransferase 1 (*CPT1*). This allows their transport from the cytosol to the mitochondrial matrix, so that the *CPT2* enzyme can dissociate acyl CoA from carnitine to obtain energy, through the oxidation of fatty acids (**Figure 4**; Houten et al., 2016).

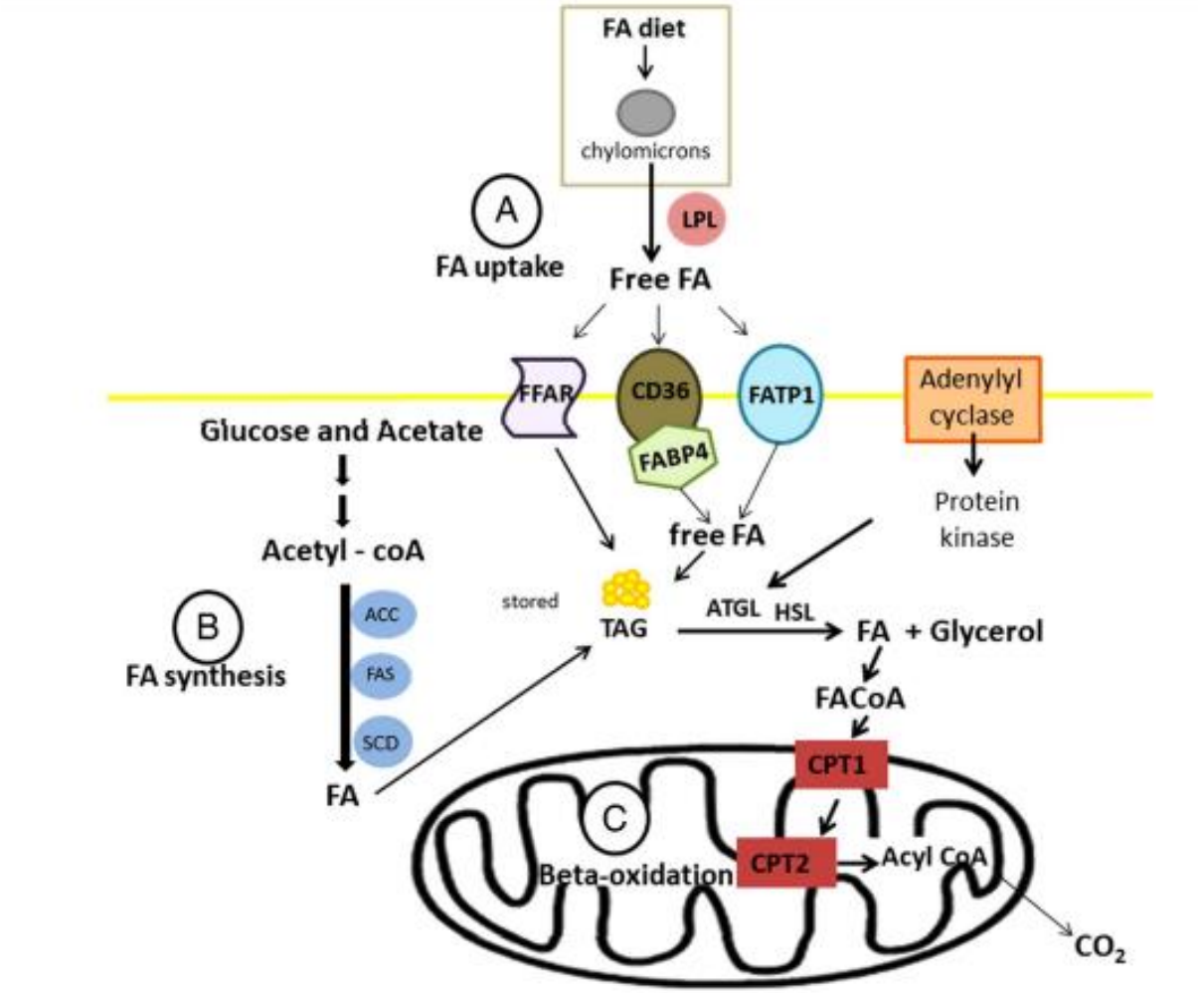

Figure 4. Adipose tissue metabolism. Fatty acid uptake (A), de novo synthesis (B) and Fatty acid oxidation (C). LPL = lipoprotein lipase; ACC = acetyl-CoA carboxylase; FAZ = fatty acid synthase, SCD = stearoyl-CoA desaturase; FFAR = free fatty acid receptors; CD36 = fatty acid translocase; FATP = fatty acid transport protein; FABP4 = fatty acid-binding protein 4; TAG = triacylglyceride; ATGL = adipose triglyceride lipase; HSL = hormone sensitive lipase; CPT = carnitine palmitoyltransferase.

Another important event that occurs in skeletal muscle tissue is fibrogenesis. As previously mentioned, adipocytes share with fibroblasts the same *pool* of stem cells (FAPs), and there are genetically controlled competitive processes that give rise to one or the other cell lineage (Du et al., 2015). Generally, the formation of fibroblasts is more intensified during late pregnancy (Du et al., 2010a), and they are recruited from the FAPs cell pool by the upregulation of cytokines and growth factors, mainly *TGF-β1* (Liu and Pravia, 2010). All *TGF-β* isoforms act on the signaling of cytoplasmic SMAD factors that trigger gene expression of fibrogenic enzymes, catalysts for collagen crosslinking (Huang et al., 2012). Collagen is the main protein of the extracellular matrix, constituted in greater abundance by types I and III in the muscle

(Light et al., 1985). According to Huang et al (2012) progenitor cells with high adipogenic potential have lower expression of *TGF-β*, since this transcription factor is expressed later compared to *ZFP423*. This allows manipulating the development to favor the deposition of intramuscular adipocytes to the detriment of the formation of connective tissue, aiming to improve qualitative aspects of the meat such as marbling and tenderness (Du et al., 2013). There is also a relationship between adipogenesis/fibrogenesis and angiogenesis (formation of new blood vessels), and adipose tissue is considered one of the most vascularized tissues, as adipocytes are surrounded by capillaries (Cao, 2010). The high vascularity of this tissue allows the supply of oxygen and nutrients necessary for its growth (Tang et al., 2016). In addition, FAPs in adipose tissue are generally associated with blood vessels, through cells with α-positive platelet-derived growth factor receptors, PDGFRα+ (Wang et al., 2017), which are considered markers of adipogenic precursors (Uezumi et al., 2010; Berry and Rodeheffer, 2013) and respond to β-adrenergic stimulation and/or high-fat diets to differentiate into adipocytes (Lee et al., 2012). Generally, FAPs are derived from the dermomyotome during prenatal development (Yang et al., 2013; Du et al., 2015). However, FAPs are also present in adipose tissue in the postnatal phase, but their density decreases as animals age (Du et al., 2013; Wang et al., 2017). Due to the sharing of the *pool* of PDGFRα+ progenitor cells, the correlation between adipogenesis and fibrogenesis is positive. In Wagyu cattle, for example, both intramuscular fat and collagen accumulation are high, with a genetically high marbling content (Duarte et al., 2013).

The excess of nutrients provided to bovine females during pregnancy also increased the deposition of fat and collagen in the skeletal muscle of the offspring (Duarte et al., 2014). In cases of nutritional restriction, Gonzalez et al. (2013) providing 40% of the nutritional requirements for beef cows, found a reduction in adipogenesis and an increase in the expression of fetal fibrogenic markers, suggesting greater formation of connective tissue in calves when there is a nutrient deficiency during pregnancy. Furthermore, maternal dietary restriction inhibits vascular epithelial growth factor expression in microvascular and aortic endothelial cells during fetal development, which decreases angiogenesis (Khorram et al., 2007) and the formation of PDGFRα+ progenitor cells (Du e Stephen, 2018).

Adipose tissue development is closely associated with capillary network development, and impaired neovascularization negatively affects the fetal adipocyte development (Wang et al., 2017). However, as adipose tissue has high plasticity, its compensatory growth generally

results in greater fat deposition in offspring from dams with nutritional deficiencies. This growth is often associated with increased food intake and lower muscle mass, which reduces energy expenditure, resulting in excessive accumulation of this energy in the form of lipids in adipose tissue, through adipocyte hypertrophy (Zhu et al., 2006; Gonzalez-Bulnes et al., 2012).

Lambs born to ewes with nutritional restriction (50% of requirements) until mid-pregnancy showed greater accumulation of subcutaneous and perirenal fat, compared to lambs from control ewes (Ford et al., 2007). Gardner et al. (2005) observed an increase in total visceral fat in the offspring of dams who experienced malnutrition during pregnancy. In these two studies, the authors found that the restricted offspring showed insulin resistance. Furthermore, Costa et al., (2021b) found that protein restriction from 100 to 200 days of gestation in beef cows reduced the formation of muscle fibers in the offspring during the postnatal phase, in addition to slightly increasing the collagen content in the skeletal muscle of the offspring. Therefore, it is clear that the importance of paying attention to maternal nutrition during pregnancy, as it affects the development of the offspring, bringing persistent consequences in the long term for the offspring.

2.1.3 Effects of arginine inclusion in maternal diet on offspring development

Arginine (ARG) is an amino acid classified as nutritionally essential for mammals during pregnancy (Wu et al., 2009), as the endogenous synthesis of ARG is not sufficient for animals that have high nutritional requirements (Wu, 2013a). This amino acid regulates metabolic pathways related to the health, growth, development, reproduction, lactation and homeostasis of animals (Flynn et al., 2002; Lassala et al., 2010).

The ARG is a common substrate for the synthesis of urea, nitric oxide (NO), polyamines (putrescine, spermidine, and spermidine), ornithine, proline, glutamate, creatine and agmatine (Wu and Morris, 1998; Wu et al., 2009; Wu et al., 2013; Bazer et al., 2015). The first step for the use of ARG by cells is the transport of this amino acid, carried out mainly by specific membrane transporters, since ARG cannot be captured in sufficient amounts of the extracellular medium by simple diffusion (Morris, 2009). Cationic transporters (*SLC7A1*, *SLC7A2*, *SLC7A3*) perform the transport of ARG to the uterine lumen and absorption by the conceptus (Bazer et al., 2015). In addition, this amino acid shares the same transporters with lysine, ornithine and

histidine (Wu et al., 2013). Therefore, it is important to pay attention to the amounts of these amino acids provided to animals, to avoid an imbalance between them.

There are several ways to use ARG in cells of different tissues, including liver, kidneys, brain, small intestine, mammary gland/macrophages and placenta (Wu and Meininger, 2009). The biologically active molecules derived from the metabolism of this amino acid perform several functions in the animal's organism: (1) It activates the *mTOR* cell signaling pathway, stimulating protein synthesis and cell proliferation in various tissues, including the placenta, uterus and fetus; (2) Prevents cell death in the face of oxidative stress, as it improves antioxidant defense systems; (3) Improves uteroplacental blood flow (**Figure 5**). Some studies have reported that ARG increased placental angiogenesis (Raghavan and Dikshit, 2004), fetal-placental blood flow (Tan et al., 2012), antioxidant capacity and improved lipid peroxidation during pregnancy (Morris, 2009).

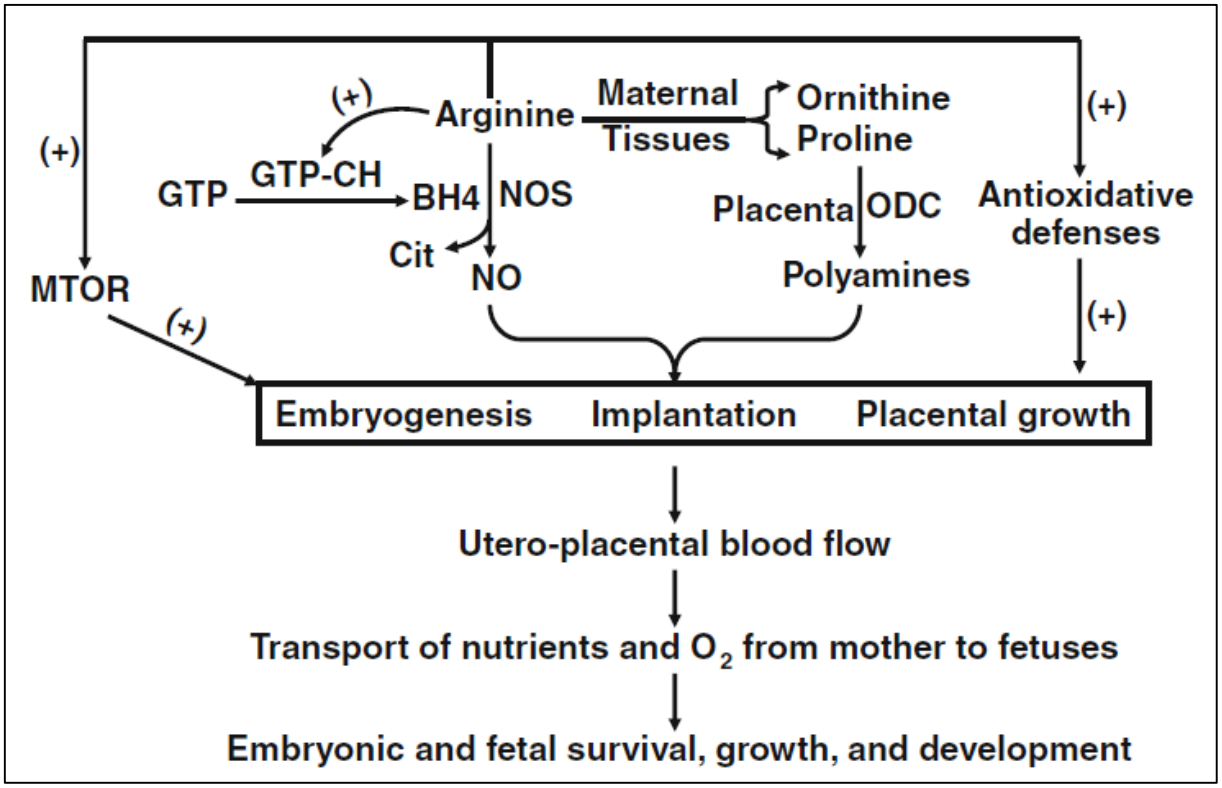

Figure 5. Role of arginine in improving embryonic and fetal production and growth in mammals. BH4 tetrahydrobiopterin, Cit citrulline, MTOR mechanistic target of rapamycin, GTP-CH GTP cyclohydrolase-I, ODC ornithine decarboxylase, NO nitric oxide, NOS nitric oxide synthase. (Wu et al., 2013).

Nitric oxide and polyamines are mainly responsible for signaling, proliferation, migration and cell remodeling processes, in addition to angiogenesis and dilation of vessels to increase blood flow (Wu et al., 2009; Wu et al., 2011a; Wu et al., 2011b). The NO synthesized from ARG (via nitric oxide synthase, *NOS*) is considered a potent vasodilator that regulates vascular tone (Martin et al., 2001) and increases fetal placental blood flow (Tan et al., 2012). In addition, it is considered a highly reactive neuromodulator and immunomodulator (Wu and Morris, 1998; Ignarro et al., 2001). Polyamines are synthesized from the hydrolysis of arginine (via arginase) and ornithine (via ornithine decarboxylase 1, *ODC1*). However, ARG in the sheep conceptus can also be converted into agmatine (via arginine decarboxylase, *ADC*) and subsequently give rise to polyamines through agmatinase activity (Figure 5; Wang et al., 2014). The functions of these compounds are involved with the regulation of gene expression, protein synthesis and angiogenesis. In addition to NO and polyamines, insulin-like growth factors (IGF) and vascular endothelial growth factors are also crucial for angiogenesis, embryogenesis, placental growth, uteroplacental blood flows and nutrient transfer from dam to fetus, as well as growth and fetal-placental development (Wu et al., 2006; Wu and Meininger, 2009). The rate of classical de novo biosynthesis of polyamines in sheep depends on the activity of the enzyme *ODC1*. However, the *ADC*/*AGMAT* functional pathway can be an alternative for the synthesis of polyamines in the reproductive tract of mammals and can compensate for the loss of *ODC1* activity, to ensure the survival and development of the conceptus (Bazer et al., 2015). The expression of genes for *NOS*, *ODC1* and the insulin-like growth factor 2 (*IGF2*), as well as the synthesis of these proteins, depend on the cell signaling that starts through the activation of *mTOR* (Nielsen et al., 1995; Kimball et al., 1999; Murakami et al., 2004), mainly induced by NO and polyamines. During pregnancy, angiogenic factors are mainly produced by maternal placental tissues in ruminant females (Reynolds et al., 1987; Reynolds and Redmer, 1988). Thus, these tissues can improve placental vascularization, with a significant effect on placental size, transport and/or blood flow, as well as on fetal growth and development (Reynolds and Redmer, 1995).

