ISABELLA DE OLIVEIRA

PRODUCTION AND PHYSIOLOGY OF PREGNANT BEEF COWS SUPPLEMENTED WITH RUMINALLY DEGRADABLE OR UNDEGRADABLE PROTEIN UNDER LOW PROTEIN BASAL DIETS DURING MID-GESTATION

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

Prof. Dr. Mateus Pies Gionbelli

Advisor

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Aprovada em 31 de maio de 2021

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A Deus por me dar forças a cada amanhecer

A minha mãe Sonia

Aos meus avós Dalva e Maurício

Dedico

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ABSTRACT

Most of the breeding herd in Brazil is managed in extensive pasture systems, where the breeding season is planned in the spring and, therefore, the second half of gestation coincides with the dry season. Consequently, poor quality of forage can lead to a reduction in protein intake and restriction of nutrients to the dam. Thus, our objective was to evaluate the effect of protein supplementation via rumen degradable protein (RDP) based supplement or via rumen undegradable protein (RUP) based supplement, which one of the main amino acids is arginine, compared to a control treatment commonly used in practice, for pregnant cows feeding on a poor basal diet in the midgestation. Thirty Tabapuã beef cows with an average weight of 532 ± 11 kg and 6 ± 0.5 years were randomly assigned to three groups with different dietary treatments from 127 ± 17 days of gestation, lasting 100 days during this feeding period. All cows were fed a single basal diet composed of whole plant corn silage and sugarcane bagasse. Control treatment cows received a nitrogenous mineral supplement (CON; estimated 7.0% CP in the diet), while, cows supplemented with RDP supplement, received a protein commercial supplement (RDP; estimated 10.0% CP in the diet), or a rumen undegradable protein supplement (RUP; estimated 15.0% CP in the diet). Cows fed RUP had higher body weight at the end of the supplementation period (at day 227 of gestation) compared to RDP and CON cows ($P \leq 0.04$). Cows fed with RDP or RUP lost 0.163 and 0.174 kg/day, respectively, while CON dams gained 0.360 kg/day during the prepartum period ($P = 0.04$). On the other hand, in the lactation phase (between days 55 to 200), cows fed RUP during mid-gestation had an average daily gain (ADG) of 0.159 kg/day, greater than the CON and RDP groups lost 0.060 and 0.078 kg/day, respectively ($P < 0.01$). When the total performance of the cows (from the beginning of the supplementation period until weaning, ~ a total production cycle) was evaluated, the dams fed with RUP were the only ones with positive values in the total ADG (0.047 kg/day), while the treatments CON and RDP lost weight (0.019 and 0.057 kg/day, respectively) ($P = 0.05$). The Rib eye area (REA) in RUP cows at the end of the supplementation period was ~15.6 and ~24.0 cm2 greater than RDP and CON cows, respectively ($P < 0.01$). The calf's body weight at birth tended to be higher when the dams were fed RUP compared to CON and RDP cows ($P = 0.09$). Cows in the CON group had higher pulsatility index (PI) at 217 days of gestation compared to RDP and RUP cows ($P = 0.02$). Therefore, supplementation with RUP during mid-gestation, reduces the mobilization of maternal body tissues, improving the performance of cows and consequently the calf's body weight at birth.

Keywords: fetal programming, maternal nutrition, RDP, RUP, body reserves

RESUMO

A maior parte do rebanho reprodutor no Brasil é manejada em sistemas extensivos de pastagem, onde a estação de monta é planejada na primavera e, portanto, a segunda metade da gestação coincide com a estação seca. Consequentemente, a má qualidade da forragem pode levar à redução da ingestão de proteínas e restrição de nutrientes materna. Assim, nosso objetivo foi avaliar o efeito da suplementação proteica via suplemento à base de proteína degradável no rúmen (RDP) ou via suplemento à base de proteína não degradável no rúmen (RUP), em que um dos principais aminoácidos é a arginina, em comparação a um tratamento controle comumente utilizado na prática, para vacas gestantes alimentandas com uma dieta basal pobre na metade da gestação. Trinta vacas de corte Tabapuã com peso médio de 532 ± 11 kg e 6 ± 0,5 anos foram distribuídas aleatoriamente em três grupos com diferentes tratamentos dietéticos a partir de 127 ± 17 dias de gestação, com duração de 100 dias neste período de alimentação. Todas as vacas foram alimentadas com uma única dieta basal composta de silagem de milho integral e bagaço de cana-de-açúcar. As vacas do tratamento controle receberam um suplemento mineral nitrogenado (CON; estimado em 7,0% PB na dieta), enquanto as vacas suplementadas com RDP, receberam um suplemento comercial de proteína (RDP; estimado 10,0% PB na dieta), ou uma proteína não degradável no rúmen suplemento (RUP; estimativa de 15,0% de PB na dieta). Vacas alimentadas com RUP apresentaram maior peso corporal ao final do período de suplementação (no dia 227 de gestação) em comparação com vacas RDP e CON ($P \leq 0,04$). Vacas alimentadas com RDP ou RUP perderam 0,163 e 0,174 kg / dia, respectivamente, enquanto as mães CON ganharam 0,360 kg / dia durante o período pré-parto ($P = 0,04$). Por outro lado, na fase de lactação (entre os dias 55 a 200), as vacas alimentadas com RUP durante o meio da gestação tiveram um ganho médio diário (GMD) de 0,159 kg / dia, maior do que os grupos CON e RDP perderam 0,060 e 0,078 kg / dia, respectivamente ($P <0,01$). Quanto ao desempenho total das vacas (desde o início do período de suplementação até o desmame, ~ um ciclo de produção total), as mães alimentadas com RUP foram as únicas com valores positivos no GMD total (0,047 kg / dia), enquanto os tratamentos CON e RDP perderam peso (0,019 e 0,057 kg / dia, respectivamente) ($P = 0,05$). A área de olho de lombo em vacas RUP no final do período de suplementação foi ~ 15,6 e ~ 24,0 cm2 maior do que as vacas RDP e CON, respectivamente ($P <0,01$). O peso corporal do bezerro ao nascer tendeu a ser maior quando as mães foram alimentadas com RUP em comparação com as vacas CON e RDP ($P = 0,09$). As vacas do grupo CON apresentaram maior índice de pulsatilidade (IP) aos 217 dias de gestação em comparação às vacas RDP e RUP ($P = 0,02$). Portanto, a suplementação com RUP no meio da gestação, reduz a mobilização dos tecidos corporais maternos, melhorando o desempenho das vacas e, consequentemente, o peso corporal do bezerro ao nascer.

Palavras-chave: programação fetal, nutrição gestacional, PDR, PNDR, reservas corporais

Produção e fisiologia de vacas de corte gestantes suplementadas com proteína degradável ou não degradável no rúmen sob dietas basais de baixa proteína durante o terço médio da gestação

Elaborado por **Isabella de Oliveira** e orientado por **Mateus Pies Gionbelli**

Geralmente as vacas de corte no Brasil iniciam a estação de monta no início da estação chuvosa. Isso faz com que elas passem o período médio da gestação na estação seca e sofram por restrições nutricionais. A suplementação proteica é uma estratégia para suprir a baixa qualidade nutricional das pastagens. E o farelo de soja protegido da degradação ruminal é uma fonte de proteína rica em arginina e que possui um papel muito importante no metabolismo e desempenho materno. Portanto o objetivo desse estudo foi avaliar o uso de um suplemento protegido da degradação ruminal, cujo um dos principais aminoácidos é a arginina, durante o terço médio da gestação para vacas de corte. Vacas alimentadas com Proteína não degradada no Rúmen (PNDR) apresentaram maior peso corporal no fim da suplementação, e maior ganho médio diário no período de lactação de 0,159 kg / dia, em relação ao Controle (CON) e ao grupo suplementado com Proteína Degradada no Rúmen (PDR), que perderam 0,060 e 0,078 kg / dia, respectivamente. As vacas do grupo PNDR também apresentaram menor perda de tecidos corporais, com Área de olho de Lombo ~ 15,6 e ~ 24,0 cm2 maior do que as vacas CON e PDR, respectivamente. As vacas dos grupos PNDR e PDR também tiveram melhor desenvolvimento de vasos sanguíneos placentários em relação ao grupo CON, que possuiu Índice de Pulsatilidade superior aos demais. Portanto os resultados sugerem que vacas gestantes suplementadas com PNDR tem melhor desempenho, conservação das reservas corporais maternas e irrigação sanguínea uterina em relação aos demais grupos experimentais, sofrendo menos com os efeitos negativos da má qualidade da dieta.

Efeito da suplementação para vacas de corte no terço médio da gestação em dietas de baixa qualidade com Proteína não Degradada no Rúmen cujo um dos principais aminoácidos é a arginina (A) em comparação a vacas não suplementadas (B)

Dissertação de mestrado em Zootecnia na UFLA, defendida em 31 de maio de 2021

SUMMARY

1. INTRODUCTION

In beef cattle production systems of tropical regions, cows usually enter the breeding period at the beginning of the rainy season, when the forage availability and quality are better (DUARTE et al., 2013a). Because of this, the dams spend the second half of gestation during the dry season, which is the time of year with less forage availability and quality. In tropical pastures during the dry season, drastic reductions in support capacity occur, reducing the production of DM and, consequently, of CP and NDT (EUCLIDES et al., 2009). As a consequence, dams reared on pasture commonly face nutritional restrictions during pregnancy, which directly impacts their metabolism and the in utero development of their progeny.

Maternal nutrition during pregnancy can cause changes in the cell signaling pathway of the fetus, which can drive the pool of undifferentiated mesenchymal cells and compromise the formation of muscle cells, adipocytes, or fibroblasts (DU et al., 2013). Since muscle cells, adipocytes, and fibroblasts are derived from the same pool of undifferentiated mesenchymal cells (DU et al., 2009), maternal nutrition during pregnancy has been reported to be one of the main factors affecting myogenesis and consequently fetal muscle development, thus influencing fetal performance of the progeny throughout their life. During the fetal phase, skeletal muscle has a lower priority in the partition of nutrients compared to vital organs, which makes it vulnerable to the nutritional deficiency of the dam (ZHU et al., 2006). Since the production of beef animals aims to maximize their performance and muscle growth combined with adequate fat deposition, interventions via maternal nutrition can be used to maximize the development of fetus skeletal muscle and to maintain adequate maternal productivity.

Cows can maintain their physiological functions in situations of fluctuations in feed supply (BLANC et al., 2006), but this could occur at the expense of changes in regular maternal metabolism, leading to the development of metabolic diseases and progenies with harmful epigenetic changes (BARKER, 1997, GODFREY; BARKER, 2000). These interventions via maternal nutrition are carried out through fetal programming, which can be defined as the result of specific changes during intrauterine development that alter the individual's developmental trajectory quantitatively and / or qualitatively during the whole life (DUARTE et al., 2013b).

Protein supplementation can be one of the ways of maternal diet improvement during the gestation. Soybean bran is a protein supplement widely used for animal feed, and has Arginine (ARG) as one of the main amino acids in its nutritional profile. The ARG is one of the most studied amino acids for pregnant cows, which is classified as nutritionally essential for the fetus. The ARG has the potential to improve the efficiency of nutrients utilization, growth, development, lactation and reproduction. Studies have shown that ARG increases placental angiogenesis (RAGHAVAN; DIKSHIT, 2004), fetal-placental blood flow (TAN et al., 2012), antioxidant capacity and improves lipid peroxidation during pregnancy (MORRIS, 2009). One of the main mechanisms developed in response to the supply of ARG is the change in placental vascularization, due to its nitric oxide precursor activity, which increases the placental-fetal blood flow and the supply of nutrients to the fetus. Current studies have evaluated the use of ARG in the ruminant diet (PEINE et al., 2018; PREZOTTO et al., 2018; SUN et al., 2017; ZHANG et al., 2016a). In these animals, it is necessary to use ruminal protection technologies that allow oral administration of this AA, because it is rapidly degraded in the rumen (CHACHER et al., 2012; ZHANG et al., 2016a). ARG also plays an important role in maternal metabolism, preserving homeostasis of the entire body during pregnancy (WU et al., 2004a).

