KAROLINA BATISTA NASCIMENTO

EFFECTS OF CRUDE PROTEIN SUPPLEMENTATION DURING BEEF COW'S MID-GESTATION ON THE OFFSPRING PERFORMANCE, PHYSIOLOGY AND METABOLISM

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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.

Advisor

Dr. Mateus Pies Gionbelli

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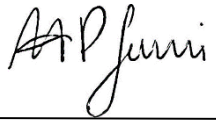

Prof. Dr. Mateus Pies Gionbelli

Advisor

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Ao meu maior incentivador e amigo, meu pai Geovaine.
Ao meu exemplo de dedicação e amor, minha mãe Joana.
Dedico.

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ABSTRACT

This work aimed to quantify the effects of protein supplementation during the mid-gestation of beef cows on the performance, metabolism, and physiology of the offspring. This study was divided into two repetitions and comprising 24 and 19 Tabapuã beef cows in each period, respectively. From 102 ± 5 to 208 ± 6 days of gestation, cows were distributed in a completely randomized design in a 2 × 2 factorial arrangements with the following treatments: two conditions of maternal nutritional management (MN) and offspring sex (OS). Control cows (**CON**; *n* = 24) were fed a basal diet with corn silage and sugarcane bagasse; while the supplemented group (**SUP**; *n* = 19) received 3.5 g/kg of body weight (BW) of a supplement with 40% crude protein (CP) per day. The offspring were evaluated from birth to 445 days of age in the following phases: cow-calf (0 – 210 days), background (255 – 320 days), growing 1 (321 – 381 days), and growing 2 (382 – 445 days) phases. Statistical differences were considered when $P < 0.10$. The SUP offspring was 3.8 kg; 16.4 kg and 30.8 kg heavier at birth, weaning, and at 445 days of age, respectively ($P \leq 0.049$). SUP calves had greater morphometric measurements at birth and weaning. SUP calves had 26% greater muscle fiber area compared to CON animals at 30 days of age ($P = 0.007$). An MN × OS interaction for regulation of *MyoD* and *FN1* genes ($P \leq 0.057$) was verified in *the Longissimus thoracis* muscle of the offspring at 7 days. Maternal supplementation also increased *mTOR* expression ($P = 0.056$) at 7 days. At 445 days of age, the SUP offspring showed lower expression of the *CPT2* gene ($P = 0.037$), while the *ACACA* and *LPL* genes were more expressed ($P \leq 0.090$). SUP-females at 445 days of age showed higher expression of *PPARG*, *FABP4*, and *SCD1* genes ($P < 0.001$). Maternal protein restriction exhibited sex-dependent responses on insulin ($P = 0.076$) and IGF-1 ($P = 0.002$) levels at weaning. However, these differences in hormone levels disappeared at 445 days of age ($P > 0.10$). The offspring from SUP dams consumed 11.3%; 9.2% and 7.9% of dry matter (DM) additional at weaning, growing 1 phase, and in the entire confinement period respectively ($P \leq 0.096$). The feed efficiency for weight gain was not affected by the MN ($P > 0.10$). There was an MN × OS interaction on the OM ($P = 0.070$) digestibility at weaning; on DM and NDF ($P \leq 0.086$) at background phase; and on DM, OM, CP, NDF, and TDN ($P \leq 0.089$) at growing 2. Overall in the growing 2 phase, the nutrients digestibility was reduced in SUP-males. The ingestive behavior of the offspring was affected by maternal nutrition. In conclusion, prenatal protein supplementation improves the offspring's performance,

favors intramuscular fat deposition, and increases the offspring's intake pattern, but reduces the nutrients digestibility of high-energy diets in males.

Keywords: Fetal programming. Gene expression. Gestational nutrition. Sexual dimorphism. Zebu.