According to some authors, ARG is abundant in the conceptus (Bazer et al., 2013; Wu, 2013b) and its concentrations in the uterine lumen increase markedly during the peri-implantation period (Gao et al., 2009; Kim et al., 2013). This amino acid is able to significantly increase the histotrophy of the ovine uterus during early pregnancy, in addition to increasing the abundance of cytoskeletal proteins (for example, the tubulin) to increase the proliferation

and size of ovine trophectoderma cells (Bazer et al., 2015). In addition, ARG is also related to the regulation of steroid hormones through polyamines, since uterine levels of putrescine and spermidine increase shortly after ovulation (Wu et al., 2013), as well as the ovarian activity of the enzyme *ODC1*, which is necessary to increase the secretion of progesterone by the corpus luteum (Bastida et al., 2002). The maintenance of pregnancy depends on a functional corpus luteum to secrete progesterone necessary for an intrauterine environment capable of supporting implantation, placentation and fetal-placental growth and development (Spencer et al., 2004).

In addition to NO, ornithine, agmatine and polyamines, ARG metabolism generates other amino acids/substances. Citrulline, for example, is produced when ARG is metabolized to generate NO (**Figure 6**). In addition, most cells are capable of converting citrulline to arginine via argininosuccinate synthase and argininosuccinate lysase, and this intracellular arginine-citrulline cycle helps maintain sufficient concentrations of arginine to support NO production (Wu et al., 2009). Thus, increases in circulating citrulline levels may reflect a compensatory increase in the production of NO in the uterine epithelium and / or endothelial cells to regulate angiogenesis and uterine blood flow (Bazer et al., 2015). Both ARG and citrulline are abundant in ovine uterine fluid (Gao et al., 2009) and allantoic fluid (Kwon et al., 2003) during the onset until mid-gestation.

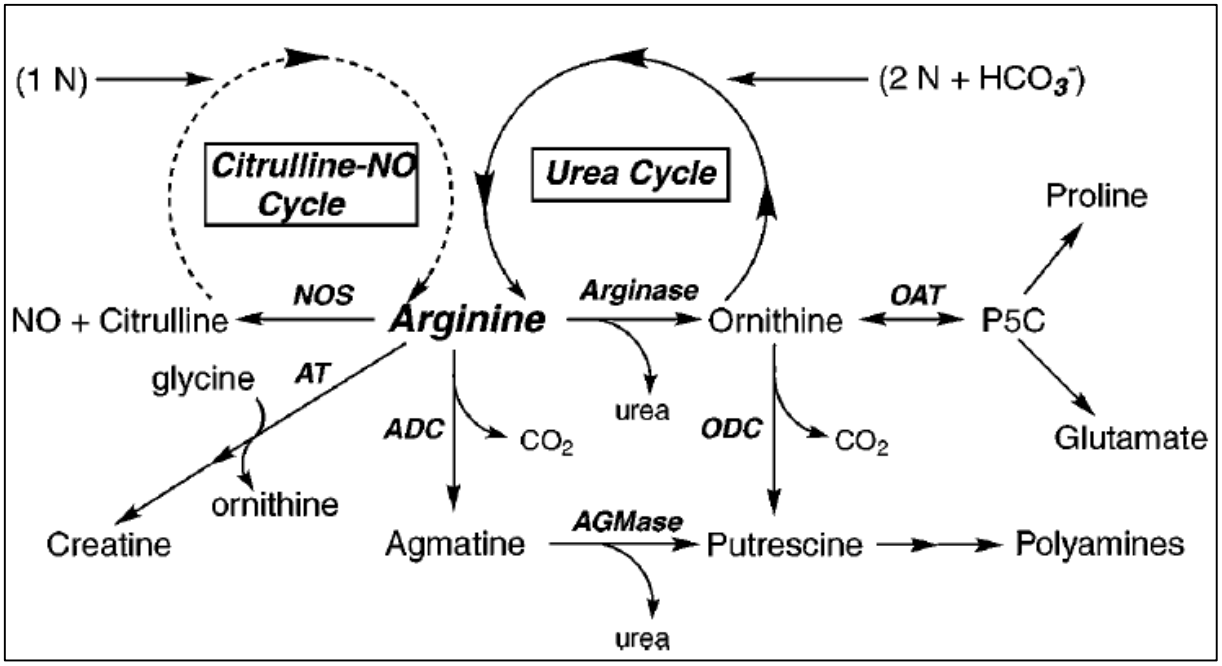

Figure 6. Summary of arginine metabolism in mammals. NOS nitric oxide synthase; AT L-arginine:glycine amidinotransferase; ADC arginine decarboxylase; AGMase agmatinase; ODC ornithine decarboxylase; OAT ornithine aminotransferase; P5C pyrroline-5-carboxylate (Morris, 2002).

Supplementation with ARG during pregnancy has been shown to be interesting to improve the supply of nutrients and oxygen to the embryo/fetus, allowing its survival, growth and development. Studies have been carried out to evaluate the effects of ARG dietary supplementation for malnourished ruminant females during pregnancy (Zhang et al., 2016a; Zhang et al., 2016b; Peine et al., 2018; Prezotto et al., 2018). For this, ruminal protection technologies are necessary to allow oral administration of ARG, as amino acids are rapidly degraded in the rumen (Chacher et al., 2012; Zhang et al., 2016b; Peine et al., 2018; Peine et al., 2020). In addition, ARG supplementation via diet for farm animals is a practical approach compared to parenteral administration (Chacher et al., 2012; Zhang et al., 2016b; Peine et al., 2020).

Zhang et al. (2016a;b) studied not only the inclusion of protected ARG (20 g / d; RP-ARG), but also N-carbamylglutamate (5 g/d; NCG), a cofactor of the enzyme carbamyl phosphate synthase-1 (a key enzyme in de novo synthesis of ARG in enterocytes), which is not extensively degraded in the rumen (Chacher et al., 2012). These authors found that the long-term dietary supplementation of these compounds (from the 35 days of gestation in sheep), increased the weight of the offspring from dams with nutritional restriction (50% of the NRC recommendations) during pregnancy, as well as resulting in higher concentrations of amino acids and polyamines in fetal blood and placental fluids. In addition, the results showed that NCG or RP-ARG provided increased activities of total antioxidant capacity and superoxide dismutase in maternal and fetal plasma and placentomas, which indicates mitigation of the adverse effects of fetal oxidative stress. The concentration of maternal cortisol was reduced when supplemented with RP-ARG or NCG in sheep with nutritional restriction. This is because RP-ARG or NCG can suppress the activity of the hypothalamic-pituitary-adrenal axis to produce less cortisol, which reduces the activation of mechanisms in response to stress (Munhoz et al., 2008). Cortisol synthesis and secretion are regulated by the pituitary hormone adrenocorticotrophin, which in turn is regulated by the hypothalamic hormone releasing corticotrophin with the synergistic action of arginine vasopressin (Papadimitriou and Priftis, 2009). Both NCG and RP-Arg supplements increased the concentration of serine in allantoic fluid and amniotic fluid. Serine is one of the main glycogenic AAs in humans (Bistrian, 2000) and sheep (Clark et al., 1976), and also participates in the synthesis of signaling molecules, including phosphatidylserine and ceramide (Bistrian, 2000). The reduction in serine

concentrations in the sheep conceptus can impair the synthesis of glucose, DNA and proteins, contributing to IUGR (Kwon et al., 2004).

Another study evaluated the effects of maternal diet restriction (60% of the requirements), with or without supplementation of 180 mg ARG/kg BW in pregnant ewes (Prezotto et al., 2018). These authors investigated the *in vitro* O_2-consumption of the liver and jejunum at 54 days of age in the offspring, in addition to evaluating the expression of proteins (neuropeptide Y, NPY; agout, AGRP; proopiomelanocortin, POMC; and neuronal nitric oxide synthase, nNOS) in the offspring hypothalamus. According to Blouet and Schwartz (2010), the hypothalamus has an important central function in detecting signals from nutrients, in order to control the use of energy throughout the body and homeostasis. Hypothalamic neurocircuits can be activated through the detection of nutrients and, consequently, regulate eating behavior, glucose homeostasis, adipose tissue and energy expenditure. Therefore, the restriction of nutrients to the pregnant female can program the energetic use of offspring visceral tissues (Buckley et al., 2005), through lasting effects on the hypothalamic circuits involved in the energetic homeostasis of the entire body (Blouet and Schwartz, 2010).

Excess nutrients to the fetus during pregnancy can also cause changes in this regard. According to Muhlhausler et al. (2006), maternal overfeeding during pregnancy caused changes in the orexigenic and anorectic peptides in the ARC of the hypothalamus, influencing the fetal fat mass, which indicates changes in the use of energy by the lambs. In addition, NO produced by neurons in the hypothalamus may have an effect on feed consumption and energy metabolism (Vincent and Kimura, 1992; Scott et al., 2009; Leshan et al., 2012). According to Joffin et al. (2012), nNOS is involved with energy metabolism and can alter feed intake in mammals (Hui and Chan, 1995), by inactivating hypothalamic leptin receptors, with positive regulation of orexigenic neuropeptides (NPY and AGRP) and negative regulation of anorectic neuropeptides (POMC; White and Martin, 1997).

Therefore, supplementation with ARG in the maternal diet was proposed to reduce the effects of IUGR (Wu et al., 2006) and as a potential substrate for NOS (Prezotto et al., 2018). In this study, dietary restriction and AGR supplementation for pregnant ewes did not influence the consumption of jejunal O_2 in the offspring. However, female lambs from dams who underwent nutritional restriction had a decrease in liver O_2 consumption. According to the authors, this suggests that the liver of the offspring may be more responsive to the programming of energy metabolism in the face of changes in the maternal diet. In addition, the authors found

a decrease in the number of cells containing POMC within the ARC and a tendency for NPY mRNA expression to decrease in the paraventricular nucleus of the offspring. The authors comment that this indicates that the abundance of NPY is responding to dietary treatment and that it may be influencing the body's energy metabolism. In addition, arginine supplementation was able to increase the total number of POMC cells that could potentially result in signaling to influence the use of liver energy. The authors also state that further studies are needed to fill the knowledge gaps that involve the effects of nutritional restriction and ARG supplementation on the use of liver and intestinal energy at different stages of development.

The restriction of fetal growth triggered by the impairment of maternal nutrition may be due to seasonality in forage production in extensive grazing systems, resulting from changes in climatic conditions. In this sense, Peine et al. (2018) evaluating RP-ARG supplementation (180 mg L-ARG/kg BW/day) for sheep with nutritional restriction (60% of nutrient requirements) during the last two thirds of gestation, observed an improvement in weight measurements body weight, average daily gain and lamb size. The intravenous infusion of AGR (81 mg AGR/kg BW/d) in pregnant sheep with nutritional restriction (60 d until parturition) also increased lamb birth weight (Lassala et al., 2010). These data suggest that ARG is a potential supplement to compensate for fetal growth restriction, in the face of improvements in the growth of offspring from dams with nutritional restriction.

ARTICLE

ABSTRACT

The objective of this study was to understand the effects of maternal supplementation rich in rumen-protected protein, containing the arginine, on the long-term growth and development characteristics of offspring. "During mid-pregnancy (127 ± 17 to 237 ± 17 days of gestation), thirty cows received one of three different supplementation treatments:" (1) Control (CON, n = 10) – basal diet (corn silage – 82.3%, DM basis; and sugarcane bagasse – 17.7%, DM basis) + commercial mineral supplement containing urea (60 g/100 kg BW); (2) Rumen-degradable protein supplement (RDP, n = 10) – basal diet + commercial protein supplement (250 g/100 kg BW) + ground corn (44 g/100 kg BW); and (3) Rumen-undegradable protein supplement (RUP, n = 10) – basal diet + commercial supplement rich in arginine (3.5 g/kg BW) + commercial mineral supplement containing urea (60 g/100 kg BW). At birth, the offspring were weighed, birth vigor scores were recorded, morphometric measurements were taken from the calves, and blood samples were collected. At 48 ± 10 days of age, biopsies of the *Longissimus thoracis* muscle were performed on the calves to evaluate muscle fiber characteristics and gene expression related to metabolism, growth, and development of skeletal muscle tissue. Out of 30 cows, only 23 calves remained in the study due to twin births and calf mortality during the cow-calf phase. At 286 ± 31 days of age, the offspring (CON, n = 9, 5 males and 4 females; RDP, n = 8, 7 males and 1 female; RUP, n = 6, 4 males and 2 females) were weighed and underwent a backgrounding phase, during which they were fed the same diet (forage-to-concentrate ratio: 50:50) for 60 days. At 306 ± 31 days of age, diet intake and apparent total-tract digestibility were assessed; at 319 ± 31 days, the offspring's feeding behavior was evaluated; and at 346 ± 31 days, the calves were weighed, and biopsies of the *Longissimus thoracis* muscle were taken. RUP calves had a higher birth weight ($P = 0.043$) compared to the other treatments; greater morphometric measurements, such as Thorax width ($P = 0.015$), Ilium bones distance ($P = 0.006$), Ilium-Ischium distance ($P = 0.011$), Height at withers ($P < 0.001$), Rump height ($P = 0.019$), and Body circumference ($P = 0.006$), compared to CON calves; and greater Withers height ($P < 0.001$) compared to RDP calves. At 346 ± 31 days of age, RUP calves showed higher expressions of the *ACACA* ($P = 0.001$), *COL3A1* ($P = 0.050$), *CPT2* ($P = 0.046$), *FASN* ($P = 0.022$), *PPARG* ($P = 0.036$), and *IGFR1* ($P = 0.017$) genes compared to CON calves. Additionally, RUP offspring showed higher expression of the *ACACA* ($P < 0.001$), *COL3A1* ($P = 0.011$), *IGFR1* ($P = 0.024$), *FABP4* ($P = 0.010$), *PPARG* (P = 0.044), *PPARA* ($P = 0.029$), and *SCD1* ($P = 0.042$) genes when compared to the RDP group. Supplementing low-quality diets with rumen-protected protein for beef cows during mid-gestation improves the performance of newborns and may influence the quality traits of the offspring's meat.

Keywords: Arginine, bypass protein, fetal programming, ruminants.

1 INTRODUCTION

The availability and quality of forages in tropical countries vary throughout the seasons. As a result, pasture-based livestock production systems in these regions concentrate deliveries and the first months of lactation for the transition period between the dry and rainy seasons (Lemos et al., 2012; Gionbelli et al., 2018). The purpose of this is to ensure a greater amount of forage of better quality to the sows during this period, since there is a greater nutritional requirement for the pregnant female in this phase, in addition to improving the reproductive aspect of the cows in the subsequent breeding seasons. However, the middle and final thirds of pregnancy coincide with a time of shortage of good quality forage, due to the dry season of the year (Silva et al., 2017; Rodrigues et al., 2020). During this period, there is a decrease in pasture dry matter production, increasing lignin contents (Detmann, 2017) and reducing non-fibrous carbohydrates (Leng, 1990) and forage nitrogen compounds (Paulino et al., 2002; Detmann et al., 2009). Therefore, pregnant cows raised on pasture are more subject to nutritional restrictions during important phases such as the middle and final third of pregnancy.

The lack of an adequate supply of nutrients during pregnancy impairs not only fetal growth, but also the development of offspring in postnatal life, through the effects of fetal programming (Hoffman et al., 2017). The consequences of maternal malnutrition vary depending on the stage of pregnancy in which the restriction occurs, and can affect the development of organs (Cruz et al., 2019), muscle tissue (Zhu et al., 2004), adipose tissue (Bispham et al., 2003; Symonds et al., 2003) and growth characteristics (Martin et al., 2007) that influence the performance and meat quality of the offspring in the postnatal phase (Wu et al., 2006; Du et al., 2010). The effects of fetal programming include changes in gene and protein expression (Du and Zhu, 2009), through epigenetic modifications (Du et al., 2010).

Maternal nutrition is capable of promoting changes in the regulation of myogenic and adipogenic markers and modifying the cell signaling route, directing the pool of undifferentiated mesenchymal cells to compromise with the formation of muscle cells, adipocytes or fibroblasts in the fetus (Du et al., 2015). Previous studies indicate that maternal nutritional restriction until the first half of pregnancy is capable of reducing the number and composition of muscle fibers (Zhu et al., 2004; Zhu et al., 2006), and that nutritional scarcity from the second half of pregnancy can affect adipogenesis and muscle hypertrophy (Du et al., 2010; Underwood et al., 2010).