We hypothesize that the use of a protein supplement rich in AAs protected from ruminal degradation (whose one of the main AA is ARG) for pregnant beef cows consuming low quality diets during mid-gestation would mitigate the negative effects of compromised maternal nutrition and bring beneficial effects on production and maternal metabolism.

Therefore, based on the high demand for AA in pregnant beef cows from the mid-gestation, as well as the beneficial potential of protein supplementation that escapes ruminal degradation, the objective of this research was to evaluate the use of a supplement rich in AA protected from ruminal degradation, whose one of the main AA is ARG, and its effects on maternal metabolism and production during gestation.

2. BACKGROUND

2.1 Influence of maternal nutrition on fetal muscle growth and development

The environmental effects at an early stage of an animal's life, especially in the womb, not only affect its development, but also have an impact on its health and performance throughout its life (BARKER et al., 1993). Fetal growth and development can be affected by

several factors, with inadequate maternal nutrition being the main cause that compromises the nutrients supply to the fetus (REYNOLDS et al., 2010). The adequate supply of nutrients to pregnant cows has been associated with the ideal development of fetal tissues, better progeny performance and meat quality. According to Wu et al. (2006), it was revealed that there are greater risks of mortality and morbidity in animals born to malnourished females during pregnancy, including intestinal and respiratory disorders, which makes supplementation at pasture, in this case, a viable tool for improving future performance of the animal (PAULINO et al., 2008).

The nutrition of beef cows can be considered as the main cost entry and the main factor that affects the productivity of the breeding systems (GIONBELLI et al., 2015b). There is an increasing demand for better production efficiency in cattle, which subjected to short cycle system and slaughtered around 18 months of age, spend about 35 to 40% of their life in the uterine environment, being nourished by the mother through the placenta (VONNAHME; LEMLEY, 2012). Because of this, the use of tools in this period such as fetal programming, which allows manipulation of fetal development, is of great importance. Fetal programming, also called programmed development or fetal development programming, is the answer to a specific challenge to the mammal's organism during a critical window of time that changes qualitatively and quantitatively the trajectory of its development, with persistent resulting effects (NATHANIELSZ; POSTON; TAYLOR, 2007; DU et al., 2009). The programming of the development of muscle tissue during the fetal phase is a priority, since there is no increase in number of muscle fibers in cattle after birth (RUSSELL; OTERUELO, 1981; PICARD et al., 2002) and during this phase is when there is an increase in the number of muscle fibers and muscle mass, which determines the carcass yield (ZHU et al., 2004). Therefore, in addition to adequate genetics, generating an adequate fetal development is also decisive for maximizing the growth potential of animals (DU et al., 2015).

Muscle growth occurs in two ways: the hyperplasia (multiplication of muscle fibers) occurs exclusively during pregnancy in cattle, with the number of fibers fixed exclusively in the gestational period; and the hypertrophy (increase in the size of muscle fibers) occurs at the end of the gestational period, and throughout the animal's postnatal life, according to its capacity. There are three types of cells: myocytes, adipocytes and fibroblasts. These three make up the basic structure of skeletal muscle, and are derived from the same pool of mesenchymal stem cells (DU et al., 2009). Myogenesis is the process of embryonic development of muscle tissue. According to Du et al., (2015) the skeletal muscle develops through primary and secondary myogenesis. Primary myofibers are formed first, followed by the formation of

secondary myofibers, responsible for most of muscle fibers in adults (BEERMANN; CASSENS; HAUSMAN, 1978). This development of skeletal muscle is initiated during the embryonic stage (COSSU; BORELLO, 1999). The growth of fetal skeletal muscle in cattle is a process regulated by interactions between undifferentiated mesenchymal cells (or stem cells) in the embryonic phase, and also by nutrients provided by the mother, which can be provided via diet or via mobilization of body reserves (JENNINGS et al ., 2016). And skeletal muscle has less priority in the nutrients partition during fetal development when compared to vital organs, such as the brain, heart and liver. As a result, skeletal muscle development is particularly vulnerable to nutrient availability (ZHU et al., 2006). Maternal nutrition may have the ability to program mesenchymal stem cells for myogenic or adipose / fibrogenic differentiation (DU et al., 2013). Most muscle fibers are formed in the fetal stage between two and seven to eight months of gestation in cattle (RUSSELL; OTERUELO, 1981) and a reduction in the number of muscle fibers formed during this stage generates irreversible negative physiological consequences for the offspring (STANNARD ; JOHNSON, 2004; ZAMBRANO et al., 2005; ZHU et al., 2006; DU et al., 2009). There is no more increase in the number of muscle fibers after birth and postnatal muscle growth is mainly due to the increase in muscle fibers size, without forming new muscle fibers (KARUNARATNE; ASHTON; STICKLAND, 2005; STICKLAND, 1978). Similarly, if the production of animals with greater potential for deposition of body fat is of interest, intrauterine intervention via gestational nutrition can be used in order to maximize the development of intramuscular adipocytes aiming at greater deposition of this tissue in the postnatal phase.

The mechanisms involved in maternal nutrition and which influence fetal myogenesis are changes in levels of Somatomedins (IGF) and in the placenta morphology and efficiency. When the nutritional supply of the pregnant cow improves, occurs a better contribution of glucose and amino acids to placenta and fetus. This fact stimulates the release of IGF, which is important in the regulation of muscle hyperplasia by stimulating myoblastic proliferation and differentiation, resulting in animals with higher number of fibers (BRIDI, unpublished data).

One of the recurring problems caused by nutritional deficiencies during pregnancy is the restriction of intrauterine fetal growth, which reduces neonatal survival and has a permanent effect of atrophy on postnatal growth and efficiency of feed use (WU et al., 2006). Insufficient availability or delivery of nutrients to the fetus alters the growth and function of the placenta, resulting in intrauterine growth restriction (ZHANG et al., 2016b). The causes of intrauterine growth restriction are multiple, but nutrition plays the most decisive role in influencing placental and fetal growth (WU et al., 2004b). Supplementation during the second trimester of

pregnancy in ruminant animals (which is the period of formation of the largest number of muscle fibers) has the ability to promote better muscle development, which increases the muscle mass and the productive efficiency of the offspring (DU et al., 2010).

2.2 Maternal metabolism and partition of nutrients during pregnancy

The control of maternal metabolism during pregnancy and lactation involves two types of application - homeostasis and homeoresis. Homeorresis is a concept developed by Bauman and Currie (1980), which suggests that there is a simultaneous endocrine regulation of tissues so that the metabolism meets its "attributions" in a coherent and balanced way. At levels that optimize the opportunity of fetus growing and surviving, without competition with maternal metabolism and with balance between them, minimizing the excessive depletion of maternal energy and protein reserves. Homeostasis involves maintaining the physiological balance or constancy of the animal's condition (BAUMAN; CURRIE, 1980).

During pregnancy cows increase their voluntary feed intake (INGVARTSEN; ANDERSEN; FOLDAGER, 1992), however, in the last weeks of gestation there is a notable reduction in this intake according to Ingvartsen and Andersen (2000), as the rumen occupies a higher proportion of abdominal cavity in relation to monogastric. When there is a shortage of feed for the pregnant mother during pregnancy, the cows manage to maintain their pregnancy, however this can occur at the expense of changes in their regular metabolism during the gestational period, leading to the development of progenies with a sparing phenotype (BARKER, 1997; GODFREY; BARKE, 2000).

In mammals, nutrients are used by tissues involved in maintaining, growing and establishing body reserves, including energy reserves (lipids), glucose reserves (glycogen) and amino acid reserves (labile protein) (BAUMAN; CURRIE, 1980). According to Wood et al. (2013), even in unfavorable conditions, the pregnant female modulates her nutrient partition in favor of the growth of the fetus.

According to Bauman and Currie (1980), two additional tissues that use a large portion of maternal nutrients are the developing fetus and the lactating mammary gland. However, these tissues differ from other tissues in the body, as they do not give any special advantage to the pregnant cow. Instead, it has high demands, so that metabolism of the pregnant or lactating cow must be changed to accommodate these needs. An inability to adjust or metabolize quickly enough to meet these needs often results in metabolic disorders. According to Gionbelli et al. (2015b), based on models of metabolic patterns for pregnancy in ruminants (BELL, 1995b; BELL; SLEPETIS; ENRHARDT, 1995a; BELL; FERRELL; FREETLY, 2005), it is expected

that during the last third of pregnancy, glucose and lactate represent 35 to 40% of fetal energy and 55% are supplied as AAs. Of the remaining 5 to 10%, most is provided by acetate, which is very little in relation to its relative abundance and importance to the maternal system of ruminant females. The uptake of glucose by the uterus increases significantly at the end of pregnancy, being responsible for at least 50% of the maternal glucose supply (BELL; FERRELL; FREETLY, 2005). Bell and Ehrhardt (1998) estimate that more than 80% of the digested crude protein is directed to the pregnant uterus, with the remainder directed to support metabolism and liquid deposition of amino acids in the mammary gland and visceral organs. According to Bauman and Currie (1980), pregnancy imposes a substantial cost on the animal, because the total nutrient requirements at the end of pregnancy are about 75% higher than in a non-pregnant animal of the same weight.

Due to the increase in metabolic demands with the advancement of pregnancy, maternal metabolism induces adaptive responses in hepatic synthesis and the use of glucose in peripheral tissues (BELL; BAUMAN, 1997). The fetal needs for oxidative metabolism are met mainly by the combined use of glucose, lactate and AAs. As ruminants have difficult to increase circulating glucose levels, it is likely that the use of AAs as precursors to gluconeogenesis is the easiest way to meet the high demand for glucose. Due to the greater need for glucose and amino acid uptake to supply the metabolism of the pregnant uterus, there is an increase in gluconeogenesis and lactate uptake in the maternal liver (FREETLY; FERRELL, 1998b), increased liver uptake of glycerol (FREETLY; FERRELL, 2000) and mobilization of amino acids from the maternal carcass (MCNEILL et al., 1997). Higher levels of ubiquitin (protein used as an indicator of protein degradation) in skeletal muscle of pregnant cows receiving a restricted diet denote that to meet the fetal requirement, the mobilization of muscle protein may be a mechanism to meet the increased energy demand imposed through pregnancy (WOOD et al., 2013). The greater supply of substrates derived from mobilization can be explained by the greater hepatic blood flow (FREETLY; FERRELL, 1998a), combined with an “incentive” of catabolic processes induced by the insulin resistance process. Insulin resistance developed in the late stages of pregnancy in protein metabolism can generate an increase in protein breakdown of extrahepatic tissues, especially in skeletal muscle, since insulin (in its normal mode of action) is responsible for protein synthesis (KOSTER; OPSOMER, 2013).

Maternal physiology during pregnancy leads to changes in the endocrine and cardiovascular systems (VONNAHME et al. 2015), in addition to changes in vascularization of the small intestine for greater absorption of nutrients (SCHEAFFER et al., 2003). When evaluating the dry or empty pregnant Jersey cow viscera, Smith and Baldwin (1973) found that

the heart was 12% larger in pregnant cows. According to Freetly and Ferrell (1998a), as the pregnancy progresses, the metabolic activity of the maternal liver also increases, since gluconeogenesis occurs mainly in the liver in mammals (NELSON; COX, 2011).