RESUMO

Este trabalho teve como objetivo quantificar os efeitos da suplementação protéica durante o terço médio da gestação de vacas de corte sobre o desempenho, metabolismo e fisiologia da progênie. O estudo foi dividido em duas repetições, e abrangeu 24 e 19 vacas de corte Tabapuã em cada período, respectivamente. Entre 102 ± 5 a 208 ± 6 dias de gestação, as vacas foram distribuídas em um delineamento inteiramente casualizado em esquema fatorial 2 × 2 com os seguintes tratamentos: duas condições de manejo nutricional materno (MN) e sexo da progênie (SP). Vacas controle (**CON**; *n* = 24) foram alimentadas com uma dieta basal, composta por silagem de milho e bagaço de cana-de-açúcar; enquanto o grupo suplementado (**SUP**; *n* = 19) recebeu um suplemento contendo 40% de proteína bruta (PB) ao nível de 3,5 g/ kg de peso corporal (PC). A progênie foi avaliada do nascimento aos 445 dias de idade, nas seguintes fases: cria (0 - 210 dias), sequestro (255 - 320 dias), crescimento 1 (321 - 381 dias) e crescimento 2 (382 - 445 dias). Diferenças estatísticas foram consideradas quando $P < 0,10$. Animais SUP foram 3,8 kg; 16,4 kg e 30,8 kg mais pesado ao nascer, desmame e 445 dias de idade, respectivamente ($P \leq 0,049$). Bezerros SUP tiveram maiores medidas morfométricas ao nascimento e desmame. Animais SUP apresentaram área de fibra muscular 26% maior que animais CON aos 30 dias de idade ($P = 0,007$). Houve interação NM × SP para regulação dos genes *MyoD* e *FN1* ($P \leq 0,057$) no *Longissimus thoracis* aos 7 dias. A suplementação materna também aumentou a expressão do gene *mTOR* ($P = 0,056$) aos 7 dias. Aos 445 dias de idade, animais SUP apresentaram menor expressão do gene *CPT2* ($P = 0,037$), mas maior expressão dos genes *ACACA* e *LPL* ($P \leq 0,090$). Fêmeas-SUP apresentaram maior expressão dos genes *PPARG*, *FABP4* e *SCD1* ($P < 0,001$) aos 445 dias de idade. A restrição protéica materna exibiu respostas dependentes do sexo nos níveis de insulina ($P = 0,076$) e IGF-1 ($P = 0,002$) ao desmame. No entanto, essas diferenças nos níveis hormonais desapareceram aos 445 dias de idade ($P > 0,10$). Filhos das mães SUP consumiram 11,3%; 9,2% e 7,9% de matéria seca (MS) adicional ao desmame, na fase de crescimento 1, e em todo o período de confinamento respectivamente ($P \leq 0,096$). A eficiência alimentar para ganho de peso não foi afetada pela NM ($P > 0,10$). Houve interação NM × SP sobre digestibilidade da MO (P = 0,070) ao desmame; da MS e FDN ($P \leq 0,086$) na fase de sequestro; e da MS, MO, PB, FDN e NDT ($P \leq 0,089$) na fase de crescimento 2. No geral, na fase de crescimento 2, a digestibilidade dos nutrientes foi reduzida em machos-SUP. O comportamento ingestivo

da prole foi afetado pela nutrição materna. Em conclusão, a suplementação pré-natal de proteína melhora o desempenho da prole, favorece a deposição de gordura intramuscular e aumenta o consumo, mas reduz a digestibilidade dos nutrientes para dietas de alta energia em machos.

Palavras-chave: Dimorfismo sexual. Expressão gênica. Nutrição gestacional. Programação fetal. Zebu.

ABBREVIATION LIST

ADG	Average daily gain
CEBP	CCAAT enhancer-binding protein
CON	Offspring from unsupplemented cows during mid-gestation
CP	Crude protein
DMI	Dry matter intake
FABP4	Adipocyte-type fatty acid-binding protein
FAPs	Fibro-adipogenic progenitor cells
FE	Feed efficiency
FP	Fecal production
GEN	Dam's index of genetic merit expected for growth traits
GH	Growth hormone
IGF-1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor 1 receptor
LMA	Longissimus muscle area
MHC	Myosin heavy chain
MN	Maternal nutrition
MRFs	Myogenic regulatory factors
mRNA	Messenger ribonucleic acid
MSC	Mesenchymal stem cells
MyoD	Myogenic differentiation 1
MyoG	Myogenin
NDF	Neutral detergent fiber
NDF*i*	Indigestible neutral detergent fiber
OM	Organic matter
OS	Offspring sex
PPARG	Peroxisome proliferator-activated receptor γ
RML	Rump muscle length
SFT	Subcutaneous fat thickness
SUP	Offspring from supplemented cows during mid-gestation
TDN	Total digestible nutrients
TGF-β	Transforming growth factor β superfamily
WNT	Wingless and Int
ZFP423	Zinc finger protein 423

Inform Graphic.