The middle third of pregnancy is the phase in which 95% of muscle fibers are formed (Zhou et al., 2021) and it coincides with the nutritional deficiency season of tropical pastures. Thus, protein supplementation can be used as a strategy to overcome the low quality of forages and ensure a greater flow of nutrients to the dam and fetus (Marquez et al., 2017). In addition, some studies point out that the supplementation of the amino acid Arginine (ARG) can be an interesting strategy (H Zhang et al., 2016b; Sun et al., 2017; Peine et al., 2018; Prezotto et al., 2018). Arginine is involved in the synthesis of nitric oxide, an important vasodilator agent that participates in the regulation of blood flow and allows an increase in vascular permeability (Martin et al., 2001; Roy et al., 2006), enabling improvements in the efficiency of nutrient absorption, embryonic and fetal development, lactation, and ovarian function (Meyer et al., 2018). However, there are knowledge gaps that need to be explored about the effects of fetal programming on metabolism and maternal/fetal performance, given these maternal supplementation strategies, especially during the phases in which dams and fetus are subject to tropical conditions of forage nutritional deficiency.

Therefore, due to the high demand for amino acids (AA) by pregnant cows raised in tropical regions, in addition to the potential benefit that ARG supplementation can promote a greater flow of nutrients between dams and fetus, the objective of this research was to identify the effects of maternal supplementation of a commercial product rich in AA protected from ruminal degradation (whose main AA is ARG), on the characteristics of growth and development of offspring in the long term.

2 MATERIAL AND METHODS

This study was performed at the Beef Cattle facilities of the Federal University of Lavras (UFLA), located in Lavras, Minas Gerais, Brazil. All management procedures for this study were approved by the UFLA Ethics Committee on Animal Use (protocol number 015/2019).

2.1 Management of cows and their diets during pregnancy

Thirty multiparous Zebu beef cows (*Bos taurus indicus;* 532 ± 11 kg BW, *6* ± 0.5 yr of age) were used in this study. Cows were submitted to a fixed-time artificial insemination protocol with semen from four Zebu males. Pregnancy was confirmed by a trained professional

using an ultrasound device (Aloka 500, 5 MHz probe, Aloka, Wallingford, CT, USA) 30 days after insemination. Additionally, cows that did not become pregnant were re-bred using two bulls. Cows with successful pregnancies were managed under the general research herd management until the onset of the experimental period. Thus, in this pre-experimental period they were allocated on a medium quality pasture (*Urochloa brizantha cv. Marandu*) and were fed a commercial mineral mixture (Probeef 800®, Cargill Animal Nutrition, Itapira, SP, Brazil) *ad libitum.* The guaranteed levels of the mineral mixture used were as follows: Calcium (max) - 190 g/kg; Calcium (min) - 140 g/kg; Cobalt (min) - 61 mg/kg; Copper (min) - 1091 mg/kg; Sulfur (min) - 16 g/kg; Fluorine (max) - 880 mg/kg; Phosphorus (min) - 80 g/kg; Iodine (min) - 55 mg/kg; Magnesium (min) - 5000 mg/kg; Manganese (min) - 4727 mg/kg; Selenium (min) - 11.8 mg/kg; Sodium (min) - 110 g/kg; and Zinc (min) - 3273 mg/kg.

At 105 days of gestation, cows were housed in individual stalls (20 m² area, 6 m² of it covered by a roof, with a concrete floor, and equipped with individual feeding and watering troughs) and were acclimated to a basal diet consisting of corn silage (82.3% DM) and sugarcane bagasse (17.7% DM) for approximately 15 days. At 127 ± 17 days of gestation, the treatments were randomly assigned to the cows, as follows: (1) **Control** (**CON**, *n* = 10) - basal diet + commercial mineral supplement containing urea (Probeef Urea®, Cargill Animal Nutrition, Itapira, SP, Brazil) at a level of 60 g/100 kg of BW (in order to simulate a production system of pregnant beef cows on pastures in tropical countries during the dry season of the year, using low supplementation) to reach at least 7.0% of CP in the total diet of the animals; (2) **Ruminally degradable protein supplement** (**RDP**, *n* = 10) - basal diet + 250g/100 kg of BW of a commercial protein supplement (Probeef Nutripec Sprint®, Cargill Animal Nutrition, Itapira, SP, Brazil) + 44 g of corn finely ground per 100 kg BW (to represent an average level of supplementation), to achieve at least 10% CP in the total diet; (3) **Ruminally undegradable protein supplement** (**RUP**, *n* = 10) - basal diet supplemented with 3.5 g/kg of BW of a commercial undegradable ruminal protein product, rich in arginine (Soypass®, Cargill Animal Nutrition, Itapira , SP, Brazil) added to a commercial mineral supplement containing urea (Probeef Urea®, Cargill Animal Nutrition, Itapira, SP, Brazil) at a level of 60 g per 100 kg of BW. Due to the interest in exploring the potential effects of fetal programming through the use of arginine, and the lack of a commercially available product containing only rumen-protected arginine in Brazil, the amount of Soypass used in the RUP treatment was calculated to ensure that at least 56% of the arginine in Soypass was protected from ruminal degradation. This

proportion was based on the study by Green et al. (2017) using beef cows as an animal model, where rumen-protected arginine had a rumen protection level of 56%. Additionally, the diet containing RUP was formulated to achieve the same proportion of RDP requirements as the RDP treatment, ensuring that any additional protein was supplied exclusively through RUP.

During the period of treatment application, the cows were fed twice daily (0700 h and 1300 h). The basal diet was provided *ad libitum*, and orts were measured daily to adjust feed intake. The cow's BW were monitored to adjust supplement amounts. From 227 days of gestation, pregnant cows were equally fed and moved to a pasture area (*Urochloa brizantha cv. Marandu*), in which they were managed as a single group. The pregnant beef cows were managed under a continuous stocking system and had *ad libitum* access to a commercial mineral mixture (Probeef 800, Cargill Animal Nutrition, Itapira, SP, Brazil).

2.2 Cow-calf management and measurements

All cows were allowed to give birth naturally. From the 30 pregnant cows utilized, one experienced an abortion, two cows gave a birth to twins (and were consequently removed from the study), and four calves died in the postnatal period due to external factors. Thus, the dataset was composed of information from 23 offsprings (16 males and 7 females). The calves were categorized as follows: 9 in the CON group (5 males and 4 females), 8 in the RDP group (7 males and 1 female), and 6 in the RUP group (4 males and 2 females).

After birth, the vigor score of the calves was assessed as described by Nascimento et al. (2024). Briefly, a single evaluator assigned a vigor score ranging from 1 to 4 to each calf. A score of 1 indicated extreme weakness and a score of 4 represented optimal vitality and health. Furthermore, their morphometric measurements were recorded at birth following the same methods also described by Nascimento et al. (2024). The measurements included thoracic and abdominal width; rib and rump depth; distances between the ischial and ileal bones, height at the withers, rump height, body length, and girth circumference. Girth circumference was measured using flexible tape, while other measurements were obtained using a hypometer (Walmur, Porto Alegre, Brazil). Moreover, the calves were weighed, identified, dewormed, and had their navels dipping in the neonatal period.

Twenty-four hours from birth, blood samples were obtained by puncturing the jugular vein of the neonates with vacutainer tubes (First Lab, São José dos Pinhais, PR, Brazil). After

collection, the blood samples were centrifuged at 3,500 × rpm for 15 minutes at 4°C to separate the serum, which was then stored at -20°C until further analysis. Commercial kits were used to determine serum concentrations of glucose (Glucose Liquiform, Labtest®, Lagoa Santa, Brazil), insulin (Monobind Inc., Lake Forest, CA, USA), and insulin-like growth factor-1 (IGF-1) (Bioassay Technology Laboratory, Yangpu Dis., Shanghai, China). Analyses were performed in the Animal Research Laboratory of the Department of Animal Science at UFLA, following the protocols provided by the commercial kits.

During the cow-calf phase, the cows and their calves were managed in the same pasture area used during the last third of gestation. The cows had *ad libitum* access to mineral supplementation (Probeef 800, Cargill Animal Nutrition, Itapira, SP, Brazil). At ~30 days of age, the calves were fed a protein-energetic supplementation (7 g/kg BW/day) through creep-feeding technique, using a commercial supplement (Probeef® Bambini Creep, Cargill Nutrição Animal, Itapira, SP, Brazil).

The calves were weighed at birth and once a month during the calf phase, to monitor the development of these animals. At 48 ± 10 days of age, biopsies of the *Longissimus thoracis* muscle were performed between the 12th and 13th ribs following disinfection of the area with 70% alcohol and shaving. Four milliliters of 2% lidocaine were administered for local anesthesia. A 1 cm incision was made with a scalpel, and approximately 1 cm^3 of muscle tissue was extracted for histological analysis. These tissue samples were immediately immersed in formalin, and after 48 hours, they were stored in 70% alcohol until further analysis. Additionally, approximately 1 g of tissue was collected for gene expression analysis. These samples were placed in 2 mL cryovials, snap-frozen in liquid nitrogen, and stored at -80°C until analysis. Post-procedure, the incision sites were sutured, and topical antibiotic, healing ointment, and anti-inflammatory agents were applied. The animals were monitored for 3 days, and any adverse reactions were recorded.

Calves were weaned at ~210 days of age. To account for variations in birth dates, calves were grouped into small groups for synchronized weaning. This approach facilitated experimental management and reduced weaning-related stress. Upon weaning, the calves were moved to a common pasture area (*Urochloa brizantha cv. Marandu*), where they remained until all animals had been weaned for the onset of the backgrounding phase.

2.3 Backgrounding management and measurements

The average age at the onset of the backgrounding phase was 277 ± 31 days. The young bulls and heifers were housed in individual feedlot pens (4 × 10 m, with a covered trough, cement flooring along the trough line, and an earthen remainder). Prior to conducting the planned measurements, the animals were submitted to a 9-day adaptation period to both the facilities and the experimental diet. During this period, the animals were fed for four consecutive days with a diet containing 75% forage and 25% concentrate, followed by 5 consecutive days with a diet containing 60% forage and 40% concentrate. After this period (at 286 ± 31 days of age), the animals began receiving the final diet for 60 days, which was used throughout the backgrounding and contained 50% forage and 50% concentrate (Table 1). In the feedlot young bulls and heifers were fed twice daily (0700 and 1300 h) and had free access to clean water. Throughout the entire feedlot period, daily feed refusals were measured to adjust the amount of diet provided accordingly.

To evaluate nutrient intake and apparent total tract digestibility of dietary components during the backgrounding, a five-day trial was conducted when the animals reached 306 ± 31 days of age. During the trial, the offered diet and feed refusals were weighed daily. In addition, representative samples of forage, concentrate, and orts were collected each day for subsequent chemical analysis. Fecal samples were collected from each animal using the hand grab technique from the rectum. These spot collections were conducted at different times each day of the trial (day 1 at 0600 hours, day 2 at 0900 hours, day 3 at 1200 hours, day 4 at 1500 hours, and day 5 at 1800 hours) to ensure that the composite samples were representative of each animal's fecal output throughout the daytime period. All collected samples (feed, orts, and feces) were stored at -20ºC until they were analyzed.

In the backgrounding phase, when the animals had 319 ± 31 days of age, a team of evaluators conducted a continuous 48-hour assessment of the offsprings to observe their behavioral patterns. Feed intake, water consumption, rumination, periods of inactivity (animal standing or sleeping), and other activities (including locomotion, socialization with other animals in adjacent pens, interaction with environmental elements, among others) were systematically recorded at 5-minute intervals throughout the ingestive behavior assessment. Observers were strategically placed to ensure that their presence did not affect the animals' natural behavior. To minimize disruption, artificial lighting in the facility was turned off during

the night and feeding behavior evaluations were performed using flashlights. Feeding behavior data were converted to continuous time by multiplying the frequency of each activity observed over a 48-hour period by the 5-minute time intervals to provide the total time spent on each activity, expressed in minutes per day.

At the end of the backgrounding period (345 ± 31 days of age), a carcass ultrasonography evaluation was performed to assess the following parameters: longissimus muscle area (LMA), subcutaneous fat thickness (SFT), rump muscle length (RML) and rump fat thickness. Ultrasonographic evaluations were performed using a high-resolution ultrasound machine (Aloka 500-V ultrasound device, Corometrics Medical Systems, Wallingford, CT) equipped with a 3.5 MHz, 17.2 cm linear transducer. For LMA and SFT measurements, the transducer was positioned between the 12th and 13th ribs along the midline of the animal's back. The Longissimus muscle area was delineated on the ultrasound image, and subcutaneous fat thickness was measured at this site. To assess RML and rump fat thickness, the transducer was placed at the junction of the biceps femoris and gluteus medius, between the ischium and the ilium, oriented parallel to the vertebral column. Rump muscle length and fat thickness were measured based on the ultrasound images obtained from this location. All images were captured and analyzed using ImageJ® software to ensure accurate measurement and quantification of the muscle and fat deposition parameters.

In addition, at 346 ± 31 days of age, a biopsy was conducted to obtain samples of the *Longissimus thoracis* muscle for histological and molecular analysis. The procedure adhered to the same methodology used for biopsies during the cow-calf phase, as previously outlined.

2.4 Laboratorial analysis

Muscle morphology. For the determination of muscle fiber number and size, the samples were dehydrated in an ascending alcohol series, cleared in xylene, and embedded in histological paraffin. A rotary microtome (RM 2265, Leica Biosystems, Nussloch, Germany) was used to obtain 3 to 6 sections, each 5.0 μm thick, which were subsequently stained with Hematoxylin-Eosin, following the method described by Pluske et al. (1996). Photomicrographs were then captured using an OLYMPUS CX31 microscope (Olympus Corp., Tokyo, Japan) at 40x magnification. Muscle fiber morphometry was analyzed using ImageJ® software (National Institutes of Health, Baltimore, MD, USA).

Gene expression. For gene expression analysis, muscle tissue samples were pulverized, and total RNA was extracted using the SV Total RNA Isolation System kit (Promega, Madison, WI, USA). RNA concentration was measured with a NanoVue spectrophotometer (GE Healthcare), and RNA integrity was confirmed by 1% agarose gel electrophoresis. The RNA was reverse transcribed into cDNA using the GoScript Reverse Transcription System kit (Promega). Primers for target and reference genes (β-actin [ACTB] and glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) were designed using PrimerQuest software (www.idtdna.com) based on GenBank sequences (Table X). RT-qPCR was conducted with a Mastercycler® realplex system (Eppendorf) and SYBR Green detection (Applied Biosystems, CA, USA). Gene expression calculations were performed using the ΔΔCt method.

Chemical analysis of feedstuffs, orts and fecal samples. All samples were dried in a forced ventilation oven (feedstuffs and refusals: 55 °C for 36 hours; fecal samples: 55 °C for 72 hours) and ground in a Wiley mill (Willye® TE-680), with bolters of 2 mm and 1 mm, for subsequent chemical analysis. The samples were analyzed according to the Brazilian National Institute of Science and Technology (INCT-CA) (Detmann et al., 2012). To determine the fecal production of the animals and thus the apparent digestibility of the total tract of the diet components, indigestible neutral detergent fiber (iNDF) was used as a marker (Valente et al., 2011). The iNDF content in feedstuffs and fecal samples was determined through *in situ* incubation for 288 hours using cannulated beef heifers, with laboratory analyses also conducted according to the protocols from the INCT-CA (Detmann et al., 2012).

2.5 Statistical Analysis

The cow and calf performance traits were analyzed according to the following linear model:

$$Y_{jklm} = \mu + T_j + S_k + P_l + \beta_1 iBW_m + \beta_2 Gen_m + \beta_3 Age_m + e_{jklm} \quad \text{[Eq. 1]}$$

where Y_{jklm} is the observed value; μ is the intercept; T_j is the fixed effect of the j^{th} level of maternal dietary treatment, with j = 1 to 3; S_k is the fixed effect of the k^{th} level of sex of the calf, with k = 1 or 2; P_l is the fixed effect of the l^{th} level of parity, with k = 1 to 3; β_1 is the

partial regression coefficient for the fixed effect of initial body weight (iBW) of the cow, and iBW_m is the iBW of the m^{th} cow; β_2 is the partial regression coefficient for the fixed effect of genetic potential of the cow (Gen) of the cow, and Gen_m is the Gen of the m^{th} cow; β_3 is the partial regression coefficient for the fixed effect of age at measurement (Age) of the cow, and Age_m is the Age of the m^{th} cow; and e_{jklm} is the random error associated with y_{jklm}, assuming $e \sim N(0, \boldsymbol{I_e}\sigma_e^2)$ where $\boldsymbol{I_e}$ represents the identity matrix of dimension equal to the number of observations.

The model used for the calf traits measured at the time of birth did not include the covariate *Age*. In addition, a logistic regression including the same fixed effects described in Eq. [1] was used for the calf trait Vigor Score, as records for this trait were binary.