2.3 Effects of ingestion of rumen undegraded protein in the rumen and amino acids on the metabolism of pregnant cows and the performance of their progeny

When pregnant cows ingest between 110 and 140% of the protein requirements for pregnancy, it is estimated that more than 80% of the digested protein is directed to the pregnant uterus (BELL; EHRHARDT, 1998), with the rest being directed to the metabolism of support, mammary gland and maternal viscera (BELL; EHRHARDT, 2000). In early or intermediate stages of pregnancy, cows can be sensitive to nutritional deficits, modify their metabolism (CAMACHO et al., 2014) and, thus, prepare the developing fetus to live in an environment of nutrient scarcity. This causes the fetus to be born with metabolic changes, due to epigenetic changes, even without differences in birth weight (WU et al., 2006; DU et al., 2010; UNDERWOOD et al., 2010; SCHOONMAKER; LADEIRA, 2014).

The rapid and extensive degradation of valuable proteins in the rumen led to the development of the concept of protection of proteins against ruminal degradation, with the main objective of increasing the supply of essential AAs to the animal and reducing nitrogen losses such as urine urea (ANNISON, 1981). The protein intake by ruminants is divided into ruminally degraded protein (RDP) and ruminally undegraded protein (RUP). The protection of proteins against ruminal degradation allows part of the AAs to arrive partially intact in the small intestine and, therefore, provide more absorbable AAs per unit of energy (KAMALAK et al., 2005). Vegetable proteins that undergo heat treatment generally have high amounts of RUP. This RUP can have a high intestinal digestibility if not damaged by heat (DAKOWSKI et al. 1996).

Therefore, in the case of ruminants, the purpose of using RUP is to decrease ruminal degradability and increase intestinal protein absorption, without decreasing digestibility. The increase of metabolizable protein is possible through the supply of the RUP (ATKINSON; TOONE; LUDDEN, 2007) in order to subsidize greater amounts of AAs to the fetus via the placenta. The use of protected proteins in the rumen, compared to the usual sources of dietary proteins, improves the supply of AAs without increasing ammonia production, resulting in better animal performance (KAUFMAN; LUPPING, 1982). Because the supply of RUP can increase the flow of metabolizable protein (MEZZOMO et al., 2011; BATISTA et al., 2016, 2017), it is expected that increases in RUP in the diet could also affect the development of the mammary gland. According to Silva et al., (2018) the increase in the amount of RUP in the diet

was responsible for a tendency to decrease the urinary excretion of N, as also observed by Batista et al. (2016). According to research by Sun et al. (2017) supplementation of 20 g / day of RP-Arg for sheep with nutrient restriction from day 35 to 110 of gestation significantly increased the weights of fetuses and most fetal organs, and markedly improved concentrations of AAs (particularly AAs from the ARG family) and polyamines in maternal and fetal plasma, indicating that feeding malnourished sheep with RP-Arg is an effective strategy (ZHANG, et al. 2016b).

One of the main amino acids present in Soybean Meal Protected from ruminal degradation is ARG. In all stages of pregnancy, an adequate supply of AAs is very important for the normal development of placenta and fetus (SUN et al., 2017). ARG is an AA considered essential, because although ruminants can synthesize it, its synthesis is not sufficient to satisfy the requirements in early stages of growth and in stages of high production (NRC, 2001). ARG is able to regulate major metabolic pathways that are critical for health, growth, reproduction, metabolism and homeostasis of animals (MORRIS, 2009). The main functions of ARG are protein synthesis and precursor activity for the synthesis of molecules such as nitric oxide, polyamines, creatine, ornithine, urea, and glutamate (WU; MORRIS, 1998). ARG is also crucial for placental angiogenesis and growth in mammals (SHEPPARD et al., 2001), it improves fetal-placental blood flow (TAN et al. 2012), has antioxidant capacity and improves lipid peroxidation during pregnancy (MORRIS, 2009).

ARG improves angiogenesis, which is the process of forming new blood vessels, or neovascularization, being essential for the growth and development of all tissues, including the placenta (HUDLIKA, 1984; FOLKMAN; KLAGSBRUN, 1987; KLAGSBRUN; D 'AMORE, 1991; REYNOLDS et al., 1992), and increase the placental-fetal blood flow and nutrients supply to the fetus. Vasculogenesis and angiogenesis are critical for adequate placental function and, therefore, for normal embryonic / fetal growth and development (DEMIR; SEVAL; HUPPERTZ, 2007, ARROYO; WINN 2008). In cows and sheep, angiogenic factors are produced mainly by maternal placental tissues (REYNOLDS et al., 1987; REYNOLDS; REDMER, 1988). Thus, these tissues can direct placental vascularization, and have a significant effect on placental size, transport and / or blood flow, influencing fetal growth and development (REYNOLDS; REDMER, 1995). When regulating vascular development and functions of the umbilical vein and placenta, ARG favors more nutrients and oxygen supply from the mother to the fetuses for survival, growth and fetal development (LIU et al. 2012). ARG is a precursor for synthesis of polyamines, which play an important role during pregnancy, and may be related to regulation of steroid hormone synthesis, as well as to embryonic, placental and fetal growth

and development (BASTIDA et al., 2002). Information on the synthesis of polyamines is essential for understanding the molecular regulation of placental and fetal growth, in addition to elucidating the mechanisms responsible for intrauterine growth retarded (KWON et al., 2003).

Another benefit of ARG supplementation is its precursor activity of nitric oxide (WU; MORRIS, 1998), since nitric oxide stimulates the development of blood vessels (WU; MORRIS, 1998). When causing vasodilation, nitric oxide is involved in regulating blood flow and increasing vascular permeability (MARTIN; JIMENEZ; MOTILVA, 2001; ROY; BHARDWAJ; YLA-HERTTUALA, 2006). It is possible that the effects of nitric oxide on blood flow regulation may have positive systemic results on nutrient absorption, embryonic and fetal development, lactation and ovarian function (MEYER et al., 2018). Nitric oxide synthase is also involved in regulation of energy metabolism (JOFFIN et al., 2012) and feed intake (HUI; CHAN, 1995) in mammals.

In pregnant rats receiving diets without ARG, there was an increase in fetal resorption, an increase in perinatal mortality and a decrease in number of live fetuses (GREENBERG et al., 1997). When there was intravenous administration of ARG in sheep with maternal nutritional restriction, an improvement in fetal growth was observed (WU et al., 2009). In addition, studies show that ARG can improve progeny growth via insulin stimulation or by other glucose metabolism pathways (FLOYD et al., 1966; SCHMIDT et al., 1992; GANNON; NUTTALL; NUTTALL, 2002). Lassala et al. (2010) found that the administration of ARG in malnourished sheep increased the birth weight of the offspring by 21% compared to the restricted animals that received only saline infusion. In addition, these authors found that there is no difference between the birth weights of the restricted progeny receiving infusion of ARG during pregnancy and the control group, in which the mothers received 100% of the nutritional requirements. In humans, the intravenous infusion of ARG for 7 days (20 g / day) in the 33rd week of pregnancy increased the birth weight (39th week) of their children by 6.4% (XIAO; LI, 2005). These findings provide a strong experimental basis for the use of ARG, however, supplementation with unprotected ARG in ruminant animals is rapidly degraded in the rumen (CHACHER et. Al, 2012). Parenteral administration of ARG for animal production is not a practical approach, and supplementation of rumen-protected ARG (RP-Arg) throughout pregnancy provides an effective approach to solving this problem, but its price is high (SUN et al., 2017).

3. HYPOTHESYS

The use of a protein supplement rich in AAs protected from rumen degradation, whose one of the main AA is the ARG, for pregnant beef cows consuming low quality diets during mid- gestation would mitigate the negative effects of compromised maternal nutrition and bring beneficial effects on production and maternal metabolism.

4. OBJECTIVES

4.1 General objectives

To evaluate the effect of a supplement rich in rumen undegradable protein, with a level of ARG that reaches the small intestine similar to levels used in previous studies, on maternal physiology and performance of Tabapuã cows during the mid-gestation submitted to a diet of low nutritional value and protein deficiency.

4.2 Specific objectives

To evaluate maternal metabolism by measuring Intake of DM and nutrients, apparent digestibility of the total tract, maternal performance, maternal tissue mobilization, uterine blood flow rates and maternal blood metabolites.

5. MATERIAL AND METHODS

5.1 Location

The experiment was carried out at Beef Cattle facilities of the Animal Science Department of Federal University of Lavras (UFLA), in Lavras, MG, Brazil. The experiment followed the standard procedures of management and animal welfare, according to the Ethics Committee on the Use of Production Animals at the Federal University of Lavras (protocol number: 015/2019).

5.2 Animals, treatments and experimental design

Thirty Zebu beef cows, Tabapuã breed, with an average weight of 532 ± 11 kg and 6 ± 0.5 years old were used. The animals were submitted to a 90 days breeding season (October to December), based on a fixed time artificial insemination (FTAI) protocol (up to three stages) followed for hand mating with bull for cows with negative pregnancy status at the first two

stages of FTAI. Pregnancy was confirmed by ultrasound (Aloka 500, 5 MHz probe, Aloka, Wallingford, CT, USA) 30 days after mating. Day 0 (zero) of pregnancy was considered the day on which the animals were inseminated of hand mated. After day 0, the animals were kept on medium- quality pastures (*Brachiaria brizantha cv. Marandu*), receiving a commercial mineral supplement (Probeef 800®, Cargill Animal Nutrition, Itapira, SP, Brazil)[1] *ad libitum* until approximately 112th day of gestation. After that, the cows were transfered to individual pens (35 m²) and underwent an adaptation period of 15 days, receiving feed and water *ad libitum*. At 127±17 days of gestation, the animals were randomly distributed into three groups with different dietary treatments and fed under that until 227±17 days of gestation. The treatment were based on a basal diet composed of a mixture between corn silage [(82.3%, dry matter (DM) basis] and sugarcane bagasse (17.7%, DM basis) in order to simulate a tropical low-quality pasture during the dry season of the year (5.5% of crude protein, CP). Then, the basal diet was supplemented at three different ways, as follows:

• **Control (CON, n = 10):** the basal diet supplemented with a mineral plus urea commercial supplement (Probeef Urea®, Cargill Animal Nutrition, Itapira, SP, Brazil)[2] at the level of 60 g / 100 kg body weight (in order to achieve at least 7.0% of CP in the total diet of the cows). This treatment represents a condition of low level of supplementation quite common offered to beef cows during the dry period of the year in tropical regions.

• **Ruminally degradable protein supplement (RDP, n = 10):** the basal diet supplemented with a protein commercial supplement (Probeef Nutripec Sprint®, Cargill Animal Nutrition, Itapira, SP, Brazil)[3] at the level of 250g / 100 kg of live weight, added 44 g of fine ground corn / 100 kg of live weight. This treatment represents a level of supplementation used in systems with medium level of technology, with the objective of reaching at least 10% of CP in the diet. Ground corn was added to this treatment in order to achieve a relationship between RDP and rumen-fermentable organic matter similar to the next (RUP) treatment in order to balance the rumen environment between these two treatments.

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[1] guarantee levels: 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; Zinc (min) - 3273 mg / kg.