Created by Karolina Batista Nascimento and supervised by Mateus Pies Gionbelli

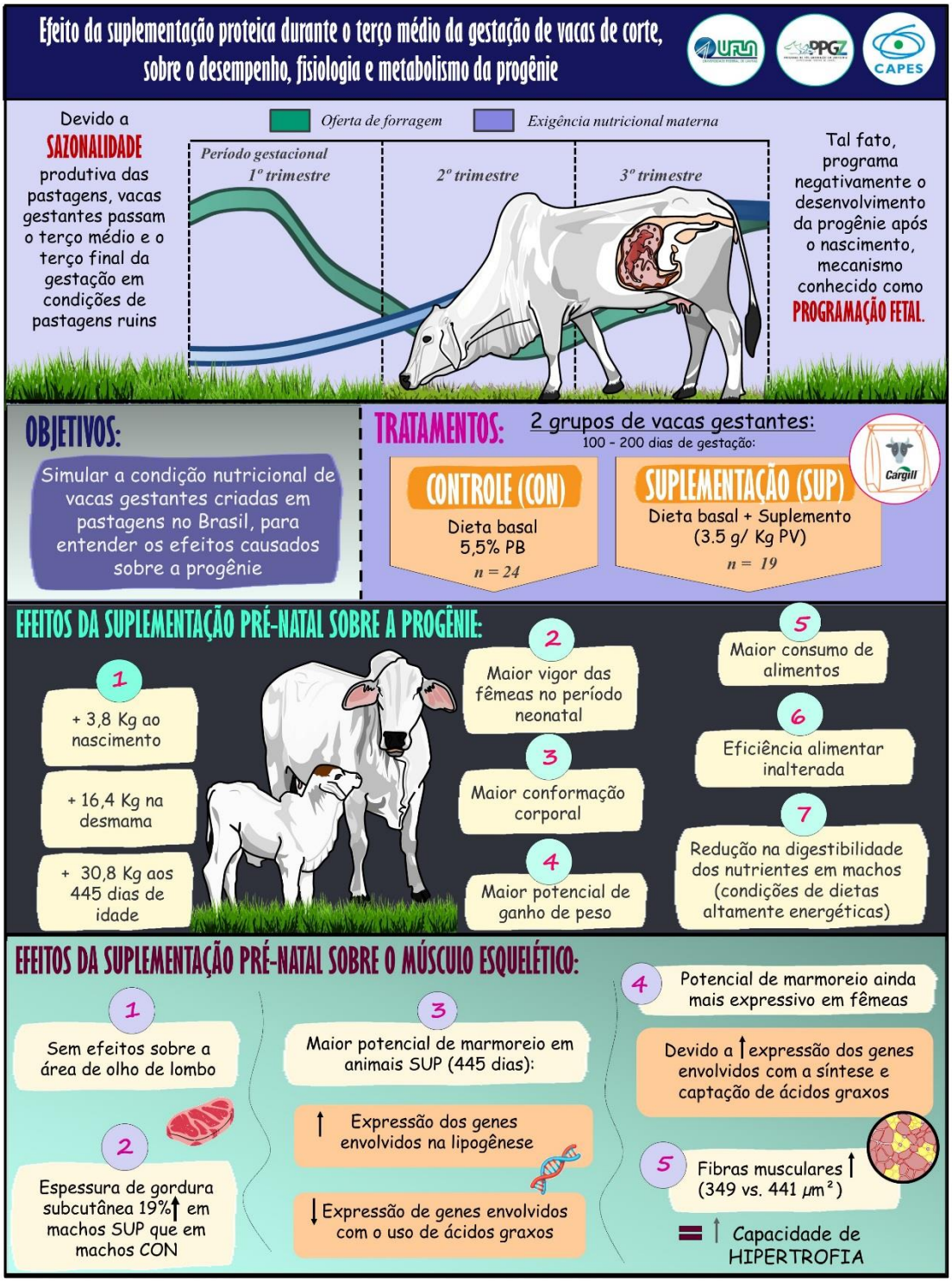

TABLE OF CONTENTS

FIRST SECTION

1. INTRODUCTION

In Brazil, tropical grasses are the main source for beef cattle production due to their higher capacity of dry matter production in tropical conditions. However, in most of the country (tropical environment), the climatic factors (rainfall, temperature, and luminosity) markedly affect the physiological and morphogenic forage processes, promoting seasonality in the net forage production and its nutritive value (Reis et al., 2013). In the dry season, the forage plant decreases its development rate and has a high senescence rate, which results in a quantitative and qualitative decrease of forage production and its nutritive value. The decrease in pasture quality during the dry season is characterized by increases in the lignification (Detmann et al., 2017), decrease in the soluble sugars and starches (Leng et al., 1990), but mainly by the low crude protein content, which corresponds to values around 5% to 8% in this period (Kabeya 2009; Rocha 2013; Lima 2018).

Due to the higher quality and availability of forage during the rainy season, the breeding and calving seasons were established at this stage in Brazil (Gionbelli et al., 2018). This practice is a strategy to improve the reproductive parameters and to attend the greater nutrients requirements for lactation after calving. Nevertheless, with this management, part of gestation, which corresponds to mid and part of late gestation, inevitably overlaps with the dry period (Rodrigues et al., 2020). These conditions not only impair fetal growth, due to the lack of nitrogen and carbon available for growth but also causes severe consequences on the offspring trails at postnatal life, through fetal programming mechanisms (Hoffman et al., 2017). The fetal programming effects can remodel the mammal's trajectory through different manners, like changes in gene and/or protein expression (Du et al., 2009), by epigenetics modifications (Du et al., 2010b), metabolic alterations (Hoffman et al., 2014), by changes in organs functions (Keomanivong et al., 2015; Zhou et al.; 2019), among others. All these changes reflect on characteristics involved with the animal's production, compromising livestock systems.