The gene expression (CT) data were analyzed according to the linear mixed model below, following the strategy proposed by Steibel et al. (2009) where the data on the target and endogenous control genes are analyzed simultaneously:

$$CT_{ijklm} = \mu + G_i + T_j + (G*T)_{ij} + S_k + (G*S)_{ik} + P_l + (G*P)_{il} + \beta_1 iBW_m + (G*\beta)_{i1} iBW_m + \beta_2 Gen_m + (G*\beta)_{i2} Gen_m + A_m + e_{ijklm}$$

[Eq. 2]

where μ, T_j, S_k, P_l, β_1, iBW_m, β_2, and Gen_m are as previously defined in Eq. [1], whereas CT_{ijklm} is the observed CT, G_i is the fixed effect of the i^{th} level of gene, with i = 1 (target) or 2 (endogenous); $(G*T)_{ij}$ is the fixed effect for the interaction between G_i and T_j; $(G*S)_{ik}$ is the fixed effect for the interaction between G_i and S_k; $(G*P)_{il}$ is the fixed effect for the interaction between G_i and P_l; $(G*\beta)_{i1}$ is the fixed effect for the interaction between G_i and β_1; $(G*\beta)_{i2}$ is the fixed effect for the interaction between G_i and β_2; A_{jklm} is the random effect of the m^{th} animal, assuming $A \sim N(0, \boldsymbol{I_A}\sigma_{S_i}^2)$, where $\boldsymbol{I_A}$ represents the identity matrix with dimensions equal to the number of animals; and e_{ijklm} is the random error associated with CT_{ijklm}, assuming $e \sim N(0, \boldsymbol{I_{Ge}}\sigma_e^2)$, where $\boldsymbol{I_{Ge}}$ represents the identity matrix with dimensions equal to the number of observations across the two genes. The interactions between G_i with T_j, S_k, P_l, β_1, and β_2, were used to assess the effects of *maternal dietary treatment*, *sex of the calf*, *parity*, *iBW*, and *Gen*, respectively, through orthogonal contrasts, as in Steibel et al. (2009). Expected CT were computed as $-\Delta CT$, such that the differences between the levels of the categorical fixed effects of interest *Treatment* were estimated as $-\Delta\Delta CT$.

Prior to final analyses, residuals were evaluated for normality. For each analysis, data points were removed one at a time until absolute Studentized residuals were lower than 3 and had non-significant ($P > 0.01$) Shapiro-Wilk's test for normality. Expected means were generated from the final models and separated using Fisher's LSD test when significant ($P < 0.05$). All analyses were performed in SAS Studio 3.81 (Enterprise Edition, SAS Institute Inc., Cary, NC, USA).

3 RESULTS

Nutrient intake data and effects of maternal supplementation during mid-gestation on maternal performance were previously reported in our complementary study (de Oliveira, 2021).

3.1 Performance and morphometric measurements of offspring

At birth, animals from cows in the RUP treatment had a higher body weight compared to animals from the CON and RDP groups ($P = 0.043$; Table 2). Morphometric data of calves were measured at birth and are presented in Table 2. Calves born to RUP cows had greater measurements of thorax width ($P = 0.015$), ilium bones distance ($P = 0.006$), ilium-ischium distance ($P = 0.011$), height at withers ($P < 0.001$), rump height ($P = 0.019$) and body circumference ($P = 0.006$), when compared to calves born to CON cows. However, RUP calves presented superior measurements compared to RDP calves only for the characteristics of body weight at birth ($P = 0.043$) and height at withers ($P < 0.001$). When comparing the RDP and CON offspring, we observed that the RDP group had greater thorax width ($P = 0.015$) and a larger Ilium bones distance ($P = 0.006$).

Maternal dietary treatments did not influence the weight gain of animals in the growing and backgrounding phases ($P > 0.242$; Table 2).

3.2 Blood parameters of offspring at birth

The Table 3 shows the blood parameters evaluated in newborn calves of cows according to the dietary treatment provided during the mid-gestation. No differences were observed for

glucose (P = 0.631), insulin (P = 0.477) or growth factor IGF-1 (P = 0.334) levels of the offspring.

3.3 Dietary fraction intake and apparent total tract digestibility of offspring

During the backgrounding phase, no differences were observed in the consumption of dry matter and other fractions of the diet ($P \geq 0.143$; Table 4), as well as the apparent digestibility of the total tract of the offspring ($P \geq 0.399$; Table 4).

3.4 Ingestive behavior of offspring

Maternal treatments during mid-gestation did not influence the ingestive behavior of the offspring during the backgrounding phase ($P \geq 0.266$, Table 5).

3.5 Measurements of growth and development of skeletal muscle tissue

The measurements related to the formation and development of muscle fibers in the offspring are presented in Table 6. The number of muscle fibers was not influenced by maternal treatment throughout the life of the offspring.

The Figure 7 shows photomicrographs of skeletal muscle tissue from progeny at different ages (48 ± 10 days or 346 ± 31 days of age), representing each of the maternal dietary treatments (CON, RDP or RUP).

3.6 Carcass ultrasound measurements of offspring

Maternal dietary treatments did not show any effects on carcass ultrasound measurements in the offspring ($P \geq 0.070$, Table 7).

3.7 mRNA Expression in skeletal muscle tissue of offspring

Gene expression in skeletal muscle tissue samples of offspring according to age is presented in Table 8. No effect of maternal supplementation type was observed on the

expression of genes involved in the growth, development and metabolism of the offspring skeletal muscle tissue up to 48 ± 10 days of age ($P \geq 0.121$).

At 346 ± 31 days of age, no gene expression differences were observed between the CON and RDP groups ($P \geq 0.173$). However, when comparing the CON and RUP groups, offspring of RUP cows showed higher expression of *ACACA* ($P = 0.001$), a gene involved in the metabolism and regulation of fatty acid biosynthesis; higher expression of *CPT2* ($P = 0.046$), a gene involved in the process of fatty acid oxidation and energy production; higher expression of *FASN* ($P = 0.022$), a gene involved in the synthesis, deposition and composition of fatty acids; *PPARG* ($P = 0.036$), gene is essential for transforming FAP cells into pre-adipocytes and then into mature adipocytes; and higher expression of *IGFR1* ($P = 0.017$), a gene of a membrane glycoprotein that mediates the biological actions of insulin-like growth factors. In addition, RUP calves showed higher expression of *COL3A1* ($P \geq 0.05$), a gene associated with the formation of type III collagen in bovine muscle tissue; when compared to other treatments.

Differences were more evident when we compared gene expression between offspring of RDP and RUP treatments. RUP calves showed greater expression of the genes previously mentioned, such as *ACACA* ($P < 0.001$) and *IGFR1* ($P = 0.024$). In addition, the RUP treatment expressed more markers such as *FABP4* and *PPARG* ($P = 0.010$; $P = 0.044$, respectively), key-regulator genes associated with fat deposition and meat quality in cattle; *PPARA* ($P = 0.029$), a gene associated with lipid metabolism and fatty acid oxidation; and *SCD1* ($P = 0.042$), a gene essential for the metabolism and composition of fatty acids in muscle and beef.

4 DISCUSSION

In tropical countries, pregnant beef cows usually spend the middle and late trimester of gestation under low-quality forage conditions (dry season of the year), which reduces the intake of DM and nutrients (Santos et al., 2022). In many cases, the main limiting factor is the lack of protein in these animals' diets. Nitrogen deficiency in the ruminal environment reduces ruminal fibrolytic activity, leading to nutritional restrictions for the animal due to a decreased passage rate and DMI (Lazzarini et al., 2009). Protein supplementation strategies can be employed in these situations to achieve productive success for both the cows (Izquierdo et al., 2022; Meneses

et al., 2024; de Oliveira, 2021) and their offspring (Bohnert et al., 2013; Carvalho et al., 2022; Nascimento et al., 2022; Palmer et al., 2022; Vedovatto et al., 2022).

In our complementary study (de Oliveira, 2021), the maternal diets provided during mid-gestation were formulated to meet different crude protein requirements (CON = 59%, RDP = 104%, and RUP = 163%). The greater fulfillment of protein requirements for RUP cows resulted in higher birth weights and larger body measurements of the calves. Consistently, a meta-analysis conducted by Barcelos et al. (2022), using data from 3,121 animals, demonstrated that pregnant beef cows with higher body weight and/or cows on high nutritional planes generally give birth to heavier calves. Thus, the higher birth weight of RUP calves in the present study may be associated with their dams having a greater body weight at the end of the treatment applied during the mid-gestation (de Oliveira, 2021). Although the offspring of RUP cows were born heavier, this performance effect did not persist over time, as no statistical differences were identified in body weight at weaning and during the backgrounding phase. However, numerically there was a significant biological difference in BW between treatments at the end of the backgrounding phase (CON = 291 kg *vs.* RDP = 304 kg *vs.* RUP = 319 kg). In addition, the RUP group exhibited higher expression of the *IGFR1* gene during the backgrounding phase compared to the other treatments. There was likely greater availability of nutrients and energy during gestation for fetal development, resulting in better performance of the RUP newborns.

Furthermore, it is important to note that the rumen undegradable protein supplement used in the RUP maternal treatment is rich in the amino acid ARG, which regulates blood flow and increases vascular permeability (Martin et al., 2001; Roy et al., 2006). This may promote improvements in nutrient absorption efficiency, embryonic and fetal development (Meyer et al., 2018). This could have contributed to a greater nutrient flow between dams and fetus, as observed in the previous study (de Oliveira, 2021), where uterine blood flow was higher for RUP cows compared to other experimental groups.

When pregnant cows are in scenarios represented by the CON treatment, where the protein requirement is not fully met, animals may trigger regulatory mechanisms to compensate for the lack of dietary N, such as mobilizing body tissues and recycling N (Batista et al., 2016; Rufino et al., 2016), in order to maintain rumen function and meet maternal-fetal demands. Consequently, the lack of maternal treatment effects on the observed characteristics, especially between the CON and RDP offspring in this study, may be related to these mechanisms. In our complementary study, we observed that CON and RDP cows showed a lower Longissimus

muscle area, indicating greater mobilization of maternal body tissues compared to the RUP group (de Oliveira, 2021).

Additionally, these compensatory processes may have resulted in the lack of statistical differences in the number and size of muscle fibers in the offspring, as well as in carcass ultrasound measurements at the end of the backgrounding phase. These findings also support the absence of maternal treatment effects on the weight gain of the offspring observed after weaning. Studies suggest that maternal nutrition can affect the recruitment of cells to the myogenic lineage (Stannard e Johnson, 2004; Zambrano et al., 2005; Zhu et al., 2006). According to reports in the literature, calves from cows that underwent protein restriction during mid-gestation showed a lower number of muscle fibers throughout life (Marquez et al., 2017; Costa et al., 2021b), which contradicts our results. Therefore, we believe that the processes of tissue mobilization and N recycling favored the individuals in the present study, especially the offspring of CON cows, through the necessary nutritional support for the formation of fetal skeletal muscle in the calves. In other words, it appears that the maternal treatments in the present study did not compromise the determination of undifferentiated mesenchymal cell (or stem cell) lineages in the offspring during the prenatal phase. Non-significant gene expression data between treatments, which are related to cellular differentiation (*C/EBPA*, *TGFB1*, *ZFP423*, *MYOD*, and *MYOG*) confirm this hypothesis.

However, our results showed that the calves from RUP cows had a higher expression of the *ACACA* gene compared to the other treatments. This gene encodes the enzyme acetyl-CoA carboxylase, which converts acetyl-CoA into malonyl-CoA. From this conversion, malonyl-CoA receives additional acetyl-CoA molecules through various reactions of the multi-enzyme fatty acid synthase complex, encoded by the *FASN* gene, resulting in the formation of long-chain fatty acids (Ladeira et al., 2016). One of these fatty acids is palmitic acid (C16:0), which can undergo elongation or desaturation processes, catalyzed by the enzyme stearoyl-CoA desaturase, encoded by the *SCD1* gene (Duckett et al., 2009; Ladeira et al., 2014). The animals in the RUP group also showed higher expression of the *SCD1*, *PPARG*, and *PPARA* genes compared to the RDP group. Additionally, we observed that RUP calves exhibited higher expression of the *FASN* gene compared to calves from the CON group. According to the literature, animals with high marbling in their meat show greater activation of acetyl-CoA carboxylase and the multi-enzyme fatty acid synthase complex (Ward et al., 2010).

The *PPARG* is expressed more intensely in adipose tissue than in muscle tissue (Kersten, 2014) and plays a crucial role in lipid metabolism, regulating genes associated with fatty acid storage, such as *FABP*, *ACSL1*, and *LPL* (Bionaz et al., 2012). According to our results, *FABP4* gene was more expressed in the offspring of RUP cows. This gene is primarily expressed in mature adipocytes, encoding proteins related to the absorption and transport of fatty acids (Gregoire et al., 1998). The *PPARA* gene also regulates the expression of long-chain fatty acid transporters; however, it also influences the expression of enzymes involved in the β-oxidation of fatty acids (Ladeira et al., 2016). Fatty acids bound to *FABP* within adipocytes are transported from the cell membrane to the site of esterification or to β-oxidation in the mitochondria (Gerbens, 2004). For β-oxidation to occur, fatty acids must undergo lipolysis and bind to carnitine molecules through the action of the enzyme carnitine palmitoyl transferase 1 (*CPT1*), allowing their transport from the cytosol to the mitochondrial matrix. Only then can the enzyme *CPT2* dissociate acyl-CoA from carnitine, enabling the oxidation of fatty acids for energy production (Houten et al., 2016). According to our study, the *CPT2* gene was more expressed in RUP calves compared to CON calves. However, we did not observe differences in the expression of genes involved in the formation of oxidative and glycolytic muscle fibers (*MYHC2a* and *MYHC2x* genes, respectively) among the evaluated treatments. The amount of intramuscular fat deposited by the animal reflects how much the adipocyte can capture fatty acids from the bloodstream, in addition to what is synthesized by *de novo* synthesis, excluding the fatty acids that are oxidized. Therefore, the results obtained in this study indicate a greater lipid turnover in the RUP offspring.

Another result observed in the RUP offspring was the higher expression of the *COLA31* gene compared to the other treatments. This gene encodes an essential structural protein in the composition of the extracellular matrix, primarily present in the connective tissues of the skin, lungs, blood vessels, and muscles (Ricard-Blum, 2011). We believe that the maternal treatment favored the expression of this gene in the RUP offspring, particularly due to the connection between the processes of angiogenesis and adipogenesis (Cao, 2010), as the RUP offspring also showed higher expression of genes related to adipogenesis.

Overall, the results of this experiment indicate that maternal supplementation with RUP, meeting 163% of the dams' crude protein requirements, improves the weight and body measurements of newborns; however, it predisposes the offspring to exhibit greater lipid turnover in muscle tissue. More studies are needed to evaluate the effects of this type of

maternal supplementation on the meat quality of the offspring, as it was not possible to slaughter the animals from this experiment for such an assessment.

5 CONCLUSION

Supplementation of low-quality forages with ruminal-protected protein for beef cows during mid-gestation is effective in improving offspring performance at birth. However, the statistical difference in body weight did not persist into the backgrounding phase. Due to the limited number of experimental units in this study, we can consider that RUP offspring have the potential for higher performance during this phase, based on the numerical data of body weight. Additionally, our gene expression results suggest that this type of maternal diet may influence the quality characteristics of the offspring's meat. Further research is needed to investigate the impacts of maternal supplementation with RUP in later stages of beef cattle production and on the qualitative aspects of the meat.

REFERENCES

Amthor, H., B. Christ, and K. Patel. 1999. A molecular mechanism enabling continuous embryonic muscle growth-a balance between proliferation and differentiation. Development. 126:1041–1053.

Astruc, T. 2014a. Carcass composition, muscle structure and contraction.

Astruc, T. 2014b. Connective tissue: Structure, function, and influence on meat quality.

Barcelos, S. de S., K. B. Nascimento, T. E. da Silva, R. Mezzomo, K. S. Alves, M. de Souza Duarte, and M. P. Gionbelli. 2022. The effects of prenatal diet on calf performance and perspectives for fetal programming studies: a meta-analytical investigation. Animals. 12:2145.

Bastida, C. M., F. Tejada, A. Cremades, and R. Peñafiel. 2002. The preovulatory rise of ovarian ornithine decarboxylase is required for progesterone secretion by the corpus luteum. Biochem. Biophys. Res. Commun. 293:106–111.

Batista, E. D., A. H. Hussein, E. Detmann, M. D. Miesner, and E. C. Titgemeyer. 2016. Efficiency of lysine utilization by growing steers. J. Anim. Sci. 94:648–655.

Bazer, F. W., J. Kim, G. Song, H. Ka, G. Wu, G. A. Johnson, J. L. Vallet, H. Rodriquez-Martinez, N. M. Soede, and W. L. Flowers. 2013. Roles of selected nutrients in development of the porcine conceptus during pregnancy. Control Pig Reprod. IX. 68:159–174.

Bazer, F. W., X. Wang, G. A. Johnson, and G. Wu. 2015a. Select nutrients and their effects on conceptus development in mammals Select nutrients and their effects on conceptus development in mammals. Anim. Nutr. doi:10.1016/j.aninu.2015.07.005.