[2] guarantee levels: Calcium (max) - 155 g / kg; Calcium (min) - 130 g / kg; Cobalt (min) - 31 mg / kg; Copper (min) -545 mg / kg; Sulfur (min) -15 g / kg; Fluorine (max) - 330 mg / kg; Phosphorus (min) -30 g / kg; Iodine (min) -27 mg / kg; Magnesium (min) - 2500 mg / kg; Manganese (min) - 2363 mg / kg; NNP Protein Eq (max) - 423 g / kg; Selenium (min) - 5.9 mg / kg; Sodium (min) -80 g / kg; Zinc (min) - 1636 mg / kg.

[3] guarantee levels: Calcium (max) - 155 g / kg; Calcium (min) - 130 g / kg; Cobalt (min) - 31 mg / kg; Copper (min) -545 mg / kg; Sulfur (min) -15 g / kg; Fluorine (max) - 330 mg / kg; Phosphorus (min) -30 g / kg; Iodine (min) -27 mg / kg; Magnesium (min) - 2500 mg / kg; Manganese (min) - 2363 mg / kg; NNP Protein Eq (max) - 423 g / kg; Selenium (min) - 5.9 mg / kg; Sodium (min) -80 g / kg; Zinc (min) - 1636 mg / kg.

• **Ruminally undegradable protein supplement (RUP, n = 10):** the basal diet supplemented with a commercial product of non-degradable rumen protein, which is rich in arginine (Soypass®, Cargill Animal Nutrition, Itapira, SP, Brazil)[4] at the level of 3.5g/ kg body weight, and a mineral plus urea commercial supplement (Probeef Urea®, Cargill Animal Nutrition, Itapira, SP, Brazil)[2] at the level of 60 g / 100 kg live weight. Notably, this treatment represents CON treatment added with Soypass supplementation in order to reach a level of supplementation of amino acids protected from ruminal degradation similar to that used in previous research that has been successful in small ruminants (PEINE et al., 2018; PREZOTTO et al., 2018). The amount of Soypass estimated to be supplied to the RUP- supp animals was based on the amount of ARG protect provided in previous treatments previous with pregnant sheep that received similar treatment (PEINE et al., 2018; PREZOTTO et al., 2018) and with non-lactating beef cows (GREEN et al., 2017) both at the level of 180 mg/kg of BW. Thus, the amount of Soypass to be supplied was calculated based on the proportion of ruminal protection of protected ARG (56%) obtained by Green et al. (2017). The estimated experimental diets (**Table 1**) were formulated according to the nutrient requirements for pregnant Zebu cows (GIONBELLI et al., 2016).

Cows were fed twice daily (07 h 00 and 13 h 00). The supplements were fed at fixed amounts according to the cows live weight. The basal diet was fed ad libitum in order to measure the increase or decrease in its intake.

From the 227th day of gestation, all cows returned to medium-quality pastures (*Brachiaria brizantha cv. Marandu*)[5], receiving commercial mineral supplement (Probeef 800, Cargill Animal Nutrition, Itapira, SP, Brazil) *ad libitum*, remaining in this condition for and after parturition, together with their calves until weaning (at 210 days of age).

The measures performed through the experiment are summarized in the **Figure 1.** The experimental procedures used to collect such measures are described below.

5.3 Weighing and monitoring the body condition score

[4] (Nutrient profile: CP- 46.5 % as is; DM- 89.0 % as is; CP- 52.0 % of DM; Soluble protein- 5.0 % of DM; RUP- 74.0 % of DM; Arginine- 6.78 % of CP; Met- 1.32 % of CP; Lys- 5.78 % of CP; Thr- 4.31 % of CP; Leu- 8.92 % of CP; Ile- 4.56 % of CP; Val-5.46 % of CP; His-2.40 % of CP; Phe- 5.60 % of CP; Trp-1.27 % of CP; Cys- 1.46 % of CP; ADF- 8.0 % of DM; NDF- 25.0 % of DM; Lignin- 1.7 % of DM; Ash- 6.6 % of DM; EE- 1.8 % of DM; NFC- 30.0 % of DM; Sugar- 14.0 % of DM; Starch- 1.0 % of DM; Soluble Fiber- 18.2 % of DM).

[5] (Nutritive value Spring (%DM): CP- 12.6; NDF- 57.9; EE- 1.92. Summer (%DM): CP- 10.8; NDF- 61.5; EE- 1.68). Fonte: HOMEM et al. (2020).

Cows were fasting weighed in the morning (07:00 am) at the beginning and the end of the supplementation period (127 to 227 days of gestation, respectively), at the prepartum period (282 days of gestation), and at the postpartum period (105 and 200 days of lactation). Pregnant body weight (BWp), empty pregnant body weight (EBWp), non-pregnant empty body weight (EBWnp), pregnancy component (PREG) and average daily gain (ADG) adjusted for pregnancy were calculated according to GIONBELLI et al. (2015), which mathematically estimated the pregnancy component (PREG) as an extra component of the cow (cow BW + PREG) to make it possible to calculate the ADG of maternal tissues. (SBWnp) = BWnp; SBW of a non-pregnant cow (SBWnp) is function of the BW of a non- pregnant cow (BWnp). (SBWp) = SBWnp + PREG; SBWp also can be expressed as the SBW of the cow if in a non-pregnant condition plus the increase of weight occurred due to the pregnancy, called pregnancy compound (PREG). For this we need to consider non-interaction between the ratio BW/SBW and DOP. (EBWnp) = SBWnp; EBW of a non-pregnant cow (EBWnp) is function of a SBWp and DOP. (EBWp) = EBWnp + PREG; EPWp also can be expressed as the EBW of the cow if in a non-pregnant condition plus the pregnancy compound. For this, we need to consider that When discounted the PREG, the relation between SBW and EBW is equal for pregnant and non-pregnant cows. (PREG) = GUdp + UDdp; PREG means the all tissues increase due to the pregnancy and is equal to the GU accretion during the pregnancy (GUdp) plus udder accretion during the pregnancy.

The body condition score (BCS) was assessed at 127 and 227 days of gestation, considering a scale of 1 to 9, (1 = very thin to 9 = very obese according to RICHARDS et al. (1986) and NICHOLSON; BUTTERWORTH (1986)) .The evaluation was carried out by two observers in a double blind scheme, in which each observer was unaware of the result of the other's evaluation, and when there was a difference greater than 1.5 points between the observers, a new evaluation was obtained by averaging the score of the two observers. The average of the BCS of the two observerds was considered as the BCS of such cow.

5.4 Dry matter intake and digestibility trial

The dry matter intake (DMI) was daily measured based on the difference between the feed provided and the leftovers.

The digestibility trial collections were performed in the middle of the supplementation period (164 - 168 days of gestation) during four consecutive days (96 h 00) according to BARBOSA (2005), PAIXÃO et al. (2007) and FERREIRA et al. (2009). The total collection of feces samples was performed, in which every time the animal defecated an aliquot of feces,

it was collected, weighed and immediately frozen (-20 ° C) throughout the collection period, and the rest of the feces was removed from the stalls, stored in containers, and weighed every 24 h 00. Throughout the digestibility test the bulky diet, the concentrate ingredients and the orts trough were also sampled daily and frozen. At the end of the collection, all samples were thawed, dried in a forced ventilation oven at 55° C for 72 hours and ground in a knife mill, with a sieve containing 1 mm sieves. Subsequently, these samples were homogenized if a sample composed of animals was prepared. The total digestibility of DM and nutrients were calculated by determining the average DM and nutrients consumed by subtracting the average DM and nutrients excreted in the feces during the same period.

5.5 Chemical-bromatological analyzes

The samples of roughage, concentrate ingredients, leftover food and feces samples were dried in a forced ventilation oven at 55 ° C for 72 hours and ground in a knife mill, with a sieve containing 1 mm sieves. The samples were analyzed at the Animal Research Laboratory of the Federal University of Lavras (UFLA), for their content of dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), and ether extract (EE). Chemical analyzes of DM, ashes, CP and EE followed the standards of the National Institute of Science and Technology in Animal Science (INCT-CA), and were described by DETMANN et al. (2012). The NDF analyzes followed patterns described by MERTENS et al. (2002), with the addition of sodium sulfite and thermostable α-amylase for the detergent.

5.6 Maternal tissue mobilization

The collection of ultrasound images for the dynamics maternal tissue was performed at the end of supplementation period (223 days of gestation) and at prepartum (270 days of gestation). Measurements were made in the region between the 12^{th} and 13^{th} ribs to assess the rib eye area (REA) and backfat thickness (BFT), and in the hip bone region on the rump to assess the fat thickness on the rump (FTR) and the length of the rump muscle (LRM). The images were collected using mineral oil as a coupler and ultrasound Aloka 210DX (Corometrics Medical Systems, Wallingford, CT), with a 3.5 MHz linear transducer measuring 17.2 cm.

5.7 Evaluation of uterine blood supply

Transrectal Doppler ultrasonography is described as an adequate and non-invasive technique for measuring the hydrodynamics of uterine vessels (BOLLWEIN et al., 2002; PANARACE et al., 2006). The evaluation of uterine irrigation by Doppler ultrasonography was

carried out at the end of the supplementation period (219 days of gestation) and in the prepartum period (267 days of gestation) by the same operator using an ultrasound equipment with mode B and Doppler mode (colored and spectral) and a linear transrectal probe of 7.0 MHz (distributed by Corometrics Medical Systems, Wallingford, CT; the veterinary, transrectal, linear, UST-5813-5 model). The uterine artery ipsilateral to the corpus luteum was located and examined to obtain the resistance index (RI), pulsatility (PI), systolic / diastolic ratio (S / D). The analysis of the Doppler indices was performed using the equipment's own analysis software.

5.8 Analysis of hormones and blood metabolites

Blood samples for hormone analysis were collected at 07:00 am on the day via puncture of the jugular vein and puncture of the coccygeal vein, approximately at 227 days of gestation and 265 days of gestation. The samples were then placed in evacuated tubes containing EDTA (to prevent blood clotting) and tubes with coagulation accelerator. After collection, the samples were immediately centrifuged at 3500 RPM for 15 minutes, and the plasma was stored in tubes at -20 ºC until analyzed.

For Insulin analysis, blood plasma samples from the animals were transported to a commercial laboratory (Carlos Chagas Laboratory, São João Del Rei, MG, Brazil) and analyzed by the ELISA method.

Commercial kits were used to analyze the other blood plasma samples, which followed the methodology described by the manufacturers. Blood glucose analysis was performed using the colorimetric method (Glucose PAP Liquiform, Labtest®, Lagoa Santa, Brazil). For the analysis of Non-Esterified Fatty Acids (NEFAs) in bovine blood, a specific commercial NEFA kit (Elabscience, Inc. Colorimetric Assay Kit) was used, based on the enzymatic method and reading by colorimetry. In the IGF-1 analyzes of the blood samples, the commercial kit was used by the ELISA test (Sigma Aldrich Quimica Brasil Ltda). And the analysis of blood Beta-hydroxybutyrate (BHA) was quantified using the enzymatic kinetic method (Randox Laboratories Ltda). And plasma N-urea was measured using the modified diacetyl method (Urea PAP Liquiform, Labtest®, Lagoa Santa Brasil).

5.9 Statistical analysis

The matrix data were analyzed using the same experimental model:

$$Y_{ijk} = \mu + D_{ij} + PI_{ijk} + e_{ijk}$$

where, Yijk is the measured variable; μ is the general average; Dij is the fixed effect of the ithr level of maternal feeding (3 levels) and calf sex; PIijk is covariate of initial weight of the kthr matrix; and eijk is the random error associated with Yijk, with e_ijk ~ N (0, σ_e ^ 2).