Among these effects, it is well established that dam nutritional planes during mid-gestation can affect the offspring's skeletal muscle development, since about 95% of muscle fibers are formed during gestation (Zhou et al., 2021). The myofibers formation is stratified into primary and secondary myogenesis, intensified during the embryonic and

fetal periods, respectively (Bonnet et al., 2010). The mid-third of gestation is the most critical period in the development of secondary fetal myogenesis (Du et al., 2009), and coincides with the nutritional shortages period faced by pregnant cow herds in tropical conditions. Thus, is necessary to study strategies to promote nutritional corrections during gestation, such as protein supplementation, to alleviate possible negative effects on the gain potential of farm animals that compose the tropical herds.

Also, there is a little-explored knowledge gap concerning the effects of fetal programming on the offspring's intake characteristics; ingestive behavior; feed efficiency, and nutrients digestibility at post-natal life. These effects require a deep approach to provide a better understanding of how animals exposed to prenatal sub-nutrition respond to the dietetic factors. Previous studies demonstrated that maternal nutrition plan during gestation causes structural, hormonal, and epigenetic changes in the hypothalamus axis of the offspring, leading to appetite deregulation (Muhlhausler et al.,2006; Stevens et al., 2010; Long et al., 2011; Prezotto et al., 2018; Smith et al., 2018). Nevertheless, this evidence was not extensively validated during the offspring's post-natal life. So far, only one study has evaluated the effects of maternal nutrition on the parameters of nutrients digestibility of the offspring (Cruz et al., 2019). Therefore, further studies are needed to understand the impacts that maternal nutrition can promote on the use of nutrients.

Moreover, there is also an interaction between maternal nutrition plan and offspring sex. However, this relationship needs to be better studied. Findings from Gionbelli et al. (2018) showed evidence of sexual dimorphism during the fetal stage, indicating a faster skeletal muscle development in males than in females, and differences in the mRNA expression of myogenic, adipogenic, and fibrogenic markers. Furthermore, Costa et al. (2021) suggested that maternal nutrition and offspring sex interactions can cause long-term changes in the offspring's skeletal muscle characteristics. Herein, the offspring's sex must be considered over fetal programming studies.