Bazer, F. W., X. Wang, G. A. Johnson, and G. Wu. 2015b. Select nutrients and their effects on conceptus development in mammals. Anim. Nutr. 1:85–95.

Bell, A. W., P. L. Greenwood, and R. A. Ehrhardt. 2005. Regulation of metabolism and growth during prenatal life. Biol. Grow. Anim. 3:3–34.

Berry, R., and M. S. Rodeheffer. 2013. Characterization of the adipocyte cellular lineage in vivo. Nat. Cell Biol. 15:302–308.

Bionaz, M., B. J. Thering, and J. J. Loor. 2012. Fine metabolic regulation in ruminants via nutrient–gene interactions: saturated long-chain fatty acids increase expression of genes involved in lipid metabolism and immune response partly through PPAR-α activation. Br. J. Nutr. 107:179–191.

Bispham, J., G. S. Gopalakrishnan, J. Dandrea, V. Wilson, H. Budge, D. H. Keisler, F. Broughton Pipkin, T. Stephenson, and M. E. Symonds. 2003. Maternal endocrine adaptation throughout pregnancy to nutritional manipulation: consequences for maternal plasma leptin and cortisol and the programming of fetal adipose tissue development. Endocrinology. 144:3575–3585.

Bistrian, B. R. 2000. Biochemical and physiological aspects of human nutrition. Am. J. Clin.

Nutr. 71:1619–1620.

Blouet, C., and G. J. Schwartz. 2010. Hypothalamic nutrient sensing in the control of energy homeostasis. Behav. Brain Res. 209:1–12.

Bohnert, D. W., L. A. Stalker, R. R. Mills, A. Nyman, S. J. Falck, and R. F. Cooke. 2013. Late gestation supplementation of beef cows differing in body condition score: Effects on cow and calf performance. J. Anim. Sci. 91:5485–5491. doi:10.2527/jas.2013-6301.

Bonnet, M., I. Cassar-Malek, Y. Chilliard, and B. Picard. 2010. Ontogenesis of muscle and adipose tissues and their interactions in ruminants and other species. Animal. 4:1093–1109.

Buckley, A. J., A. L. Jaquiery, and J. E. Harding. 2005. Nutritional programming of adult disease. Cell Tissue Res. 322:73–79.

Bunger, M., H. M. van den Bosch, J. van der Meijde, S. Kersten, G. J. E. J. Hooiveld, and M. Muller. 2007. Genome-wide analysis of PPARα activation in murine small intestine. Physiol. Genomics. 30:192–204.

Cao, Y. 2010. Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nat. Rev. Drug Discov. 9:107–115.

Carvalho, E. B., T. C. Costa, L. P. Sanglard, K. B. Nascimento, J. A. M. Meneses, M. C. Galvão, N. V. L. Serão, M. S. Duarte, and M. P. Gionbelli. 2022. Transcriptome profile in the skeletal muscle of cattle progeny as a function of maternal protein supplementation during mid-gestation. Livest. Sci. 263:104995.

Caton, J. S., M. S. Crouse, K. J. McLean, C. R. Dahlen, A. K. Ward, R. A. Cushman, A. T. Grazul-Bilska, B. W. Neville, P. P. Borowicz, and L. P. Reynolds. 2020. Maternal periconceptual nutrition, early pregnancy, and developmental outcomes in beef cattle. J. Anim. Sci. 98:skaa358.

Caton, J. S., M. S. Crouse, L. P. Reynolds, T. L. Neville, C. R. Dahlen, A. K. Ward, and K. C. Swanson. 2019. Maternal nutrition and programming of offspring energy requirements. Transl. Anim. Sci. 3:976–990.

Caton, J. S., and B. W. Hess. 2010. Maternal plane of nutrition: Impacts on fetal outcomes and postnatal offspring responses. In: Proc. 4th Grazing Livestock Nutrition Conference. BW Hess, T. DelCurto, JGP Bowman and RC Waterman (eds.) West. Sect. Am. Soc. Anim. Sci., Champaign, Ill. p. 104–122.

Caton, J. S., J. J. Reed, R. P. Aitken, J. S. Milne, P. P. Borowicz, L. P. Reynolds, D. A. Redmer, and J. M. Wallace. 2009. Effects of maternal nutrition and stage of gestation on body weight, visceral organ mass, and indices of jejunal cellularity, proliferation, and vascularity in pregnant ewe lambs. J. Anim. Sci. 87:222–235. doi:10.2527/jas.2008-1043.

Chacher, B., D. M. Wang, H. Y. Liu, and J. X. Liu. 2012. Degradation of L-arginine and N-carbamoyl glutamate and their effect on rumen fermentation in vitro. Ital. J. Anim. Sci. 11:374–377. doi:10.4081/ijas.2012.e68.

Chargé, S. B. P., and M. A. Rudnicki. 2004. Cellular and molecular regulation of muscle regeneration. Physiol. Rev. 84:209–238.

Clare, C. E., A. H. Brassington, W. Y. Kwong, and K. D. Sinclair. 2019. One-carbon metabolism: linking nutritional biochemistry to epigenetic programming of long-term development. Annu. Rev. Anim. Biosci. 7:263–287.

Clark, M. G., O. H. Filsell, and I. G. Jarrett. 1976. Gluconeogenesis in isolated intact lamb liver cells. Effects of glucagon and butyrate. Biochem. J. 156:671–680.

Corah, L., and M. McCully. 2007. Declining quality grades: A review of factors reducing marbling deposition in beef cattle. Certif. Angus Beef News Releases-Research.

Costa, Thais Correia, M. Du, K. B. Nascimento, M. C. Galvão, J. A. M. Meneses, E. B. Schultz, M. P. Gionbelli, and M. de S. Duarte. 2021a. Skeletal muscle development in postnatal beef cattle resulting from maternal protein restriction during mid-gestation. Animals. 11:860.

Costa, Thaís Correia, M. P. Gionbelli, and M. de S. Duarte. 2021b. Fetal programming in ruminant animals: understanding the skeletal muscle development to improve meat quality. Anim. Front. 11:66–73.

Crouse, M. S., J. Caton, and A. K. Ward. 2020. 390 Micronutrients, One-Carbon Metabolism, and Epigenetics: Potential Developmental and Production Outcomes. J. Anim. Sci. 98:170.

da Cruz, W. F. G., J. P. Schoonmaker, F. D. de Resende, G. R. Siqueira, L. M. Rodrigues, G. D. R. Zamudio, and M. M. Ladeira. 2019. Effects of maternal protein supplementation and inclusion of rumen-protected fat in the finishing diet on nutrient digestibility and expression of intestinal genes in Nellore steers. Anim. Sci. J. 90:1200–1211.

Desvergne, B., and W. Wahli. 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr. Rev. 20:649–688.

Detmann, E. 2017. Nutrition of grazing beef cattle focused on efficiency of N utilization. In: Anais X SIMPEC and V International Symposium of Beef Cattle Production.

Detmann, E., M. F. Paulino, H. C. Mantovani, S. de C. V. Filho, C. B. Sampaio, M. A. de Souza, Í. Lazzarini, and K. S. C. Detmann. 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using Michaelis-Menten kinetics. Livest. Sci. 126:136–146. doi:10.1016/j.livsci.2009.06.013. Available from: http://dx.doi.org/10.1016/j.livsci.2009.06.013

Detmann, E., M. A. de Souza, S. de C. Valadares Filho, A. C. de Queiroz, T. T. Berchielli, E. de O. S. Saliba, L. da S. Cabral, D. dos S. Pina, M. M. Ladeira, and J. A. G. Azevedo. 2012. Métodos para análise de alimentos. Visconde do Rio Branco, MG Suprema. 214.

Du, M., Y. Huang, A. K. Das, Q. Yang, M. S. Duarte, M. V Dodson, and M.-J. Zhu. 2013. Meat Science and Muscle Biology Symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. J. Anim. Sci. 91:1419–1427.

Du, M., and P. Stephen. 2018. Optimizing livestock production efficiency through maternal nutritional management and fetal developmental programming. 7:5–11. doi:10.2527/af.2017-0122.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88:E51–E60.

Du, M., J. Tong, J. Zhao, K. R. Underwood, M. Zhu, S. P. Ford, and P. W. Nathanielsz. 2010. Fetal programming of skeletal muscle development in ruminant animals. J. Anim. Sci. 88. doi:10.2527/jas.2009-2311.

Du, M., B. Wang, X. Fu, Q. Yang, and M.-J. Zhu. 2015. Fetal programming in meat production. Meat Sci. 109:40–47.

Du, Min, X. Yan, J. F. Tong, J. Zhao, and M. J. Zhu. 2010a. Maternal obesity, inflammation, and fetal skeletal muscle development. Biol. Reprod. 82:4–12.

Du, Min, J. Yin, and M. J. Zhu. 2010b. Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscle. Meat Sci. 86:103–109.

Du, M., and M. J. Zhu. 2009. Fetal programming of skeletal muscle development. Appl Musc Biol Meat Sci. 81–96.

Du, M., M. J. Zhu, G. A. Olson, B. W. Hess, W. J. Means, and S. P. Ford. 2005. Nutrient restriction in cows alters the number and volume of fetal myofibers. In: JOURNAL OF DAIRY SCIENCE. Vol. 88. AMER DAIRY SCIENCE ASSOC 1111 N DUNLAP AVE, SAVOY, IL 61874 USA. p. 388.

Duarte, M. S., M. P. Gionbelli, P. V. R. Paulino, N. V. L. Serão, C. S. Nascimento, M. E. Botelho, T. S. Martins, S. C. V Filho, M. V Dodson, and S. E. F. Guimaraes. 2014. Maternal overnutrition enhances mRNA expression of adipogenic markers and collagen deposition in skeletal muscle of beef cattle fetuses. J. Anim. Sci. 92:3846–3854.

Duarte, M. S., P. V. R. Paulino, A. K. Das, S. Wei, N. V. L. Serao, X. Fu, S. M. Harris, M. V Dodson, and M. Du. 2013. Enhancement of adipogenesis and fibrogenesis in skeletal muscle of Wagyu compared with Angus cattle. J. Anim. Sci. 91:2938–2946.

Duckett, S. K., S. L. Pratt, and E. Pavan. 2009. Corn oil or corn grain supplementation to steers grazing endophyte-free tall fescue. II. Effects on subcutaneous fatty acid content and lipogenic gene expression. J. Anim. Sci. 87:1120–1128.

Fève, B. 2005. Adipogenesis: cellular and molecular aspects. Best Pract. Res. Clin. Endocrinol. Metab. 19:483–499.

Flynn, N. E., C. J. Meininger, T. E. Haynes, and G. Wu. 2002. The metabolic basis of arginine nutrition and pharmacotherapy. Biomed. Pharmacother. 56:427–438.

Ford, S. P., B. W. Hess, M. M. Schwope, M. J. Nijland, J. S. Gilbert, K. A. Vonnahme, W. J. Means, H. Han, and P. W. Nathanielsz. 2007. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. J. Anim. Sci. 85:1285–1294. doi:10.2527/jas.2005-624.

Fowden, A. L. 2006. Intrauterine Programming of Physiological Systems: Causes and Consequences. Physiology. 21:29–37. doi:10.1152/physiol.00050.2005. Available from: http://physiologyonline.physiology.org/cgi/doi/10.1152/physiol.00050.2005

Gao, H., G. Wu, T. E. Spencer, G. A. Johnson, X. Li, and F. W. Bazer. 2009. Select nutrients in the ovine uterine lumen. I. Amino acids, glucose, and ions in uterine lumenal flushings of cyclic and pregnant ewes. Biol. Reprod. 80:86–93.

Gardner, D. S., K. Tingey, B. W. M. Van Bon, S. E. Ozanne, V. Wilson, J. Dandrea, D. H. Keisler, T. Stephenson, and M. E. Symonds. 2005. Programming of glucose-insulin metabolism in adult sheep after maternal undernutrition. Am. J. Physiol. Integr. Comp. Physiol. 289:R947–R954.

Gerbens, F. 2004. Genetic control of intramuscular fat accretion. In: Muscle development of livestock animals: physiology, genetics and meat quality. CABI Publishing Wallingford UK. p. 343–362.

Ghaben, A. L., and P. E. Scherer. 2019. Adipogenesis and metabolic health. Nat. Rev. Mol. cell Biol. 20:242–258.

Gionbelli, T. R. S., C. M. Veloso, P. P. Rotta, S. C. Valadares Filho, B. C. Carvalho, M. I. Marcondes, C. S. Cunha, M. A. S. Novaes, L. D. Prezotto, and M. S. Duarte. 2018. Foetal development of skeletal muscle in bovines as a function of maternal nutrition, foetal sex and gestational age. J. Anim. Physiol. Anim. Nutr. (Berl). 102:545–556.

Glatz, J. F. C., J. J. F. P. Luiken, and A. Bonen. 2010. Membrane fatty acid transporters as regulators of lipid metabolism: implications for metabolic disease. Physiol. Rev.

Gnanalingham, M. G., A. Mostyn, M. E. Symonds, and T. Stephenson. 2005. Ontogeny and nutritional programming of adiposity in sheep: potential role of glucocorticoid action and uncoupling protein-2. Am. J. Physiol. Integr. Comp. Physiol. 289:R1407–R1415.

Gonzalez-Bulnes, A., C. Ovilo, C. J. Lopez-Bote, S. Astiz, M. Ayuso, M. L. Perez-Solana, R. Sanchez-Sanchez, and L. Torres-Rovira. 2012. Gender-specific early postnatal catch-up growth after intrauterine growth retardation by food restriction in swine with obesity/leptin resistance. Reproduction. 144:269.

Gonzalez, J. M., L. E. Camacho, S. M. Ebarb, K. C. Swanson, K. A. Vonnahme, A. M. Stelzleni, and S. E. Johnson. 2013. Realimentation of nutrient restricted pregnant beef cows supports compensatory fetal muscle growth. J. Anim. Sci. 91:4797–4806.

Greenwood, P. L., A. S. Hunt, J. W. Hermanson, and A. W. Bell. 2000. Effects of birth weight and postnatal nutrition on neonatal sheep: II. Skeletal muscle growth and development. J. Anim. Sci. 78:50–61.

Greenwood, P. L., R. M. Slepetis, A. W. Bell, and J. W. Hermanson. 1999. Intrauterine growth retardation is associated with reduced cell cycle activity, but not myofibre number, in ovine fetal muscle. Reprod. Fertil. Dev. 11:281–291.

Gregoire, F. M., C. M. Smas, and H. S. Sul. 1998. Understanding adipocyte differentiation. Physiol. Rev. 78:783–809.

Grifone, R., J. Demignon, J. Giordani, C. Niro, E. Souil, F. Bertin, C. Laclef, P.-X. Xu, and P. Maire. 2007. Eya1 and Eya2 proteins are required for hypaxial somitic myogenesis in the mouse embryo. Dev. Biol. 302:602–616.

Gupta, R. K., R. J. Mepani, S. Kleiner, J. C. Lo, M. J. Khandekar, P. Cohen, A. Frontini, D. C. Bhowmick, L. Ye, and S. Cinti. 2012. Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. Cell Metab. 15:230–239.

Hoffman, M. L., S. A. Reed, S. M. Pillai, A. K. Jones, K. K. McFadden, S. A. Zinn, and K. E. Govoni. 2017. Physiology and endocrinology symposium: the effects of poor maternal nutrition during gestation on offspring postnatal growth and metabolism. J. Anim. Sci. 95:2222–2232.

Houten, S. M., S. Violante, F. V Ventura, and R. J. A. Wanders. 2016. The biochemistry and physiology of mitochondrial fatty acid β-oxidation and its genetic disorders. Annu. Rev. Physiol. 78:23–44.

Huang, Y., J.-X. Zhao, X. Yan, M.-J. Zhu, N. M. Long, R. J. McCormick, S. P. Ford, P. W. Nathanielsz, and M. Du. 2012. Maternal obesity enhances collagen accumulation and cross-linking in skeletal muscle of ovine offspring. PLoS One. 7:e31691.

Hui, S.-C. G., and T.-Y. Chan. 1995. Mechanisms mediating NG-nitro-L-arginine methyl ester-induced hypophagia in mice. Eur. J. Pharmacol. 283:141–150.

Ignarro, L. J., G. M. Buga, L. H. Wei, P. M. Bauer, G. Wu, and P. del Soldato. 2001. Role of the arginine-nitric oxide pathway in the regulation of vascular smooth muscle cell proliferation. Proc. Natl. Acad. Sci. 98:4202–4208.

Izquierdo, V., M. Vedovatto, E. A. Palmer, R. A. Oliveira, H. M. Silva, J. M. B. Vendramini, and P. Moriel. 2022. Frequency of maternal supplementation of energy and protein during late gestation modulates preweaning growth of their beef offspring. Transl. Anim. Sci. 6:txac110.