For each of the characteristics analyzed, the effects on the model, with the exception of D, were removed when P-value> 0.10. Before final analyzes, Studentized residues were removed when they did not appear within ± 3 standard deviations, and normality (P value> 0.05) was assessed using the Shapiro-Wilk test. The least squares means were separated using the Fisher's test with the least significant difference. The results were considered significant when the P value ≤ 0.05. All analyzes were performed using SAS 9.2 (Institute of Statistical Analysis of the Institute, Inc., Cary, NC, USA).

6. RESULTS

6.1 Intake and apparent total tract digestibility

There was no interaction between maternal nutrition (MN) and calf sex (CS) for DMI and nutrient intake (P ≥ 0.22; **Table 2**). MN affected DMI and nutrient intake (P <0.05). Cows receiving RUP consumed ~ 2.41 kg / d and 1.17 kg / d DM more than cows in the CON and RDP group, respectively (P <0.01). When intake was expressed as g / kg of SBW, the cows of the RUP treatment maintained the highest (P <0.01) DMI 18.2 g / kg, followed by the cows of the RDP group consuming 15.7 g / kg of SBW, and of the group CON consuming 13.9 g / kg of SBW of MS. The cows in the CON group consumed ~ 15% OM in kg / d less compared to the RDP groups, and ~ 27% OM in kg / d less compared to the RUP group (P <0.01). As expected, the cows in the RUP supplemented group showed higher (P <0.01) CP intake (2.42 kg / d; 4.83 g / kg of SBW), compared to RDP and CON cows (1.86 kg / d; 3.67 g / kg of SBW; 1.47 kg / d; 3.07 g / kg of SBW), respectively. The NDF intake was higher (P ≤ 0.02) for RUP-treated cows, with RDP and CON cows consuming the least amounts. The RUP and RDP treatments obtained higher NFC intake compared to the CON group (P ≤ 0.03). The EE intake was higher in cows supplemented with RDP, followed by cows supplemented with RUP, and CON cows were those that consumed less EE both in kg / d and in g / kg of SBW (P ≤ 0.01). The total digestible nutrients (TDN) were affected by MN (P ≤ 0.01), in which the RUP group cows had an NDT intake of 2.02 kg / d higher than the CON group cows, and 1.08 kg / d higher than those of the CON group. RDP cows. The group supplemented with RUP when represented by g / kg of SBW, also obtained higher TDN intake (P ≤ 0.01) than the treatments RDP and CON (12.8 vs 10.6 and 9.11 g / kg of SBW), respectively. There was no effect of MN, CS, and

interaction between MN and CS on the intake of dry forage matter and the rate of forage replacement (P ≥ 0.26). The apparent digestibility coefficients demonstrated effects of MN, the NFC showed higher apparent digestibility coefficients for both supplemented groups RUP and RDP, with values 819 and 808 g / kg, respectively, in relation to the CON group with 744 g / kg (P = 0.05). In relation to EE, the RDP group had a higher digestibility coefficient, followed by RUP E CON (P <0.01). There was a trend towards higher apparent digestibility coefficients of DM (P = 0.06) and OM (P = 0.08) for the RUP group, followed by the RDP group, and with lower coefficients in the CON group.

6.2 Performance measurements

The **Table 3** shows the results of body weight, performance and adjustment of maternal components of non-supplemented (CON) or supplemented (RDP or RUP) cows and with male or female offspring during the second third of gestation (127 to 227 days), prepartum (282 days), mid-lactation (105 days) and weaning (200 days). There were no effects of MN, CS or MN×CS interaction on BWp, EBWp, EBWnp, BCS at day 127 of gestation ($P \geq 0.13$), except for the PREG which was ~0.89 kg heavier when the offspring was male ($P < 0.01$). As for the performance measurements of cows at the end of the period, that is, on the 227th day of gestation, greater BWp, EBWp and EBWnp were observed when fed with RDP or RUP, but supplementation with RUP still showed better results ($P \leq 0.04$). Additionally, in this same period of gestation, a tendency for PREG to be heavier when the offspring was male was observed ($P = 0.07$; Table 3). Body weights in cows at 282 days of gestation, during mid-lactation and weaning were not affected by MN, CS, or MN×CS interaction ($P \geq 0.23$).

Performance measures such as ADG, EBG and maternal EBG of cows were affected only by MN during the supplementation period ($P \leq 0.03$). Thus, cows fed with RUP showed additional ADG of ~0.36 and ~0.65 kg/d compared to cows fed with RDP and CON group, respectively ($P < 0.01$; **Figure 2**). EBG had the same pattem as ADG, where cows fed RUP 758 showed additional gains of ~0.33 and ~0.60 kg/d compared to RDP and CON groups, respectively ($P < 0.01$; Figure 2). As for maternal EBG, cows fed RUP had additional gains of ~0.26 and ~0.52 kg/d compared to cows that received the RDP and CON diet; the latter group showed losses of 0.06 kg/d (Figure 2).

Average daily gain measurements throughout the different stages of the production cycle were only observed effects of MN, where cows supplemented with RDP or RUP showed losses of 0.163 and 0.174 kg/day, while CON dams gained 0.360 kg/day since the end of the supplementation period to the prepartum ($P = 0.04$). On the other hand, in the lactation phase

between days 55 to 200, cows fed RUP during mid-gestation, had an ADG of 0.159 kg/day, while the CON and RDP groups lost 0.060 and 0.078 kg/day, respectively ($P < 0.01$; Table 3). The total performance of the cows, that is, from the beginning of the supplementation period until weaning, it was noticed that the dams fed with RUP were the only ones with positive values in the total ADG with 0.047 kg/day, while the treatments CON and RDP lost 0.019 and 0.057 kg/day, respectively ($P = 0.05$). As a result, the total gain was 17.0 kg in cows fed RUP, and losses of 8.08 and 21.0 kg in dams fed with CON and RDP diets, respectively ($P = 0.05$; Table 3).

Body weight of pre- and postpartum cows and calf health score at birth were not affected by MN, CS, nor MN×CS interaction ($P \geq 0.18$**;** Table 3), except, the calf's BW, where males had ~2.96 kg additional at birth ($P = 0.05$). Also, a trend was observed when the cows were fed RUP, where the calves had more weight at birth with ~3.19 and ~3.99 kg compared to the offspring from cows fed RDP and CON diet, respectively ($P = 0.09$; Table 3).

6.3 Dynamics of variation of maternal body tissues

Body composition measurements in cows were only affected by MN and CS for the variables of REA and REA adjusted to 100 kg BW, and MN×CS interaction for FTR during the supplementation period ($P < 0.01$; **Table 4**). Cows fed with RUP between 127 to 227 days of gestation, showed ~15.6 and ~24.0 cm^2 higher REA compared to cows fed with RDP and CON, respectively ($P < 0.01$). Similarly for REA adjusted to 100 kg BW, it was ~3.4 and ~5.1 cm^2 higher when the cows received RUP compared to RDP and CON groups, respectively ($P < 0.01$). The effects related to CS, cows with female offspring had ~16.8 and ~3.4 cm^2 greater REA and REA adjusted to 100 kg BW, respectively ($P < 0.01$; Table 4). The MN×CS interaction showed that feeding with RUP in cows with male offspring had a higher FTR at the end of the supplementation period ($P < 0.01$).

6.4 Uterine blood flow indexes

The uterine blood flow Doppler ultrasound measurements showed differences in the pulsatility index (PI; $P \leq 0.05$) and a tendency for resistance index (IR) related to MN during days 219 and 267 of gestation ($P = 0.07$ and 0.09, respectively) as shown in **Figure 3**. While for the effects of CS, only a trend towards PI at day 217 of gestation ($P < 0.08$). Cows fed with RDP or RUP had ~18 and ~24% less PI at day 217 of gestation compared to CON cows ($P = 0.02$; Figure 3), and for day 267 of gestation the PI was ~34 and ~26% lower in cows fed with

RDP or RUP compared to the CON group (P = 0.05). Effects related to CS, cows with male offspring had a trend of ~15% more PI at day 217 of gestation.

6.5 Maternal blood metabolites

The plasma urea concentration tended to be affected only by MN (P = 0.06), in which the cows in the CON group had a higher amount of circulating urea (16.9 mg / dL) at the end of the supplementation period (227 days of gestation) when compared to RDP and RUP cows, which presented 15.3 mg / dL and 15.1 mg / dL of urea circulating in the bloodstream, respectively (**Table 5**). The other blood parameters, such as glucose, insulin, beta-hydroxybutyrate, IGF-1, and NEFA, did not show effects of MN at the end of the supplementation period (P ≥ 0.31), nor in the prepartum period (P ≥ 0.41; Table 5). Fetal sex did not affect any blood parameters.

7. DISCUSSION

Brazilian beef cattle are distributed in a greater proportion in the Midwest region of the country, where the breeding herd is managed in extensive pasture systems (FERRAZ; DE FELÍCIO, 2010). Annually, most herds enter the breeding season in the spring and, therefore, the second trimester of pregnancy coincides with the dry season and low quality of forage (LEMOS et al., 2012), where the protein it is considered the main limiting nutrient (DELCURTO et al., 2000). In view of this, a way to get around this situation is through protein supplementation, which has the potential to improve the intake and digestibility of low-quality forages, leading to an increase in the flow of nutrients to the gestating cow and the fetus (MARQUEZ et al., 2017).

In our study, MN affected the intake of DM, OM, TDN, and nutrients, and showed superior results with the RUP treatment, except for the intake of EE. And this increase in the intake of nutrients due to the increase in protein supplementation has been previously reported by SOTELO et al., (2018). In our study, we obtained a higher intake of CP, and TDN, these results are in accordance with the results of MARQUEZ et el. (2017), in which a higher CP and TDN intake was observed in the cows of the MID group (P = 0.002), which were supplemented in the middle of gestation (30 to 180 days of gestation), when compared with treatments with no supplemented (CON) and supplemented late (LATE). It was expected that protein supplementation could improve the intake of low-quality forage (OWENS et al., 1991), but in our study, there were no influences of MN on the intake of forages, however, our result was also observed in LOPES et al. (2020). The higher intake of DM and nutrients generated a higher

energy intake, which may explain the results of an increase in BWp, EBWp, EBWnp, and ADG at the end of the supplementation period.

An adequate nutritional status of the cow during gestation can have a direct impact on the long-term performance of the offspring through developmental programming (FUNSTON et al., 2010; REYNOLDS et al., 2010). On the other hand, BW and to a greater extent BCS are related to the nutritional status of the cow during pregnancy and have a great influence on productive responses such as reproductive performance (COOKE et al., 2009). In this sense, the lack of differences in body weight variables (BWp, EBWp and EBWnp) and BSC before starting the supplementation period indicated that the cows entered the same nutritional status, considered adequate for the present study. It is likely that during early gestation, the supply and quality of forage were adequate to meet the MN requirements of the cows in this trial. On the other hand, in this same period of gestation, the forage intake is not yet impaired by the physical effect of the growth of the fetus and maternal tissues on the rumen size (FORBES, 1968), corroborated with the PREG estimate was lower at the beginning supplementation period (Table 3). Although supplementation with RDP or RUP did not increase from BCS at the end of the experimental period (day 227 of gestation), body weight gains, as well as, the performance measured through ADG was higher compared with the CON group. In contrast, weight loss per day in cows from RDP or RUP group in the prepartum (227 to 282 days of gestation) may be a response to supplement withdrawal, since in this period all cows returned to a medium-quality pasture, receiving mineral supplement. On the other hand, during lactation (55 to 200 days of lactation) the RUP group cows had weight gains per day, in contrast to the daily weight loss observed in the CON and RDP dams (Table 4). It is likely that supplementation with RUP, which is rich in arginine, during gestation may increase the clearance of uterine fluids in the first postpartum days, leading to an accelerated uterine involution and a faster return of rumen size, which can increase feed intake and consequently the performance in these animals. Studies in mares supplemented with arginine during pregnancy have shown that due to the increased uterine arterial blood flow, uterine fluids during the postpartum period decrease rapidly and accelerate uterine involution (MORTENSEN et al., 2011; KELLEY et al., 2013; MESA et al., 2015).