In this sense, this research was done to simulate a real condition faced by pregnant cows raised in the tropical condition, fed with a poor diet during the mid-gestation, proposing a nutritional correction performed through protein supplementation to improve the offspring development. Therefore, this study aimed to:

(1) Evaluate changes in the body weight; body composition; and morphometric measures promoted by maternal nutrition;

(2) Identify the mechanisms that interfere with the changes in the skeletal muscle tissue of the offspring through analysis of gene expression and histological assay;

(3) Investigate endocrine changes in the offspring induced by prenatal nutrition;

(4) Evaluate the effect of maternal nutrition during pregnancy on feed intake, nutrient digestibility, ingestive behavior, and feeding efficiency of the offspring;

(5) Verify with fetal programming effects on all target variables described above are sex-dependent;

The knowledge of this information would provide a solid basis for the real impact that extensive management in tropical conditions during gestation has on beef cattle productivity, while making clear the mechanisms that govern such changes in the long term.

LITERATURE REVIEW

FETAL PROGRAMMING IN BEEF CATTLE

2.1. Fetal programming of muscle, adipose and connective tissues

2.1.1. Gene regulation of myogenesis, adipogenesis, and fibrogenesis

Myogenesis during the pre-natal life is a crucial event because there is no new muscle fibers formation in the postnatal life (Zhu et al., 2004). In this sense, the majority of muscle growth occurs by hypertrophy in post-natal life, through the increase in the muscle fiber diameter (Zhu et al., 2006).

The schematic representation of myogenesis is described in **Figure 1**. During fetal life, myogenesis is regulated by different signals from the neural tube and notochord, as well as by the molecules *Wingless and Int* (*Wnt*) and *Sonic hedgehog* (*Shh*), which induce the expression of the myogenic regulatory factors (MRF's) (Chargé & Rudnicki, 2004). The MRF's are transcriptional factors with a basic Helix-Loop-Helix (bHLH) domain (Carlsen & Gundersen, 2000), which identify and bind (as dimers) to the E-box sequence present in the promoter region of the muscle-specific genes in DNA, activating its expression (Blackwell & Weintraub, 1990). *WNT* (which is *β-catenin* dependent) and the *SHH* acts through *Pax 3/7* - transcriptional factors members of the paired box protein family, and through glioma-associated oncogenes (*Gli*) expressions, which induces the *MyF5* and *MyoD* expressions (Du et al., 2009).