Joe, A. W. B., L. Yi, A. Natarajan, F. Le Grand, L. So, J. Wang, M. A. Rudnicki, and F. M. V Rossi. 2010. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat. Cell Biol. 12:153–163.

Joffin, N., F. Niang, C. Forest, and A.-M. Jaubert. 2012. Is there NO help for leptin? Biochimie. 94:2104–2110.

Kassar-Duchossoy, L., E. Giacone, B. Gayraud-Morel, A. Jory, D. Gomès, and S. Tajbakhsh. 2005. Pax3/Pax7 mark a novel population of primitive myogenic cells during development. Genes Dev. 19:1426–1431.

Kersten, S. 2014. Integrated physiology and systems biology of PPARα. Mol. Metab. 3:354–371.

Khorram, O., N. Khorram, M. Momeni, G. Han, J. Halem, M. Desai, and M. G. Ross. 2007. Maternal undernutrition inhibits angiogenesis in the offspring: a potential mechanism of programmed hypertension. Am. J. Physiol. Integr. Comp. Physiol. 293:R745–R753.

Kim, J., G. Song, G. Wu, and F. W. Bazer. 2012. Functional roles of fructose. Proc. Natl. Acad. Sci. 109:E1619–E1628.

Kim, J., G. Song, G. Wu, H. Gao, G. A. Johnson, and F. W. Bazer. 2013. Arginine, leucine, and glutamine stimulate proliferation of porcine trophectoderm cells through the MTOR-

RPS6K-RPS6-EIF4EBP1 signal transduction pathway. Biol. Reprod. 88:111–113.

Kimball, S. R., L. M. Shantz, R. L. Horetsky, and L. S. Jefferson. 1999. Leucine regulates translation of specific mRNAs in L6 myoblasts through mTOR-mediated changes in availability of eIF4E and phosphorylation of ribosomal protein S6. J. Biol. Chem. 274:11647–11652.

Kuang, S., K. Kuroda, F. Le Grand, and M. A. Rudnicki. 2007. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell. 129:999–1010.

Kuhn, E., N. Binart, and M. Lombes. 2012. Brown, white, beige: the color of fat and new therapeutic perspectives for obesity... In: Annales d'endocrinologie. Vol. 73. p. S2-8.

Kwon, H., S. P. Ford, F. W. Bazer, T. E. Spencer, P. W. Nathanielsz, M. J. Nijland, B. W. Hess, and G. Wu. 2004. Maternal nutrient restriction reduces concentrations of amino acids and polyamines in ovine maternal and fetal plasma and fetal fluids. Biol. Reprod. 71:901–908.

Kwon, H., G. Wu, F. W. Bazer, and T. E. Spencer. 2003. Developmental Changes in Polyamine Levels and Synthesis in the Ovine Conceptus1. Biol. Reprod. 69:1626–1634. doi:10.1095/biolreprod.103.019067. Available from: https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod.103.019067

Ladeira, M. M., L. C. Santarosa, M. L. Chizzotti, E. M. Ramos, O. R. M. Neto, D. M. Oliveira, J. R. R. Carvalho, L. S. Lopes, and J. S. Ribeiro. 2014. Fatty acid profile, color and lipid oxidation of meat from young bulls fed ground soybean or rumen protected fat with or without monensin. Meat Sci. 96:597–605.

Ladeira, M. M., J. P. Schoonmaker, M. P. Gionbelli, J. C. O. Dias, T. R. S. Gionbelli, J. R. R. Carvalho, and P. D. Teixeira. 2016. Nutrigenomics and beef quality: A review about lipogenesis. Int. J. Mol. Sci. 17:918.

Ladeira, M. M., J. P. Schoonmaker, K. C. Swanson, S. K. Duckett, M. P. Gionbelli, L. M. Rodrigues, and P. D. Teixeira. 2018. Nutrigenomics of marbling and fatty acid profile in ruminant meat. Animal. 12:s282–s294.

Lassala, A., F. W. Bazer, T. a Cudd, S. Datta, D. H. Keisler, M. C. Satterfield, T. E. Spencer, and G. Wu. 2010. Parenteral Administration of L -Arginine Prevents Fetal Growth Restriction in Undernourished Ewes 1 , 2. J. Nutr. 140:1242–1248. doi:10.3945/jn.110.125658.retardation.

Lassar, A. B., R. L. Davis, W. E. Wright, T. Kadesch, C. Murre, A. Voronova, D. Baltimore, and H. Weintraub. 1991. Functional activity of myogenic HLH proteins requires hetero-oligomerization with E12/E47-like proteins in vivo. Cell. 66:305–315.

Latres, E., A. R. Amini, A. A. Amini, J. Griffiths, F. J. Martin, Y. Wei, H. C. Lin, G. D. Yancopoulos, and D. J. Glass. 2005. Insulin-like growth factor-1 (IGF-1) inversely regulates atrophy-induced genes via the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/mTOR) pathway. J. Biol. Chem. 280:2737–2744.

Lazzarini, I., E. Detmann, C. B. Sampaio, M. F. Paulino, S. de C. Valadares Filho, M. A. de Souza, and F. A. Oliveira. 2009. Intake and digestibility in cattle fed low-quality tropical

forage and supplemented with nitrogenous compounds. Rev. Bras. Zootec. 38:2021–2030.

Lee, Y.-H., A. P. Petkova, E. P. Mottillo, and J. G. Granneman. 2012. In vivo identification of bipotential adipocyte progenitors recruited by β3-adrenoceptor activation and high-fat feeding. Cell Metab. 15:480–491. doi:10.1016/j.cmet.2012.03.009.

Lemos, B. J. M., F. M. de Souza, K. K. G. Moreira, T. P. Guimarães, M. L. R. Pereira, S. F. Ferreira, and R. M. da Silva. 2012. Suplementação de bovinos de corte em pastejo. PubVet. 6:Art-1455.

Leng, R. A. 1990. Factors affecting the utilization of 'poor-quality'forages by ruminants particularly under tropical conditions. Nutr. Res. Rev. 3:277–303.

Leshan, R. L., M. Greenwald-Yarnell, C. M. Patterson, I. E. Gonzalez, and M. G. Myers. 2012. Leptin action through hypothalamic nitric oxide synthase-1–expressing neurons controls energy balance. Nat. Med. 18:820–823.

Light, N., A. E. Champion, C. Voyle, and A. J. Bailey. 1985. The role of epimysial, perimysial and endomysial collagen in determining texture in six bovine muscles. Meat Sci. 13:137–149.

Liu, R.-M., and K. A. G. Pravia. 2010. Oxidative stress and glutathione in TGF-β-mediated fibrogenesis. Free Radic. Biol. Med. 48:1–15.

Liu, Y., W. Lv, B. Yu, T. Ju, F. Yang, M. Jiang, Z. Liu, L. Xu, W. Sun, J. Huang, D. Li, and J. Yin. 2013. S-adenosylmethionine-induced adipogenesis is accompanied by suppression of Wnt/β-catenin and Hedgehog signaling pathways. Mol. Cell. Biochem. 382:59–73. doi:10.1007/s11010-013-1718-3.

Long, N. M., K. A. Vonnahme, B. W. Hess, P. W. Nathanielsz, and S. P. Ford. 2009. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine. J. Anim. Sci. 87:1950–1959.

Maltin, C. A., C. C. Warkup, K. R. Matthews, C. M. Grant, A. D. Porteig, and M. I. Delday. 1997. Pig Muscle Fibre Characteristics as a Source of Variation in Eating Quality. 41.

Marquez, D. C., M. F. Paulino, L. N. Rennó, F. C. Villadiego, R. M. Ortega, D. S. Moreno, L. S. Martins, D. M. De Almeida, M. P. Gionbelli, and M. R. Manso. 2017. Supplementation of grazing beef cows during gestation as a strategy to improve skeletal muscle development of the offspring. animal. 11:2184–2192.

Martin, J. L., K. A. Vonnahme, D. C. Adams, G. P. Lardy, and R. N. Funston. 2007. Effects of dam nutrition on growth and reproductive performance of heifer calves. J. Anim. Sci. 85:841–847.

Martin, M. J., M. D. Jimenez, and V. Motilva. 2001. New issues about nitric oxide and its effect on the gastrointestinal tract. Curr. Pharm. Des. 7:881–908.

Megeney, L. A., and M. A. Rudnicki. 1995. Determination versus differentiation and the MyoD family of transcription factors. Biochem. Cell Biol. 73:723–732.

Meneses, J. A. M., K. B. Nascimento, M. C. Galvão, G. M. Moreira, L. H. L. Chalfun, S. P.

de Souza, G. D. Ramírez-Zamudio, M. M. Ladeira, M. S. Duarte, and D. R. Casagrande. 2024. Protein supplementation during mid-gestation affects maternal voluntary feed intake, performance, digestibility, and uterine blood flow of beef cows. J. Anim. Physiol. Anim. Nutr. (Berl).

Mersmann, H. J., and S. B. Smith. 2005. Development of white adipose tissue lipid metabolism. In: Biology of growing animals. Vol. 3. Elsevier. p. 275–302.

Meyer, A. M., S. I. Klein, M. Kapphahn, D. V Dhuyvetter, R. E. Musser, and J. S. Caton. 2018. Effects of rumen-protected arginine supplementation and arginine-HCl injection on site and extent of digestion and small intestinal amino acid disappearance in forage-fed steers. Transl. Anim. Sci. 2:205–215.

Meyer, A. M., J. J. Reed, K. A. Vonnahme, S. A. Soto-Navarro, L. P. Reynolds, S. P. Ford, B. W. Hess, and J. S. Caton. 2010. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. J. Anim. Sci. 88:2410–2424. doi:10.2527/jas.2009-2220.

Miyamoto, J., S. Hasegawa, M. Kasubuchi, A. Ichimura, A. Nakajima, and I. Kimura. 2016. Nutritional signaling via free fatty acid receptors. Int. J. Mol. Sci. 17:450.

Morris, S. M. 2002. R EGULATION OF E NZYMES OF THE U REA C YCLE AND A RGININE M ETABOLISM. 87–105. doi:10.1146/annurev.nutr.22.110801.140547.

Morris, S. M. 2009. Recent advances in arginine metabolism: Roles and regulation of the arginases. Br. J. Pharmacol. 157:922–930. doi:10.1111/j.1476-5381.2009.00278.x.

Moseti, D., A. Regassa, and W.-K. Kim. 2016. Molecular Regulation of Adipogenesis and Potential Anti-Adipogenic Bioactive Molecules. Int. J. Mol. Sci. 17. doi:10.3390/ijms17010124.

Muhlhausler, B. S., J. A. Duffield, and I. C. McMillen. 2007. Increased maternal nutrition stimulates peroxisome proliferator activated receptor-γ, adiponectin, and leptin messenger ribonucleic acid expression in adipose tissue before birth. Endocrinology. 148:878–885.

Muhlhausler, Beverly Sara, Clare Lesley Adam, P. A. Findlay, J. A. Duffield, I. C. McMillen, B S Muhlhausler, C L Adam, P. A. Findlay, J. A. Duffield, and I. C. Mcmillen. 2006. Increased maternal nutrition alters development of the appetite-regulating network in the brain. FASEB J. 20:E556–E565.

Munhoz, C. D., B. Garcia-Bueno, J. L. M. Madrigal, L. B. Lepsch, C. Scavone, and J. C. Leza. 2008. Stress-induced neuroinflammation: mechanisms and new pharmacological targets. Brazilian J. Med. Biol. Res. 41:1037–1046.

Murakami, M., T. Ichisaka, M. Maeda, N. Oshiro, K. Hara, F. Edenhofer, H. Kiyama, K. Yonezawa, and S. Yamanaka. 2004. mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol. Cell. Biol. 24:6710–6718.

Nakamura, Y., E. Hinoi, T. Iezaki, S. Takada, S. Hashizume, Y. Takahata, E. Tsuruta, S. Takahashi, and Y. Yoneda. 2013. Repression of adipogenesis through promotion of Wnt/β-catenin signaling by TIS7 up-regulated in adipocytes under hypoxia. Biochim. Biophys. Acta. 1832:1117–1128. doi:10.1016/j.bbadis.2013.03.010.

Nascimento, K. B., M. C. Galvão, J. A. M. Meneses, G. M. Moreira, G. D. Ramírez-Zamudio, S. P. de Souza, L. D. Prezotto, L. H. L. Chalfun, M. de S. Duarte, and D. R. Casagrande. 2022. Effects of maternal protein supplementation at mid-gestation of cows on intake, digestibility, and feeding behavior of the offspring. Animals. 12:2865.

Nascimento, K. B., M. C. Galvão, J. A. M. Meneses, G. D. Ramírez-Zamudio, D. G. Pereira, P. V. R. Paulino, D. R. Casagrande, T. R. S. Gionbelli, M. M. Ladeira, and M. S. Duarte. 2024. Maternal protein supplementation during mid-gestation improves offspring performance and metabolism in beef cows. J. Anim. Sci. 102:skae058.

Nielsen, F. C., L. Østergaard, J. Nielsen, and J. Christiansen. 1995. Growth-dependent translation of IGF-II mRNA by a rapamycin-sensitive pathway. Nature. 377:358–362.

Oldham, J. M., J. A. K. Martyn, M. Sharma, F. Jeanplong, R. Kambadur, and J. J. Bass. 2001. Molecular expression of myostatin and MyoD is greater in double-muscled than normal-muscled cattle fetuses. Am. J. Physiol. Integr. Comp. Physiol. 280:R1488–R1493.

DE OLIVEIRA, I. 2021. Production and Physiology of Pregnant Beef Cows Supplemented with Ruminally Degradable or Undegradable Protein under Low Protein Basal Diets during Mid-Gestation.

Owens, F. N., D. R. Gill, D. S. Secrist, and S. W. Coleman. 1995. Review of some aspects of growth and development of feedlot cattle. J. Anim. Sci. 73:3152–3172.

Palmer, E. A., M. Vedovatto, R. A. Oliveira, J. Ranches, J. M. B. Vendramini, M. H. Poore, T. Martins, M. Binelli, J. D. Arthington, and P. Moriel. 2022. Timing of maternal supplementation of dried distillers grains during late gestation influences postnatal growth, immunocompetence, and carcass characteristics of Bos indicus-influenced beef calves. J. Anim. Sci. 100:skac022.

Papadimitriou, A., and K. N. Priftis. 2009. Regulation of the hypothalamic-pituitary-adrenal axis. Neuroimmunomodulation. 16:265–271.

Paulino, M. F., J. T. Zervoudakis, E. Moraes, E. Detmann, and S. de C. VALADARES FILHO. 2002. Bovinocultura de ciclo curto em pastagens. Simpósio Produção Gado Corte. 3:153–196.

Peine, J. L., G. Jia, M. L. Van Emon, T. L. Neville, J. D. Kirsch, C. J. Hammer, S. T. O'Rourke, L. P. Reynolds, and J. S. Caton. 2018. Effects of maternal nutrition and rumen-protected arginine supplementation on ewe performance and postnatal lamb growth and internal organ mass. J. Anim. Sci. 96:3471–3481. doi:10.1093/jas/sky221.

Peine, J. L., T. L. Neville, E. E. Klinkner, K. E. Egeland, P. P. Borowicz, A. M. Meyer, L. P. Reynolds, and J. S. Caton. 2020. Rumen-protected arginine in ewe lambs: effects on circulating serum amino acids and carotid artery hemodynamics. J. Anim. Sci. 98:skaa196.

Picard, B., and M. Gagaoua. 2020. Muscle fiber properties in cattle and their relationships with meat qualities: An overview. J. Agric. Food Chem. 68:6021–6039.

Pino, L. M. del, A. Arana, L. Alfonso, J. A. Mendizábal, and B. Soret. 2017. Adiposity and adipogenic gene expression in four different muscles in beef cattle. PLoS One. 12:1–19.

Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. Anim. Sci. 62:145–158.

Prezotto, L. D., J. F. Thorson, P. P. Borowicz, J. L. Peine, M. Bedenbaugh, S. M. Hileman, C. A. Lents, J. S. Caton, and K. C. Swanson. 2018. Influences of maternal nutrient restriction and arginine supplementation on visceral metabolism and hypothalamic circuitry of offspring. Domest. Anim. Endocrinol. 65:71–79. doi:10.1016/j.domaniend.2018.06.001. Available from: https://doi.org/10.1016/j.domaniend.2018.06.001

Pyatt, N. A., L. L. Berger, D. B. Faulkner, P. M. Walker, and S. L. Rodriguez-Zas. 2005a. Factors affecting carcass value and profitability in early-weaned Simmental steers: I. Five-year average pricing. J. Anim. Sci. 83:2918–2925.

Pyatt, N. A., L. L. Berger, D. B. Faulkner, P. M. Walker, and S. L. Rodriguez-Zas. 2005b. Factors affecting carcass value and profitability in early-weaned Simmental steers: II. Days on feed endpoints and sorting strategies. J. Anim. Sci. 83:2926–2937.