Studies with protein supplementation from mid-gestation have increased the cows' body weight gain while grazing low-quality forages. However, the results for BCS during this gestation period and the prepartum are inconsistent (LARSON et al., 2009; MULLINIKS et al., 2012; RODRIGUES et al., 2020; SOTELO et al., 2018; STALKER et al., 2006). Variations in the results of previous studies may be related to differences in the amount and type of protein

supplied, total protein intake in the diet and long-term nutritional antecedents of the cow herd (BROADHEAD et al., 2019).

On the other hand, the highest BW of calves at birth was only evidenced for cows supplemented with RUP, which shows that fetal development not only depends on the protein content provided in the diet, but also on specific amino acids in the supplement (VAUGHAN; FOWDEN, 2016; ZHANG et al., 2016). This was corroborated by Peine et al. (2018) in ewes that received three nutritional planes from the second trimester of gestation (CON, 100% of requirements; RES, 60% of requirements and RES-ARG, 60% of requirements plus supplement with rumen-protected arginine). Results from this study showed that although ewes supplemented with arginine were nutritionally restricted, newborn lambs were heavier compared to the RES group, and with a similar weight to the CON group. Arginine is a functional amino acid that, provided in the diet, fulfills various physiological functions through the metabolites nitric oxide, ornithine and proline resulting from the degradation of this organic compound (MANTA-VOGLI et al., 2020). Nitric oxide is a vasodilator and promoter of angiogenesis, while ornithine and proline are substrates for the synthesis of polyamines that are regulators of gene expression, protein synthesis and angiogenesis in the uterus and placenta (BAZER et al., 2015). Therefore, greater development and function of the placenta through supplementation with this amino acid in the diet results in an improvement in embryonic and fetal growth and survival (LIN et al., 2014).

Embryonic gender-specific difference may have differences in fetal growth through changes in gene expression (KWONG et al., 2006), DNA methylation pattern (DOBBS et al., 2013), and placental and fetal perfusion (PRIOR et al., 2013). The RI and PI rates are related to placental vascular development and fetal umbilical artery, and high values of these measurements indicate a lower blood flow (BOLLWEIN et al., 2002; PANARACE et al., 2006). Therefore, it is likely that the lowest BW at birth of female calves regardless of the MN plane used on the cows in this trial is related to a low blood flow evidenced with an increase in PI on day 227 of gestation (Figure 3). A study in humans indicated that the restriction on the growth of female neonates is due to a lower uteroplacental blood flow as a consequence of an increased production of renin-angiotensin that causes greater vasoconstriction (WANG et al., 2012). On the other hand, the literature indicates that the gender difference influences transcripts of genes related to glucose transport in the placenta, thus, it was found that the expression of *SCL2A1* (GLUT1) increased when the fetus was male. Additionally, the expression of *IGF1R* in the placenta increases during gestation when the offspring is male and remains constant in female offspring (O'CONNELL et al., 2013; ROSENFELD, 2015). This

demonstrates that probably the male calves in the present study received a greater amount of nutrients and, therefore, had a greater growth potential exhibited in BW at birth. In view of this, an increase in fetal growth demands a greater amount of nutrients; therefore, this may explain why the male pregnant cows had lower REA and REA adjusted to 100 kg of BW at the end of the experimental period, which may be due to a greater mobilization of lean tissue (Table 4).

The literature reports that MN restriction during mid- to late gestation reduces the weight of offspring at birth (GREENWOOD; CAFE, 2007; LARSON et al., 2009; RODRIGUES et al., 2020; STALKER et al., 2007). This may be a consequence of decreased expression of endothelial nitric oxide synthase in the placenta, which is the enzyme that catalyzes the production of nitric oxide from arginine (LEKATZ et al., 2010; VONNAHME; LEMLEY, 2012). The hypothesis of these authors, is that the nutritional restriction causes a greater vascular RI and PI, therefore, it affects the flow of nutrients and consequently the fetal growth. This was corroborated in our study, where CON cows had higher PI and RI values at day 217 and 267 of gestation (Figure 3) and, consequently, lower BW of calves at birth (Table 3). However, there were no differences for PI and RI in cows fed with RDP or RUP, because we expected that supplementation with RUP, which has arginine as one of the main amino acids, would increase the production of nitric oxide and thus increase blood flow, represented in lower values of PI and RI compared to the RDP treatment. Nevertheless, nitric oxide is not the only contributor to changes in blood flow in the placenta, also the vascular endothelial growth factor (VEGF) is a potent factor associated with fetal and placental vascular development (VONNAHME et al., 2015; VONNAHME et al., 2007). It is likely that other nutrients ingested in the diet with RDP may stimulate VEGF, but we do not know whether this may have happened in the present study.

Regarding the plasma concentrations of hormones and blood metabolites, the cows in the CON group tended to present a higher amount of circulating urea at the end of the supplementation period, compared to the RDP and RUP groups. This higher level of circulating urea in the CON group can be attributed to a greater mobilization of carcass amino acids to generate glucose in the liver through gluconeogenesis. During the mobilization of amino acids, these amino acids go through the deamination process, which generates the release of urea into the bloodstream to return to the rumen by recycling nitrogen or to be eliminated in the urine. Studies in rats have shown that protein metabolism has a biphasic course, with an anabolism phase during early gestation with deposition of muscle tissue in the dam, and a catabolic phase in late gestation, where the body's protein reserves are used to support the exponential growth of the fetus (NAISMITH; MORGAN, 1976; REMESAR et al., 1987). Additionally, under

conditions of MN restriction, the oxidation rate of amino acids increases to provide fuel for the placenta during gestation (MANTA-VOGLI et al., 2020). A study carried out in pregnant ewes showed that nutritional restriction increases the endogenous and exogenous amino acid deamination rate from maternal tissues for the synthesis and supply of glucose to the fetus (NOLAN; LENG, 1970). Additionally, in these same ewes, the urea excretion rate was lower, indicating greater body retention and recycling of urea to the digestive tract. Also, under conditions of MN restriction, fetal gluconeogenesis is induced and increased (BELL; EHRHARDT, 2002; LEURY et al., 1990), where glucose is replaced by amino acids as the main substrate (FAICHNEY; WHITE, 1987), resulting in an increase in fetal urea synthesis due to the high catabolism of amino acids (DONKIN; HAMMON, 2005; HODGSON et al., 1982). On the other hand, the placenta is an important site for the excretion of fetal urea, and this can clear high concentrations of urea in situations of feed restriction (SIMMONS et al., 1974). In this sense, the higher concentration of plasma urea observed in CON cows in the present study (Table 5) may be the result of a high clearance of fetal urea to maternal circulation, as well as an increase in the amino acid deamination of the muscle tissue that can be corroborated with the lowest BW, REA and REA adjusted to 100 kg BW in this group of dams (Tables 3 and 4).

No differences were found between treatments for NEFA and Betahydroxybutyrate. The NEFA are non-esterified fatty acids, which come from the mobilization of body fat, and Betahydroxybutyrate is the by-product of the oxidation of fatty acids in the liver for energy generation, and both are linked to the mobilization of adipose tissue in the carcass. Both parameters were not affected by MN, indicating that the mobilization of carcass fat between the three treatment groups was the same. These results can be reaffirmed by the results obtained in the carcass ultrasound in this study, in which there were also no differences in the parameters of fat thickness between the nutritional treatments. During the gestational period, the cow is unable to use long-chain fatty acids, which are the fatty acids derived from the mobilization of adipose tissue to nourish the placenta, so the pregnant cow generally uses little energy that comes from the mobilization of body fat. Sotelo et al. (2018) indicated that protein supplementation from mid-gestation does not promote changes on BCS, nor changes in plasma levels of β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA). Although free fatty acids (FFA) are essential for the development of fetal-placental tissues (HERRERA; ORTEGA-SENOVILLA, 2014), in animals with an epitheliochorial placenta like ruminants, these substances appear to be relatively poorly permeable (VAUGHAN; FOWDEN, 2016). On the other hand, studies in sheep with a mid- to late pregnancy have observed the presence of

transporters of short- or long-chain fatty acids in the placentomes, however, there was little evidence of the transfer of these fatty acids through the placenta (MA et al., 2011; ZHU et al., 2010). In addition, Carver and Hay Jr. (1995), demonstrated that sheep with prolonged hypoglycemia during the second half of gestation, FFA uptake was not evident, whereas, at BHB and acetoacetate were decreased. This shows that the contribution of fatty acids in obtaining energy for the fetus in situations of nutritional restriction is not significant (VAUGHAN; FOWDEN, 2016), and therefore can explain the lack of difference in the levels of BHB and NEFA, as well as the BCS of CON cows compared to the RDP and RUP groups in the present study (Tables 5 and 3).

However, despite the fact that pregnant cows have a lesser capacity to use fatty acids mobilized from adipose tissue, cows generally mobilize mainly amino acids from muscle tissue during pregnancy, to generate glucose in the liver through gluconeogenesis. Since glucose is the main source of energy that the pregnant uterus uses during pregnancy. Glucose is known to be present in low concentrations in the bloodstream of ruminants, especially in high roughage diets of low nutritional quality, as in this study. In these cases, the cow needs to use real protein to meet its nutritional needs, part of this protein comes from dietary protein, and the rest from the mobilization of amino acids in the maternal carcass. This was observed in our study since there was a difference between treatments for maternal muscle mass. And that the cows of the RUP group obtained better results of protein content in the carcass, for presenting a larger REA, indicating that they mobilized less amino acids of the carcass, in relation to the other experimental groups.

Contrary to what was expected, no difference was observed between the CON group and the supplemented groups for circulating glucose levels, and consequently also for insulin and IGF-1 in blood plasma. This fact can be explained by the fact that the cows in the CON group mobilized amino acids from the carcass to produce glucose via gluconeogenesis, and because of this there was no difference in blood glucose concentration between the cows in the CON group, and the RDP and RUP, and consequently, insulin and IGF-1 concentrations were also similar.

8. CONCLUSIONS

The supplementation of RUP for beef cows fed low- quality forage basal diets in the midgestation is able to reverse negative effects of the compromised maternal nutrition and bring beneficial effects on the performance and metabolism of the cows. Cows supplemented with

RUP shows high performance, DM intake, maternal body reserves conservation and blood parameters, in addition to not suffering the negative effects of poor diet quality on uterine blood supply, during the second half of gestation.

Table 1 – Estimated daily intake of DM and nutritional fractions, estimated nutritional composition of the diet, and the estimated meeting requirements of diets.

Item	Treatment		
	CON	**RDP**	**RUP**
Estimated daily intake of DM and nutritional fractions			
DM, kg	6.97	8.57	8.94
TDN, kg	3.89	4.88	5.45
OMDR, kg	3.59	4.53	4.53
CP, g	488	853	1340
RDP, g	382	666	666
RUP, g	106	187	674
RUPd, g	64	117	564
RDP/OMDR (g/kg)	107	147	147
Estimated nutritional composition of diets (%)			
TDN	55.79	62.59	67.03
OMDR	51.47	52.91	50.72
CP	7.00	9.95	14.99
RDP	5.48	7.77	7.45
RUP	1.52	2.18	7.54
RUPd	0.92	1.37	6.31
Estimated meeting requirements of diets (%*)			
TDN	83%	104%	116%
CP	59%	104%	163%
RDP	69%	120%	120%
RUP	39%	70%	251%

CON = control; RDP = Rumen degradable protein; RUP = Rumen undegradable protein; DM (dry matter), TDN (total digestible nutrients), OMDR (Digestible organic matter in the rumen), CP (crude protein), RDP (rumen degradable protein), RUP (rumen undegradable protein), RUPDd (digestible rumen undegradable protein).
*According to Gionbelli et al. (2016)

Table 2 – Intake and apparent total tract digestibility of dry matter and its fractions of beef cows supplemented either with RDP or RUP during midgestation.