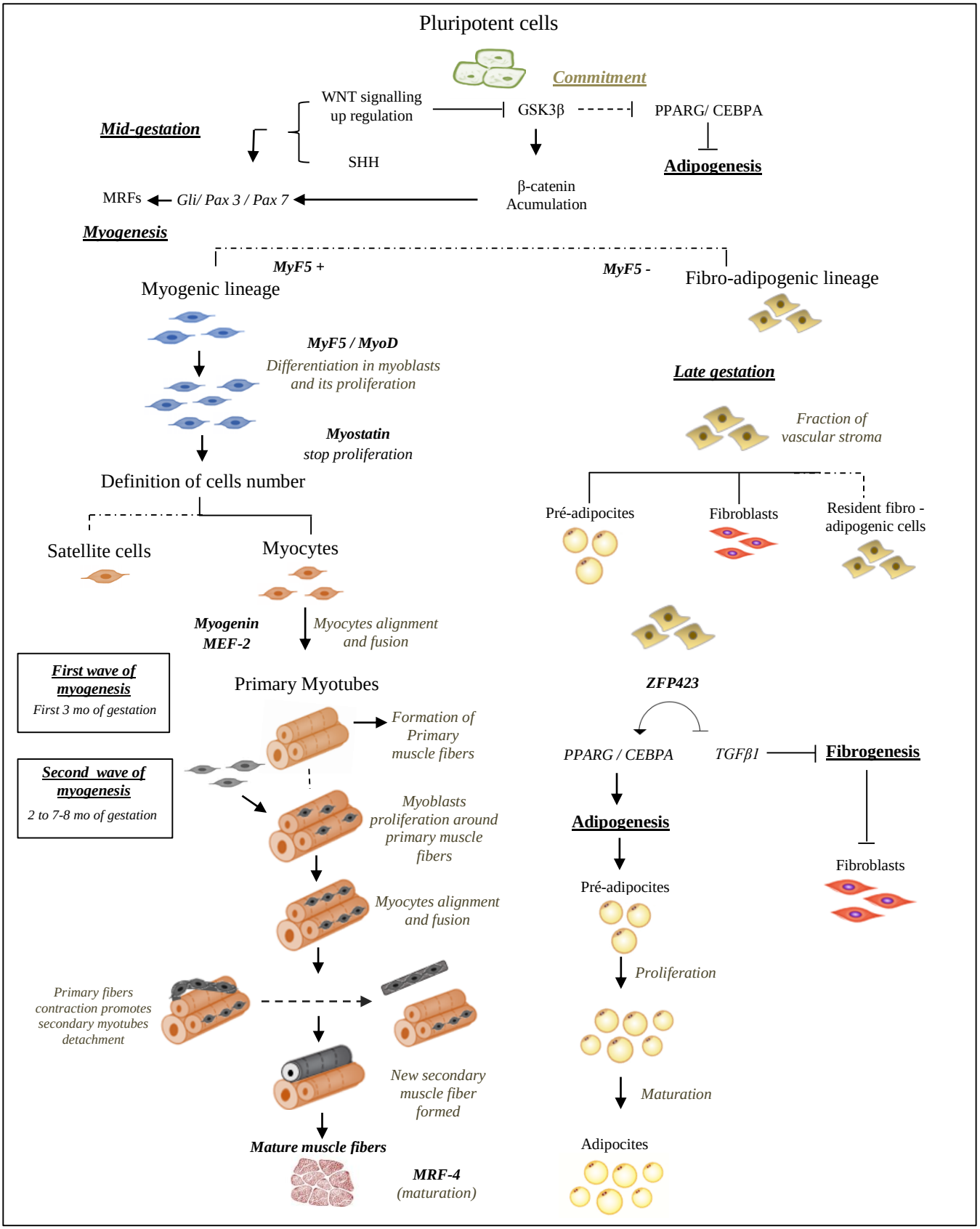

Figure 1. Relationship between myogenesis, adipogenesis, and fibrogenesis. Elaborated considering the works from Christodoulides et al. (2009); Du et al. (2010a); Hui 2012; Huang et al., (2012); Du et al. (2015) and Ladeira et al. (2016).

MyF5 and *MyoD* are the first MRFs expressed for the commitment of progenitor cells to the myogenic lineage (Silva & Carvalho, 2007), with *MyF5* expressed early compared to *MyoD* (Hui, 2012). Cells positive for *MyF5*, which are highly specific to committed skeletal myoblastic cells, give rise to myoblasts, while cells negative for this MRF may originate others cells types, as white adipoblasts, chondrocytes, and fibroblasts (Ladeira et al., 2016).

Myoblasts are fusiform cells with one central nucleus with the ability to enter in cell cycle and increase in number (Hui, 2012). Thus, myoblasts proliferate and then they undergo modifications to acquire the level of specialization necessary to form a mature muscle cell (Hui, 2012). This step of proliferation that precedes the differentiation, is very important for meat production, once more cells can originate more muscle fiber in the fetal stage (Du et al., 2015). At this point, *myostatin*, which is a member of the transforming growth factor β superfamily (*TGFβ*), acts as an inhibitor of myogenic cell proliferation (McPherron & Lee, 1997). *Myostatin* stops myoblast proliferation through a negative regulation in the progression from G1 to S phase of the cell cycle, and also stimulates cell differentiation through *myogenin* (*MyoG*) regulation, beyond to protect undifferentiated myoblasts from cell apoptosis (Joulia et al., 2003).

After proliferation, the myoblast undergoes modifications to acquire the level of specialization necessary to form a mature muscle cell (Hui, 2012), which are regulated by *myogenin* and *MRF-4*, considered secondary MRFs (Jennings et al., 2016). *Myogenin* regulates the formation of myotubes, acting directly on the differentiation process, while *MRF-4* seems to be more related to myotubes maturation (Zhang et al., 1998). Additionally, transcription factors, members of the *MEF-2* family, are essential for myoblasts differentiation (Lu et al., 2000). The myogenic proteins with bHLH domain have to combine with *MEF-2* to bind in with A/T-rich sequence in muscle gene regulatory sites, thus MRFs and *MEF-2* cooperate to activate the muscle gene expression. There is also positive feedback among them to increase and stabilize their expression in a proportion that myoblast enters in the differentiation pathway (Lu et al., 2000).

After exiting the cell cycle, myoblasts align and fuse to form multinucleated embryonic myotubes (Jennings et al., 2016). These structures are formed between 1-3 months' post-conception in primary myogenesis and act as a scaffold for the formation of fetal myotubes from the second myogenic wave, which occurs around 3-7 months after conception (Du et al., 2009).