Raghavan, S. A. V, and M. Dikshit. 2004. Vascular regulation by the L-arginine metabolites, nitric oxide and agmatine. Pharmacol. Res. 49:397–414.

Ravi Kambadur, R. K., A. Bishop, M. S. Salerno, S. McCroskery, and M. S. Mridula Sharma. 2004. Role of myostatin in muscle growth. In: Muscle development of livestock animals: physiology, genetics and meat quality. CABI Publishing Wallingford UK. p. 297–316.

Redmer, D. A., J. M. Wallace, and L. P. Reynolds. 2004. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Domest. Anim. Endocrinol. 27:199–217. doi:10.1016/j.domaniend.2004.06.006.

Reynolds, L. P., P. P. Borowicz, J. S. Caton, M. S. Crouse, C. R. Dahlen, and A. K. Ward. 2019. Developmental programming of fetal growth and development. Vet. Clin. North Am. Food Anim. Pract. 35:229–247.

Reynolds, L. P., and J. S. Caton. 2012. Role of the pre-and post-natal environment in developmental programming of health and productivity. Mol. Cell. Endocrinol. 354:54–59.

Reynolds, L. P., J. S. Caton, D. A. Redmer, A. T. Grazul-Bilska, K. A. Vonnahme, P. P. Borowicz, J. S. Luther, J. M. Wallace, G. Wu, and T. E. Spencer. 2006. Evidence for altered placental blood flow and vascularity in compromised pregnancies. J. Physiol. 572:51–58.

Reynolds, L. P., D. S. Millaway, J. D. Kirsch, J. E. Infeld, and D. A. Redmer. 1987. Angiogenic activity of placental tissues of cows. J. Reprod. Fertil. 81:233–240.

Reynolds, L. P., and D. A. Redmer. 1988. Secretion of angiogenic activity by placental tissues of cows at several stages of gestation. J. Reprod. Fertil. 83:497–502.

Reynolds, L. P., and D. A. Redmer. 1995. Utero-placental vascular development and placental function. J. Anim. Sci. 73:1839–1851. doi:10.2527/1995.7361839x.

Reynolds, L. P., A. K. Ward, and J. S. Caton. 2017. Epigenetics and developmental programming in ruminants: Long-term impacts on growth and development. In: Biology of domestic animals. CRC Press. p. 85–121.

Ricard-Blum, S. 2011. The collagen family. Cold Spring Harb. Perspect. Biol. 3:a004978.

Rodrigues, L. M., J. P. Schoonmaker, F. D. Resende, G. R. Siqueira, O. R. M. Neto, M. P. Gionbelli, T. R. S. Gionbelli, and M. M. Ladeira. 2020. Effects of protein supplementation on Nellore cows' reproductive performance, growth, myogenesis, lipogenesis and intestine development of the progeny. Anim. Prod. Sci. 61:371–380.

Roy, H., S. Bhardwaj, and S. Ylä-Herttuala. 2006. Biology of vascular endothelial growth factors. FEBS Lett. 580:2879–2887.

Rufino, L. M. de A., E. Detmann, D. Í. Gomes, W. L. S. dos Reis, E. D. Batista, S. de C. Valadares Filho, and M. F. Paulino. 2016. Intake, digestibility and nitrogen utilization in cattle fed tropical forage and supplemented with protein in the rumen, abomasum, or both. J. Anim. Sci. Biotechnol. 7:1–10.

Russell, R. G., and F. T. Oteruelo. 1981. An ultrastructural study of the dufferentiation of skeletal muscle in the bovine fetus. Anat. Embryol. (Berl). 162:403–417.

Santos, M. M. dos, T. C. Costa, G. D. Ramírez-Zamudio, K. B. Nascimento, M. P. Gionbelli, and M. de S. Duarte. 2022. Prenatal origins of productivity and quality of beef. Rev. Bras. Zootec. 51:e20220061.

Scott, M. M., J. L. Lachey, S. M. Sternson, C. E. Lee, C. F. Elias, J. M. Friedman, and J. K. Elmquist. 2009. Leptin targets in the mouse brain. J. Comp. Neurol. 514:518–532.

Shimano, H. 2001. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog. Lipid Res. 40:439–452.

da Silva, A. G., M. F. Paulino, E. Detmann, H. J. Fernandes, L. da Silva Amorim, R. E. M. Ortega, V. V. de Carvalho, J. A. da Costa Lima, F. H. de Moura, and M. B. Monteiro. 2017. Energetic-protein supplementation in the last 60 days of gestation improves performance of beef cows grazing tropical pastures. J. Anim. Sci. Biotechnol. 8:1–9.

Spalding, K. L., E. Arner, P. O. Westermark, S. Bernard, B. A. Buchholz, O. Bergmann, L. Blomqvist, J. Hoffstedt, E. Näslund, and T. Britton. 2008. Dynamics of fat cell turnover in humans. Nature. 453:783–787.

Spencer, T. E., G. A. Johnson, F. W. Bazer, and R. C. Burghardt. 2004. Implantation mechanisms: insights from the sheep. Reproduction. 128:657–668.

Stannard, S. R., and N. A. Johnson. 2004. Insulin resistance and elevated triglyceride in muscle: more important for survival than 'thrifty' genes? J. Physiol. 554:595–607. doi:10.1113/jphysiol.2003.053926. Available from: http://doi.wiley.com/10.1113/jphysiol.2003.053926

Steibel, J. P., R. Poletto, P. M. Coussens, and G. J. M. Rosa. 2009. A powerful and flexible linear mixed model framework for the analysis of relative quantification RT-PCR data. Genomics. 94:146–152.

Stickland, N. C. 1978. A quantitative study of muscle development in the bovine foetus (Bos indicus). Anat. Histol. Embryol. 7:193–205.

Sun, L., H. Zhang, Y. Fan, Y. Guo, G. Zhang, H. Nie, and F. Wang. 2017. Metabolomic profiling in umbilical venous plasma reveals effects of dietary rumen-protected arginine or N-carbamylglutamate supplementation in nutrient-restricted Hu sheep during pregnancy. Reprod. Domest. Anim. 52:376–388. doi:10.1111/rda.12919.

Symonds, M. E., G. Gopalakrishnan, J. Bispham, S. Pearce, J. Dandrea, A. Mostyn, M. M. Ramsay, and T. Stephenson. 2003. Maternal nutrient restriction during placental growth, programming of fetal adiposity and juvenile blood pressure control. Arch. Physiol. Biochem. 111:45–52.

Tajbakhsh, S., D. Rocancourt, G. Cossu, and M. Buckingham. 1997. Redefining the genetic hierarchies controlling skeletal myogenesis: Pax-3 and Myf-5 act upstream of MyoD. Cell. 89:127–138.

Tan, B., X. Li, G. Wu, X. Kong, Z. Liu, T. Li, and Y. Yin. 2012. Dynamic changes in blood flow and oxygen consumption in the portal-drained viscera of growing pigs receiving acute administration of L-arginine. Amino Acids. 43:2481–2489.

Tang, Q. Q., and M. D. Lane. 2012. Adipogenesis: from stem cell to adipocyte. Annu. Rev. Biochem. 81:715–736.

Tang, Y., S.-W. Qian, M.-Y. Wu, J. Wang, P. Lu, X. Li, H.-Y. Huang, L. Guo, X. Sun, and C.-J. Xu. 2016. BMP4 mediates the interplay between adipogenesis and angiogenesis during expansion of subcutaneous white adipose tissue. J. Mol. Cell Biol. 8:302–312.

Uezumi, A., S. Fukada, N. Yamamoto, S. Takeda, and K. Tsuchida. 2010. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat. Cell Biol. 12:143–152.

Underwood, K. R., J. F. Tong, P. L. Price, A. J. Roberts, E. E. Grings, B. W. Hess, W. J. Means, and M. Du. 2010. Nutrition during mid to late gestation affects growth, adipose tissue deposition, and tenderness in cross-bred beef steers. Meat Sci. 86:588–593. doi:10.1016/j.meatsci.2010.04.008.

Valente, T. N. P., E. Detmann, A. C. de Queiroz, S. de C. Valadares Filho, D. I. Gomes, and J. F. Figueiras. 2011. Evaluation of ruminal degradation profiles of forages using bags made from different textiles. Rev. Bras. Zootec. 40:2565–2573.

Vedovatto, M., V. Izquierdo, E. Palmer, R. A. Oliveira, H. M. Silva, J. M. B. Vendramini, and P. Moriel. 2022. Monensin supplementation during late gestation of beef cows alters maternal plasma concentrations of insulin-like growth factors 1 and 2 and enhances offspring preweaning growth. Transl. Anim. Sci. 6:txac105.

Vincent, S. R., and H. Kimura. 1992. Histochemical mapping of nitric oxide synthase in the rat brain. Neuroscience. 46:755–784.

Vonnahme, K. A., M. J. Zhu, P. P. Borowicz, T. W. Geary, B. W. Hess, L. P. Reynolds, J. S. Caton, W. J. Means, and S. P. Ford. 2007. Effect of early gestational undernutrition on angiogenic factor expression and vascularity in the bovine placentome. J. Anim. Sci. 85:2464–2472. doi:10.2527/jas.2006-805.

Wang, B., X. Fu, X. Liang, Z. Wang, Q. Yang, T. Zou, W. Nie, J. Zhao, P. Gao, and M.-J.

Zhu. 2017. Maternal retinoids increase PDGFRα+ progenitor population and beige adipogenesis in progeny by stimulating vascular development. EBioMedicine. 18:288–299.

Wang, X., W. Ying, K. A. Dunlap, G. Lin, M. C. Satterfield, R. C. Burghardt, G. Wu, and F. W. Bazer. 2014. Arginine decarboxylase and agmatinase: an alternative pathway for de novo biosynthesis of polyamines for development of mammalian conceptuses. Biol. Reprod. 90:81–84.

Ward, R. E., B. Woodward, N. Otter, and O. Doran. 2010. Relationship between the expression of key lipogenic enzymes, fatty acid composition, and intramuscular fat content of Limousin and Aberdeen Angus cattle. Livest. Sci. 127:22–29.

Wertz, A. E., L. L. Berger, P. M. Walker, D. B. Faulkner, F. K. McKeith, and S. L. Rodriguez-Zas. 2002. Early-weaning and postweaning nutritional management affect feedlot performance, carcass merit, and the relationship of 12th-rib fat, marbling score, and feed efficiency among Angus and Wagyu heifers. J. Anim. Sci. 80:28–37.

Wertz, E., L. L. Berge, P. M. Walker, D. B. Faulkner, F. K. McKeith, and S. Rodriguez-Zas. 2001. Early weaning and postweaning nutritional management affect feedlot performance of Angus× Simmental heifers and the relationship of 12th rib fat and marbling score to feed efficiency. J. Anim. Sci. 79:1660–1669.

White, B. D., and R. J. Martin. 1997. Evidence for a central mechanism of obesity in the Zucker rat: role of neuropeptide Y and leptin. Proc. Soc. Exp. Biol. Med. 214:222–232.

Wu, G. 2013a. Functional amino acids in nutrition and health. doi:10.1007/s00726-013-1500-6.

Wu, G. 2013b. Amino acids: biochemistry and nutrition. CRC Press.

Wu, Guoyao, F. W. Bazer, R. C. Burghardt, G. A. Johnson, S. W. Kim, D. A. Knabe, P. Li, X. Li, J. R. McKnight, and M. C. Satterfield. 2011. Proline and hydroxyproline metabolism: implications for animal and human nutrition. Amino Acids. 40:1053–1063.

Wu, G., F. W. Bazer, T. A. Davis, S. W. Kim, P. Li, J. Marc Rhoads, M. Carey Satterfield, S. B. Smith, T. E. Spencer, and Y. Yin. 2009. Arginine metabolism and nutrition in growth, health and disease. Amino Acids. 37:153–168. doi:10.1007/s00726-008-0210-y.

Wu, G, F. W. Bazer, G. A. Johnson, D. A. Knabe, R. C. Burghardt, T. E. Spencer, X. L. Li, and J. J. Wang. 2011. TRIENNIAL GROWTH SYMPOSIUM: Important roles for L-glutamine in swine nutrition and production1,2. J. Anim. Sci. 89:2017–2030. doi:10.2527/jas.2010-3614.

Wu, G., F. W. Bazer, M. C. Satterfield, and X. Li. 2013. Impacts of arginine nutrition on embryonic and fetal development in mammals Impacts of arginine nutrition on embryonic and fetal development in mammals. doi:10.1007/s00726-013-1515-z.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Board-invited review: Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316–2337. doi:10.2527/jas.2006-156.

Wu, G., and C. J. Meininger. 2009. Nitric oxide and vascular insulin resistance. Biofactors.

35:21–27.

Wu, G., and S. M. J. Morris. 1998. Arginine metabolism: nitric oxide and beyond. Biochem. J. 336 (Pt 1:1–17.

Yang, Q.-Y., J.-F. Liang, C. J. Rogers, J.-X. Zhao, M.-J. Zhu, and M. Du. 2013. Maternal obesity induces epigenetic modifications to facilitate Zfp423 expression and enhance adipogenic differentiation in fetal mice. Diabetes. 62:3727–3735.

Zambrano, E., P. M. Martinez-Samayoa, C. J. Bautista, M. Deas, L. Guillen, G. L. Rodriguez-Gonzalez, C. Guzman, F. Larrea, and P. W. Nathanielsz. 2005. Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. J. Physiol. 566:225–236.

Zhang, H, L. W. Sun, Z. Y. Wang, M. T. Deng, G. M. Zhang, R. H. Guo, T. W. Ma, and F. Wang. 2016a. Dietary N-carbamylglutamate and rumen-protected L-arginine supplementation ameliorate fetal growth restriction in undernourished ewes. J. Anim. Sci. 94:2072–2085.

Zhang, H, L. W. Sun, Z. Y. Wang, M. T. Deng, G. M. Zhang, R. H. Guo, T. W. Ma, and F. Wang. 2016b. Supplementation Ameliorate Fetal Growth Restriction in Undernourished Ewes 1 , 2. 2072–2085. doi:10.2527/jas2015-9587.

Zhang, Hao, L. Sun, Z. Wang, M. Deng, H. Nie, G. Zhang, M. Tiewei, and F. Wang. 2016. N-carbamylglutamate and L-arginine improved maternal and placental development in underfed ewes. Reproduction. 151:623–635. doi:10.1530/REP-16-0067.

Zhou, X., Q. Yan, L. Liu, G. Chen, S. Tang, Z. He, and Z. Tan. 2021. Maternal Undernutrition Alters The Skeletal Muscle Development and Methylation of Myogenic Factors in Progeny: A Randomized Controlled Trial.

Zhu, M.-J., S. P. Ford, P. W. Nathanielsz, and M. Du. 2004. Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle. Biol. Reprod. 71:1968–1973.

Zhu, M. J., S. P. Ford, W. J. Means, B. W. Hess, P. W. Nathanielsz, and M. Du. 2006. Maternal nutrient restriction affects properties of skeletal muscle in offspring. J. Physiol. 575:241–250. doi:10.1113/jphysiol.2006.112110. Available from: http://doi.wiley.com/10.1113/jphysiol.2006.112110

Table 1. Ingredients and chemical composition of feeds used during the backgrounding phase of the offspring.

Item	Inclusion/Composition
Ingredients, % of DM	
Corn silage	50.0
Ground corn	38.4
SNAP N protein[1]	11.6
Chemical composition, g/kg of DM	
Corn silage	
Dry matter	370.5
Organic matter	927.5
Crude protein	77.5
Ash and protein-free neutral detergent fiber	437.1
Non-fibrous carbohydrate	385.0
Ether extract	27.9
Ground corn	
Dry matter	862.6
Organic matter	986.4
Crude protein	68.1
Ash and protein-free neutral detergent fiber	113.8
Non-fibrous carbohydrate	745.6
Ether extract	58.9
Mineral protein supplement[1]	
Dry matter	882.6
Organic matter	829.8
Crude protein	457.7
Ash and protein-free neutral detergent fiber	107.4
Non-fibrous carbohydrate	227.4
Ether extract	37.3

[1]Protein, mineral and vitamin concentrate: Moisture (Max.) = 120.00 g/kg, Crude Protein (Min.) = 463.29 g/kg, NPN Equiv. Protein (Min.) = 148.90 g/kg, Ether Extract (Min.) = 10.60 g/kg, Mineral Matter (Max.) = 200.00 g/kg, Crude Fiber (Max.) = 150, 00, ADF (Max.) = 130.00 g/kg, Calcium (Min.) 36.30 g/kg, Calcium (Max.) 38.0 g/kg, Phosphorus (Min.) 11.76 g/kg , Sodium (Min.) 15.76 g/kg, Potassium (Min.) 21.81g/kg, Magnesium (Min.) 15.80 g/kg, Sulfur (Min.) 6.930 mg/kg, Zinc (Min.) 279.00 mg/kg, Copper (Min.) 74.16 mg/kg, Fluorine (Max.) 65.40 mg/kg, Manganese (Min.) 234.00 mg/kg, Cobalt (Min.) 6 .39 mg/kg, Iodine (Min.) 4.48 mg/kg, Selenium (Min.) 1.23 mg/kg, Folic Acid (Min.) 6.36 mg/kg, Biotin (Min.) 4600 µg /kg, Pantothenic Acid (Min.) 61.10 mg/kg, Vitamin A (Min.) 12,870 IU/kg, Vitamin B12 (Min.) 523.58 µg/kg, Vitamin B6 (Min.) 37.2 mg /kg, Vitamin D3 (Min.) 1,788 IU/kg Vitamin E (Min.) 193.00 IU/kg and Monensin Sodium 650.00 mg/kg.