Item	Maternal nutrition			Calf sex		SEM	P-value		
	CON	RDP	RUP	Female	Male		MN	CS	MN×CS
Total intake of dry matter and its fractions									
Dry matter, kg/day	6.67^{c}	7.91^{b}	9.08^{a}	7.96	7.81	0.436	<0.01	0.72	0.46
Dry matter, g/kg of SBW	13.9^{c}	15.7^{b}	18.2^{a}	15.7	16.1	0.86	<0.01	0.64	0.99
Organic matter, kg/day	6.33^{c}	7.37^{b}	8.63^{a}	7.51	7.37	0.418	<0.01	0.73	0.46
Organic matter, g/kg of SBW	13.2^{b}	14.6^{b}	17.2^{a}	14.8	15.2	0.82	<0.01	0.65	0.99
Crude protein, kg/day	1.47^{c}	1.86^{b}	2.42^{a}	1.98	1.85	0.138	<0.01	0.33	0.90
Crude protein, g/kg of SBW	3.07^{c}	3.67^{b}	4.83^{a}	3.92	3.79	0.252	<0.01	0.61	0.78
Neutral detergent fiber, kg/day	3.77^{b}	3.85^{b}	4.79^{a}	4.07	4.20	0.249	<0.01	0.63	0.22
Neutral detergent fiber, g/kg of SBW	7.89^{b}	7.64^{b}	9.62^{a}	8.06	8.70	0.533	0.02	0.24	0.81
Non-fibrous carbohydrates, kg/day	0.921^{b}	1.17^{a}	1.20^{a}	1.14	1.05	0.0824	0.01	0.30	0.86
Non-fibrous carbohydrates, g/kg of SBW	1.92^{b}	2.31^{a}	2.39^{a}	2.25	2.16	0.160	0.03	0.62	0.90
Ether extract, kg/day	0.211^{c}	0.398^{a}	0.270^{b}	0.302	0.284	0.0191	<0.01	0.24	0.81
Ether extract, g/kg of SBW	0.441^{c}	0.784^{a}	0.545^{b}	0.596	0.585	0.336	<0.01	0.75	0.88
Total digestible nutrients, kg/day	4.38^{c}	5.32^{b}	6.40^{a}	5.46	5.27	0.400	<0.01	0.66	0.45
Total digestible nutrients, g/kg of SBW	9.11^{b}	10.6^{b}	12.8^{a}	10.6	11.1	0.865	<0.01	0.62	0.97
Roughage intake as a function of cow's supplementation									
Roughage dry matter intake, kg/day	6.49	6.47	7.27	6.81	6.68	0.415	0.26	0.75	0.45
Roughage dry matter intake, g/kg of SBW	13.5	12.9	14.6	13.2	14.1	1.007	0.48	0.38	0.74
Roughage substitution rate (g/kg)	-	74.2	-60.3	8.92	0.319	74.33	0.43	0.90	0.96
Apparent total tract digestibility									
Dry matter, g/kg	623^{B}	662^{A}	682^{A}	675	648	21.2	0.06	0.54	0.70
Organic matter, g/kg	642^{B}	676^{AB}	702^{A}	692	665	20.4	0.08	0.53	0.66
Crude protein, g/kg	826	809	837	837	816	13.2	0.25	0.22	0.87
Neutral detergente fiber, g/kg	534	544	607	582	555	31.2	0.19	0.94	0.38
Non-fibrous carbohydrates, g/kg	744^{b}	808^{a}	819^{a}	797	788	26.7	0.05	0.72	0.97
Ether extract, g/kg	792^{b}	887^{a}	810^{b}	829	830	12.0	<0.01	0.79	0.86
Total digestible nutrientes, g/kg	653^{B}	678^{AB}	701^{A}	693	670	18.0	0.10	0.57	0.61

CON = control; RDP = Rumen degradable protein; RUP = Rumen undegradable protein; MN = Maternal nutrition; CS = Calf sex.

$^{a\text{-}c}$Means followed by different superscripts are different (P<0.05).

$^{A\text{-}B}$Means followed by different superscripts are different (P<0.10).

Table 3 – Performance measurements of beef cows supplemented either with RDP or RUP during midgestation.

Item	Maternal nutrition			Calf sex		SEM	P-value		
	CON	RDP	RUP	Female	Male		MN	CS	MN×CS
Measurements at 127 days of gestation (initial weights)									
Cow BWp, kg	507	550	526	535	520	17.4	0.16	0.41	0.25
Cow EBWp, kg	451	490	469	477	462	16.1	0.16	0.39	0.27
Cow EBWnp, kg	444	484	460	471	455	16.0	0.16	0.36	0.21
PREG, kg	6.68	6.70	6.64	6.21^{b}	7.14^{a}	0.185	0.97	<0.01	0.66
Cow BCS	5.28	5.76	5.33	5.55	5.37	0.223	0.21	0.46	0.12
Measurements at 227 days of gestation (end of supplementation period)									
Cow BWp, kg	546^{b}	574^{ab}	611^{a}	571	583	16.2	0.01	0.51	0.63
Cow EBWp, kg	489^{b}	514^{ab}	550^{a}	512	523	15.1	0.01	0.50	0.61
Cow EBWnp, kg	457^{b}	482^{ab}	510^{a}	478	488	15.4	0.03	0.58	0.81
PREG, kg	31.1	32.6	33.4	30.3^{B}	34.6^{A}	2.41	0.65	0.06	0.52
Cow BCS	5.34	5.91	5.84	5.78	5.62	0.254	0.18	0.56	0.66
Measurements at 282 days of gestation (prepartum) and lactation (up to weaning)									
Cow BWp at 282 days of gestation, kg	578	580	606	578	598	14.3	0.28	0.23	0.62
Cow BW at 105 days of lactation, kg	512	502	512	504	513	16.6	0.89	0.58	0.99
Cow BW at 200 days of lactation (weaning), kg	518	503	539	509	530	19.4	0.33	0.29	0.62
Maternal average daily gain (ADG) and total gain during different phases of the production cycle									
Supplementation period ADG (127 to 227 DG), kg/day	0.182^{b}	0.470^{ab}	0.837^{a}	0.440	0.547	0.161	0.01	0.56	0.67
Prepartum ADG (227 to 282 DG), kg/day	0.360^{a}	-0.163^{b}	-0.174^{b}	-0.099	0.114	0.171	0.04	0.31	0.65
Lactation ADG (55 to 200 days of lactation), kg/day	-0.060^{b}	-0.078^{b}	0.159^{a}	0.001	0.012	0.055	<0.01	0.85	0.28
TOTAL ADG (127 DG to 200 days of lactation), kg/day	-0.019^{b}	-0.057^{b}	0.047^{a}	-0.022	0.003	0.033	0.05	0.41	0.28
TOTAL gain (127 DG to 200 days of lactation), kg	-8.08^{b}	-21.0^{b}	17.0^{a}	-8.85	0.75	12.1	0.05	0.41	0.28
Calf birth weight and health score									
Calf birth weight, kg	30.3^{B}	31.1^{B}	34.3^{A}	30.4^{B}	33.3^{A}	1.33	0.08	0.05	0.51
Calf birth health score (1-5 scale)	3.24	3.81	3.69	3.71	3.45	0.305	0.19	0.38	0.55

CON = control; RDP = Rumen degradable protein; RUP = Rumen undegradable protein; MN = Maternal nutrition; CS = Calf sex.

$^{a-b}$Means followed by different superscripts are different (P<0.05). $^{A-B}$Means followed by different superscripts are different (P<0.10).

Table 4 – Dynamics of variation of maternal body tissues of beef cows supplemented either with RDP or RUP during midgestation.

Item	Maternal nutrition			Calf sex		SEM	P-value		
	CON	RDP	RUP	Female	Male		MN	CS	MN×CS
Measurements at 223 days of gestation (end of supplementation period)									
Rib eye area (REA), cm²	87.7^{b}	96.2^{b}	112^{a}	107^{a}	90.1^{b}	3.63	<0.01	<0.01	0.11
Rib eye area (REA), cm²/100kg	17.0^{b}	18.7^{b}	22.1^{a}	21.0^{a}	17.6^{b}	0.779	<0.01	<0.01	0.12
Length of the rump muscle (LRM), cm	101	103	118	110	104	6.95	0.13	0.44	0.23
Backfat thickness (BFT), mm	2.11	2.07	2.62	2.57^{A}	1.97^{B}	0.302	0.34	0.08	0.90
Fat thickness on the rump (FTR), mm	2.08	2.16	2.34	2.05	2.35	0.244	0.68	0.26	<0.01
Measurements at 270 days of gestation (pre-partum)									
Rib eye area (REA), cm²	77.7	70.6	87.6	80.1	77.3	12.3	0.62	0.83	0.67
Rib eye area (REA), cm²/100kg	13.6	12.1	15.2	13.8	13.4	1.92	0.53	0.84	0.31
Length of the rump muscle (LRM), cm	85.0	88.6	106	89.6	96.7	9.66	0.21	0.49	0.23
Backfat thickness (BFT), mm	2.30	1.90	2.24	2.30	2.00	0.365	0.66	0.43	0.70
Fat thickness on the rump (FTR), mm	2.49	2.28	2.21	2.38	2.27	0.334	0.73	0.77	0.86

CON = control; RDP = Rumen degradable protein; RUP = Rumen undegradable protein; MN = Maternal nutrition; CS = Calf sex.

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

$^{A-B}$Means followed by different superscripts are different (P<0.10).

Table 5 – Blood metabolites of beef cows supplemented either with RDP or RUP during midgestation.

Item	Maternal nutrition			SEM	P-value
	CON	RDP	RUP		
Measurements at 227 days of gestation (end of supplementation period)					
Glucose, mg/dL	88.0	87.2	87.0	1.49	0.87
Insulin, µUI/ml	26.7	37.1	27.0	5.29	0.31
Betahydroxybutyrate, mmol/L	0.147	0.158	0.204	0.0369	0.50
IGF-1, ng/ml	7.89	8.29	7.07	1.018	0.69
NEFA, µmol/L	86.4	103.0	88.6	9.18	0.34
Urea, mg/dL	16.9^{A}	15.3^{B}	15.1^{B}	0.61	0.06
Measurements at 265 days of gestation (pre-partum)					
Glucose, mg/dL	75.3	75.3	74.3	1.30	0.81
Insulin, µUI/ml	4.46	4.83	4.59	0.466	0.85
Betahydroxybutyrate, mmol/L	0.278	0.347	0.348	0.0437	0.41
IGF-1, ng/ml	7.58	8.98	8.05	1.489	0.69

CON = control; RDP = Rumen degradable protein; RUP = Rumen undegradable protein.

$^{A-B}$Means followed by different superscripts are different ($P<0.10$).

Figure 1 – Summary of the experimental procedures and measurement timepoints.

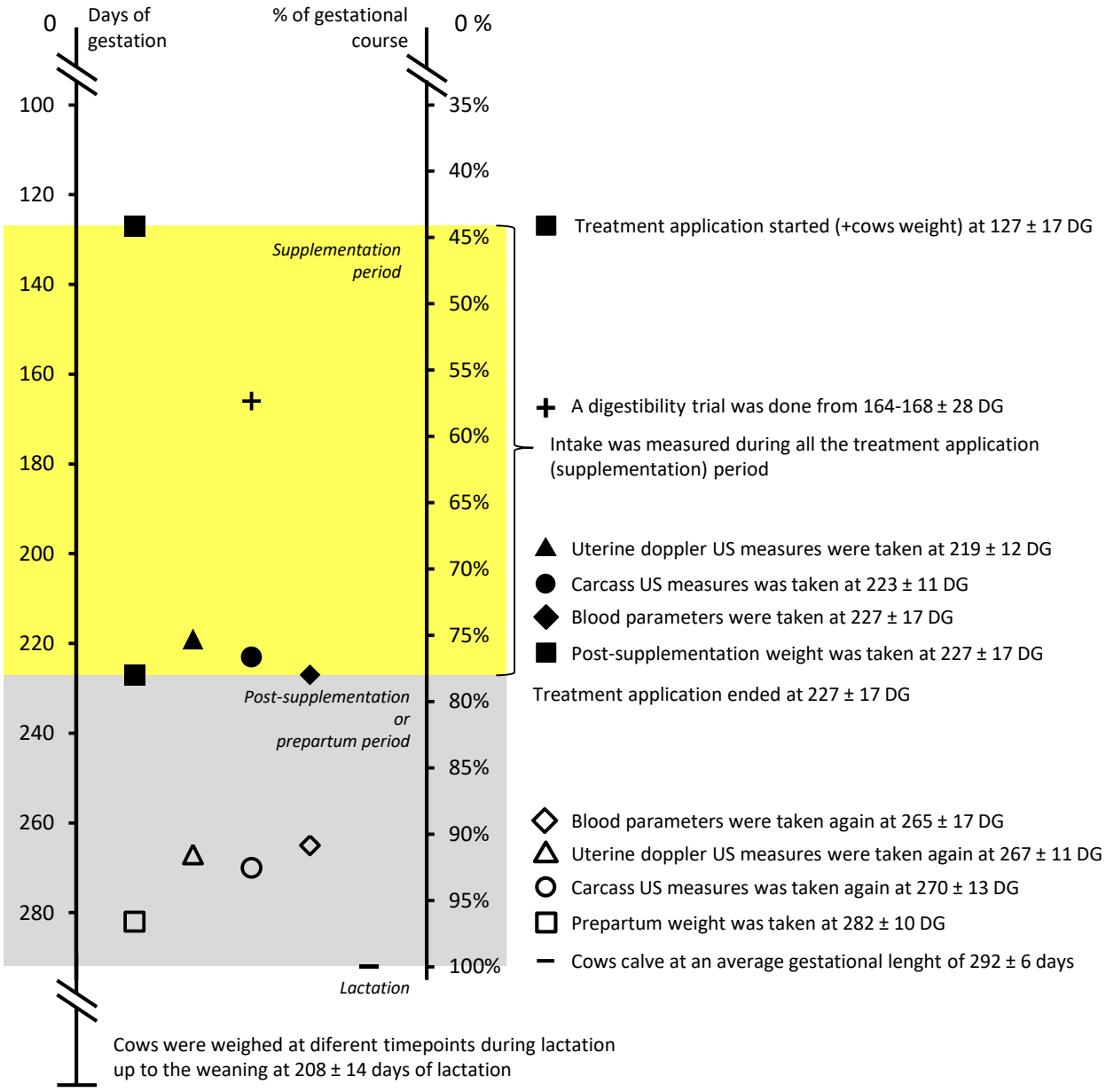
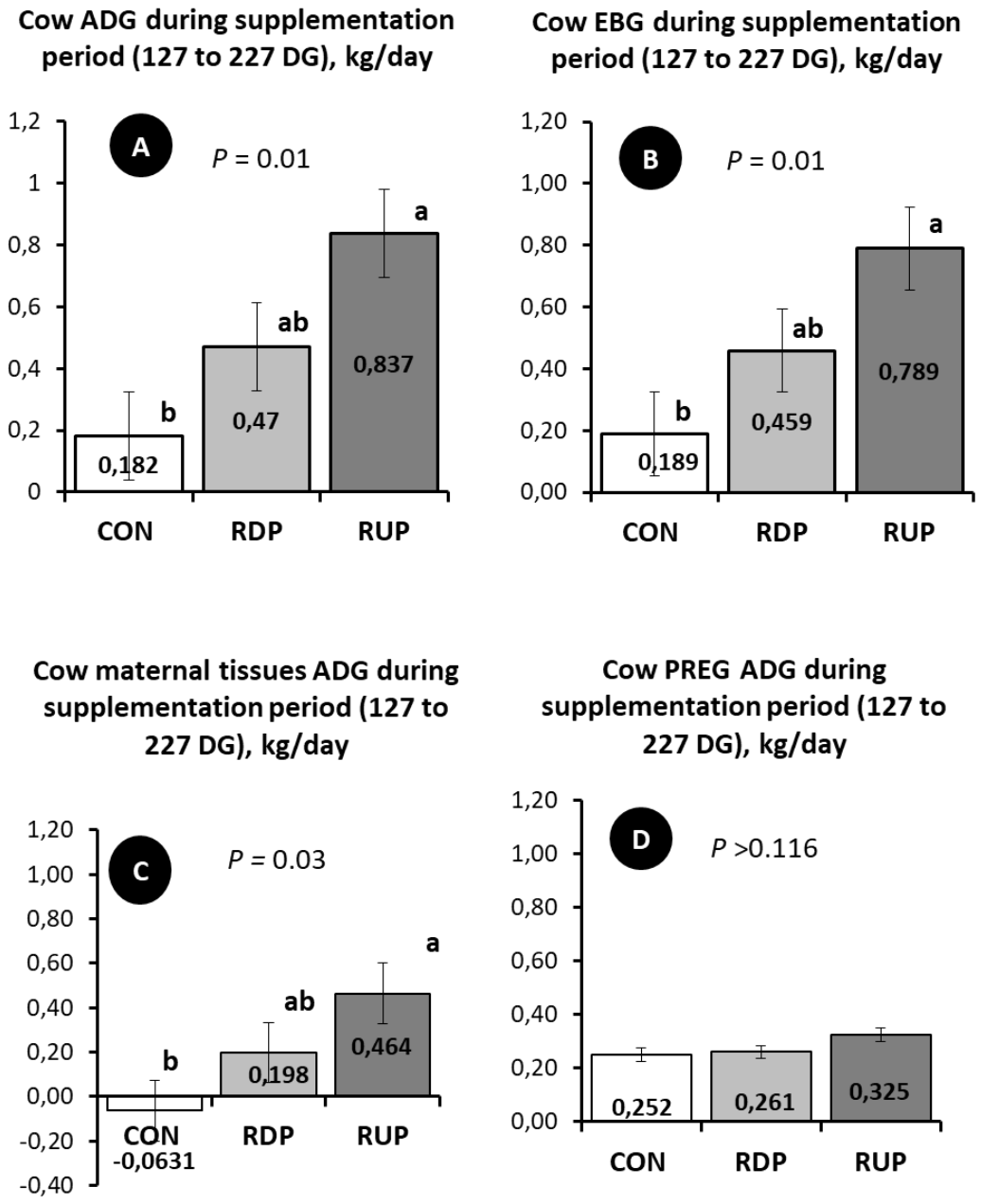
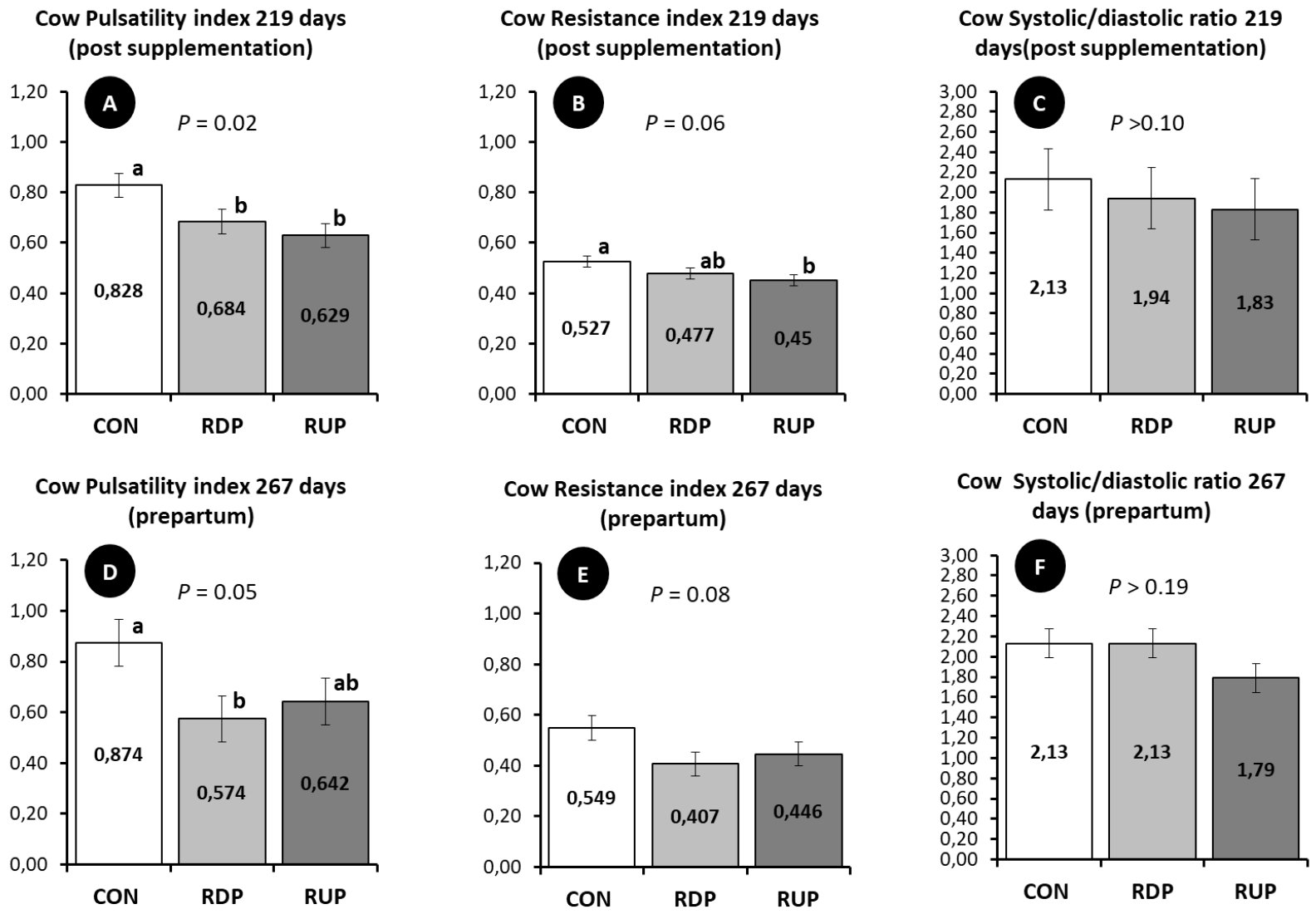


Figure 2 – Maternal weight gain and pregnant compounds weight gain between 127 and 227 days of gestation of beef cows supplemented either with RDP or RUP.



[a-b]Means followed by a different superscript are different (P<0.05)

Figure 3 – Uterine blood flow measurements of beef cows supplemented either with RDP or RUP during midgestation.



[a-b]Means followed by a different superscript are different (P<0.05)

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