Table 2. Effects of maternal treatment on offspring body weight, vigor score, morphometric measurements, average daily gain.

Trait	Maternal treatment			SEM	*P*-value
	CON	RDP	RUP		
Measurements at birth					
Birth weight, kg	29.4^{B}	29.8^{B}	35.3^{A}	1.99	0.043
Vigor score; arbitrary units	3.24	3.62	3.81	0.23	0.200
Thorax width, cm	13.0^{B}	15.5^{A}	15.4^{A}	0.82	0.015
Abdomen width, cm	13.1	14.9	15.4	0.94	0.084
Rib deep, cm	24.0	23.6	25.2	0.79	0.188
Rump deep, cm	23.2	24.3	26.7	1.41	0.088
Ischial bones distance, cm	8.67	8.85	9.29	0.81	0.758
Ilium bones distance, cm	13.2^{B}	15.5^{A}	14.9^{A}	0.61	0.006
Ilium-Ischium distance, cm	17.6^{B}	19.5^{AB}	20.9^{A}	0.87	0.011
Height at withers, cm	66.0^{B}	67.5^{B}	71.8^{A}	0.85	<0.001
Rump height, cm	72.1^{B}	74.6^{AB}	77.9^{A}	1.67	0.019
Body length, cm	58.7	58.2	61.3	3.06	0.599
Body circumference, cm	68.3^{B}	71.5^{AB}	74.4^{A}	1.48	0.006
Measurements from birth to weaning					
Weaning weight, kg	206	211	214	11.8	0.837
Average daily gain, kg/d	0.850	0.873	0.864	0.05	0.928
Measurements at backgrounding phase					
Initial body weight, kg	231	245	255	11.4	0.288
Final body weight, kg	291	304	319	14.2	0.242
Average daily gain, kg/d	1.24	1.18	1.29	0.07	0.419

$^{A-B}$Means followed by different superscripts are different ($P < 0.05$).

Abbreviations: CON = Control; RDP = Ruminally degradable protein supplement; RUP = Ruminal non-degradable protein supplement.

Table 3. Effects of maternal treatment on blood insulin concentration, glucose and IFG-I levels at birth.

Trait	Maternal treatment			SEM	*P*-value
	CON	RDP	RUP		
Glucose, mg/dL	136	127	137	8.31	0.631
Insulin, μUI/mL	9.60	9.13	5.16	3.13	0.477
IGF-1, ng/mL	110	127	147	20.0	0.334

Abbreviations: CON = Control; RDP = Ruminally degradable protein supplement; RUP = Ruminal non-degradable protein supplement.

Table 4. Effects of maternal treatment on the diets components intake and the offspring apparent total-tract digestibility during the backgrounding phase.

Trait	Maternal treatment			SEM	*P*-value
	CON	RDP	RUP		
Total intake of dry matter and its fractions					
Dry matter, kg/day	6.72	6.68	7.39	0.54	0.456
Organic matter, kg/day	6.44	6.49	7,01	0.46	0.497
Protein crude, kg/day	0.889	0.897	0.970	0.06	0.480
Neutral detergent fiber, kg/day	2.39	2.41	2.60	0.16	0.451
Ash and protein-free neutral detergent fiber, kg/day	2.27	2.28	2.47	0.16	0.143
Non-fibrous carbohydrates, kg/day	3.15	3.17	3.42	0.23	0.527
Ether extract, kg/day	0.233	0.235	0.256	0.02	0.421
Total digestible nutrients, kg/day	4.12	4.12	3.91	0.50	0.892
Apparent total tract digestibility					
Dry matter, g/kg	559	576	527	39.2	0.497
Organic matter, g/kg	540	559	504	51.5	0.653
Crude protein, g/kg	385	482	453	54.9	0.399
Neutral detergent fiber, g/kg	330	379	362	40.5	0.642
Ash and protein-free neutral detergent fiber, g/kg	295	347	329	42.5	0.639
Non-fibrous carbohydrates, g/kg	828	849	830	18.7	0.633
Total digestible nutrients, g/kg	612	633	575	48.7	0.534

Abbreviations: CON = Control; RDP = Ruminally degradable protein supplement; RUP = Ruminal non-degradable protein supplement.

Table 5. Effects of maternal treatment on the offspring ingestive behavior during the backgrounding phase.

Trait	Maternal treatment			SEM	*P*-value
	CON	RDP	RUP		
Eating, min/d	196	211	206	15.4	0.694
Drinking water, min/d	11.7	9.18	8.97	1.63	0.266
Ruminating, min/d	386	417	393	22.7	0.410
Idleness, min/d	685	647	660	29.5	0.538
Other activities, min/d	166	156	172	13.7	0.614

Abbreviations: CON = Control; RDP = Ruminally degradable protein supplement; RUP = Ruminal non-degradable protein supplement.

Table 6. Effects of maternal treatment on fiber area of *Longissimus thoracis* muscle collected though biopsies 48 ± 10 days or 346 ± 31 days of age.

Trait	Maternal treatment			SEM	*P*-value
	CON	RDP	RUP		
48 ± 10 days					
Fibers number	21.1	24.1	26.6	4.39	0.433
Fiber area, μm^2	425	394	333	94.8	0.609
346 ± 31 days					
Fibers number	13.0	11.5	13.3	1.37	0.549
Fiber area, μm^2	1560	1981	1470	192	0.105

Abbreviations: CON = Control; RDP = Ruminally degradable protein supplement; RUP = Ruminal non-degradable protein supplement.

Table 7. Effects of maternal treatment on muscle development and fat thickness during the backgrounding phase.

Trait	Maternal treatment			SEM	*P*-value
	CON	RDP	RUP		
Longissimus muscle area, cm^2	56.7	55.9	56.2	3.42	0.974
Longissimus muscle area, cm^2/100kg	18.6	17.8	17.3	0.48	0.070
Subcutaneous fat thickness, mm	0.286	0.318	0.264	0.03	0.331
Rump muscle area, cm^2	7.55	7.84	7.98	0.43	0.637
Rump muscle area, cm^2/100kg	2.49	2.49	2.44	0.11	0.889
Rump fat thickness, mm	0.460	0.527	0.488	0.07	0.723

Abbreviations: CON = Control; RDP = Ruminally degradable protein supplement; RUP = Ruminal non-degradable protein supplement.

Table 8. Effects of maternal treatment on mRNA expression at 48 ± 10 days or 346 ± 31 days of age on the offspring *Longissimus thoracis*.

Gene	CON-RDP		CON-RUP		RDP-RUP	
	Log2FC [CI]	***P*-value**	**Log2FC [CI]**	***P*-value**	**Log2FC [CI]**	***P*-value**
48 ± 10 days						
C/EBPA	1.62 [-4.73, 1.49]	0.293	-0.27 [-2.95, 2.40]	0.836	1.35 [-1.53, 4.23]	0.344
COL3A1	-1.92 [-5.28, 1.44]	0.250	-1.40 [-4.29, 1.49]	0.328	0.52 [-2.59, 3.63]	0.733
FABP4	-2.27 [-5.59, 1.05]	0.173	-1.25 [-4.10, 1.61]	0.379	1.02 [-2.05, 4.09]	0.501
IGFR1	-2.03 [-5.31, 1.24]	0.214	0.71 [-2.11, 3.53]	0.611	2.74 [-0.29, 5.77]	0.075
mTOR	-0.34 [-3.49, 2.81]	0.827	-0.32 [-3.03, 2.39]	0.810	0.02 [-2.90, 2.93]	0.990
MYOD	-1.64 [-4.73, 1.44]	0.283	0.11 [-2.54, 2.76]	0.932	1.76 [-1.09, 4.61]	0.217
MYOG	1.10 [-2.21, 4.40]	0.501	0.15 [-2.70, 2.99]	0.916	-0.95 [-4.01, 2.11]	0.529
PPARG	0.03 [-3.18, 3.23]	0.987	-0.71 [-3.47, 2.04]	0.599	-0.74 [-3.70, 2.22]	0.612
TGFB1	-1.18 [-4.38, 2.01]	0.453	-0.05 [-2.80, 2.70]	0.971	1.13 [-1.82, 4.09]	0.438
ZFP423	-2.00 [-5.22, 1.21]	0.212	0.32 [-2.45, 3.09]	0.816	2.32 [-0.65, 5.30]	0.121
346 ± 31 days						
ACACA	1.14 [-1.00, 3.28]	0.285	-3.31 [-5.15,-1.46]	0.001	-4.44 [-6.43,-2,46]	<0.001
ACOX	-0.69 [-3.11, 1.73]	0.562	-0.38 [-2.46, 1.71]	0.713	0.31 [-1.92, 2.55]	0.775
AMPKa2	-0.31 [-2.73, 2.10]	0.791	1.28 [-0.80, 3.37]	0.217	1.60 [-0.64, 3.84]	0.154
COL3A1	0.91 [-1.47, 3.29]	0.440	-2.03 [-4.07, 0.02]	0.050	-2.94 [-5.14,-0.74]	0.011
CPT1A	-0.58 [-3.36, 2.20]	0.673	-1.67 [-4.07, 0.72]	0.163	-1.09 [-3.67, 1.48]	0.390
CPT2	-0.59 [-2.67, 1.48]	0.562	-1.82 [-3.61,-0.04]	0.046	-1.23 [-3.15, 0.69]	0.201
FABP4	1.66 [-1.19, 4.51]	0.242	-1.91 [-4,37, 0.54]	0.121	-3.57 [-6.21,-0.94]	0.010
FASN	-0.81 [-3.13, 1.52]	0.482	-2.37 [-4.37,-0.37]	0.022	-1.57 [-3.72, 0.59]	0.147
IGFR1	0.03 [-2.07, 2.13]	0.978	-2.23 [-4.04,-0.42]	0.017	-2.26 [-4.20,-0.32]	0.024
INSR	-0.30 [-2.49, 1.88]	0.778	-0.91 [-2.79, 0.97]	0.329	-0.61 [-2.63, 1.41]	0.542
LPL	0.32 [-2,58, 3.22]	0.822	-1.84 [-4.34, 0.66]	0.142	-2.16 [-4.84, 0.52]	0.110
mTOR	0.04 [-2.11, 2.19]	0.970	-1.66 [-3.51, 0.20]	0.077	-1,70 [-3.69, 0.29]	0.091
MYHC1	0.07 [-2.46, 2.60]	0.956	0.74 [-1.44, 2.91]	0.494	0.67 [-1.67, 3.01]	0.564
MYHC2a	0.04 [-2.78, 2.85]	0.980	1.13 [-1.29, 3.55]	0.348	1.09 [-1.51, 3.69]	0.397
MYHC2x	0.10 [-2.39, 2.59]	0.936	0.93 [-1.21, 3.07]	0.382	0.83 [-1.47, 3.13]	0.466
PDK4	-0.47 [-3.91, 2.97]	0.781	0.56 [-2.39, 3.52]	0.698	1.03 [-2.15, 4.21]	0.510
PPARα	1.23 [-0.97, 3.43]	0.260	-1.06 [-2.95, 0.83]	0.261	-2.29 [-4.32,-0.26]	0.029
PPARG	0.07 [-2.41, 2.56]	0.953	-2.30 [-4.44,-0.16]	0.036	-2.37 [-4.67,-0.07]	0.044
SCD1	1.53 [-2.44, 5.49]	0.436	-2.29 [-5.69, 1.12]	0.180	-3.81 [-7.47,-0.15]	0.042
SLC2a4	-0.32 [-2.60, 1.97]	0.779	0.40 [-1.56, 2.37]	0.676	0.72 [-1.39, 2.84]	0.490
SREBF1	1.15 [-1.02, 3.32]	0.286	-0.45 [-2.32, 1.41]	0.623	-1.60 [-3.61, 0.40]	0.113
UCP3	-0.47 [-3.20, 2.26]	0.729	-0.12 [-2.47, 2.23]	0.916	0.34 [-2.18, 2.87]	0.781

Log2FC = Log2 fold change. CI = 95% Confidence Interval.

P-value < 0.05 = Means are different.

Abbreviations: CON = Control; RDP = Ruminally degradable protein supplement; RUP = Ruminal non-degradable protein supplement; *ACACA = Acetyl-CoA carboxylase 1; ACOX = Acyl-coenzyme A oxidase 1;; AMPKa2 = Protein kinase AMP-activated catalytic subunit alpha 2; CPT1A = Carnitine palmitoyltransferase 1A; CPT2 = Carnitine O-palmitoyltransferase 2; COL3A1 = Collagen type III; C/EBPA = Enhacer Biding protein alpha constitutive α; FABP4 = Fatty acid binding protein 4; FASN = Fatty acid synthase; IGFR1 = Insulin-like growth factor 1 receptor; INSR = Insulin receptor; LPL = Lipoprotein lipase; mTOR = Mechanistic target of rapamycin kinase; MyHC I = Myosin heavy chain type I; MyHC IIa = Myosin heavy chain type IIa; MyHC IIx = Myosin heavy chain type IIx; MyoD = Myogenic differentiation 1; MyoG = Myogenin; PDK4 = Pyruvate dehydrogenase kinase 4; PPARA= Peroxisome proliferator-activated receptor α; PPARG = Peroxisome proliferator-activated receptor G; SREBF1 = Sterol regulatory element-binding factor 1; SREBP2 = Sterol regulatory element-binding protein 2; SCD1 = Stearoyl-CoA desaturase 1; TGFB1 = Transforming growth factor beta 1; UCP3 = Uncoupling protein 3; ZFP423 = Zinc finger protein 423*.

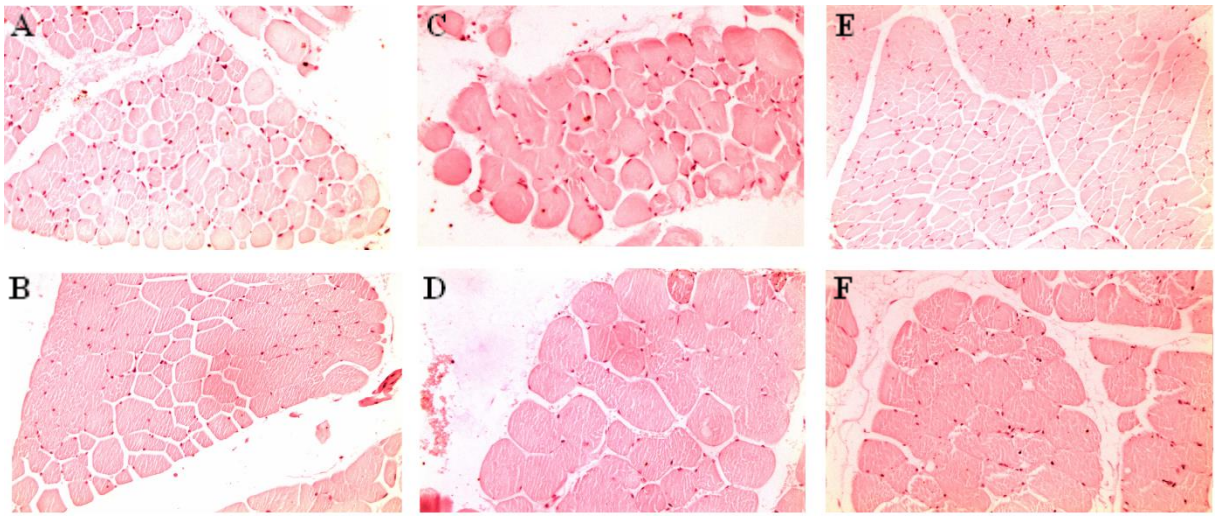

Figure 7. Photomicrographs of skeletal muscle tissue of offspring according to maternal treatment and at different ages (A: CON treatment, 48 ± 10 days of age; B: CON treatment, 346 ± 31 days of age; C: RDP treatment, 48 ± 10 days of age D: RDP treatment, 346 ± 31 days of age; E: RUP treatment, 48 ± 10 days of age; F: RUP treatment, 346 ± 31 days of age).