The secondary myogenesis occurs from the proliferation and fusion of fetal myoblasts located close to the embryonic myotubes, generating myotube clusters with the same basal lamina (Ross et al., 1987). The fetal myotubes, while being elongated, remain connected to the embryonic myotubes only for a short period and later become independent (Biressi et al., 2007), obtaining their basal lamina (Ross et al., 1987).

Myoblasts that do not enter into the differentiation process remain quiescent and are located between the sarcolemma and the basement membrane of mature muscle fibers (Morgan & Partridge, 2003) at the G0 phase of the cell cycle (Yan et al., 2013). These mononucleate myogenic cells are denominated satellite cells and have an important role in muscle hypertrophy and regeneration after injury in postnatal life (Asakura et al., 2001). In hypertrophy processes, satellite cells proliferate and fuse with pre-existing muscle fibers to donate their nuclei and synthesize new proteins (Silva & Carvalho, 2007). In conditions of muscle damage, satellite cells activate, proliferate, and fuse to form new myotubes that will replace the affected muscle tissue (Morgan & Partridge, 2003).

Another important event for the meat market that begins during pre-natal life is adipogenesis (**Figure 1**). This process refers to the formation of adipocytes that starts with the commitment of progenitor cells into pre-adipocytes (determination), which proliferate and differentiate into cells capable of accumulating lipids (Tseng et al., 2010). The *peroxisome proliferator-activated receptor γ* (*PPARG*) and *CCAAT enhancer-binding protein α* (*CEBPA*) are considered as the main transcription factors that regulate adipogenesis (Christodoulides et al., 2009). In early adipogenesis, activation of *PPARG* and *CEBPA* is induced by the expression of *the CCAAT enhancer-binding protein β* (*CEBPB)* and δ (*CEBPD*) factors (Lee et al., 2019). After this event, *PPARG* and *CEBPA* remain expressed through a feedback process that subsequently induces the expression of genes related to adipocyte differentiation and maturation (Christodoulides et al., 2009).

The mesenchymal stem cells (MSC) from mesoderm can give rise to different cell types, including cells of muscle, adipose, and connective tissue (Ladeira et al., 2016). Adipogenesis starts in the mid-third gestation, overlapping with secondary myogenesis in this phase (Du et al., 2009). The balance between myogenesis and adipogenesis is controlled by *the WNT/β-catenin* complex (Christodoulides et al., 2009). Activation of this pathway favors the differentiation of MSCs into myoblasts, and a reduction in the differentiation of these cells into pre-adipocytes, due to the inhibition of *PPARG* and *CEBPA* expression (Christodoulides et al., 2009).

The down-regulation of *PPARG* occurs through the ability of *WNT* to inhibit the synthesis of *glycogen kinase-3 β* (*GSK-β*) that activates *PPARG* (Du et al., 2009). Also, *SHH* signaling favors the myogenic lineages and damage adipogenesis (Du et al., 2010). Seems that *SHH* induces the expression of *GATA2* and *COUP-TFII*, which dry the *PPARG* stock and bind to the promoters of *PPARG* and *C/EBPA*, downregulating their expression, respectively (Du et al., 2010a).

Fetal adipogenesis is intensified in the last third of gestation, and unlike myogenesis, adipocyte proliferation still occurs after birth (Du et al., 2013). The formation of different fat deposits starts at different points over gestation (Du et al., 2013). The visceral adipocytes are formed first in the mid-gestation, followed by intensification of subcutaneous adipogenesis in mid-to-late pregnancy. Concerning intramuscular adipocytes, they are formed at the end of gestation, but they continue with hyperplasia until approximately 250 days of postnatal life in cattle (Du et al., 2013).

The formation of intramuscular fat deposits, which is considered a specialized connective tissue, and fibrogenesis are correlated events that derive from a unique pool of fibro-adipogenic progenitor cells (FAP) present in the stromal-vascular fraction within skeletal muscle (Du et al., 2013). Although fibrogenesis happens in the post-natal period, this process is more pronounced during the late period of pregnancy, when primordial perimysium and epimysium of muscle bundles are formed, overlapping with intramuscular adipocytes formation (Ladeira et al., 2016), to give rise to the basic structure of skeletal muscle.

Intramuscular adipogenesis and connective tissue formation are competitive processes (**Figure 1**), and therefore an opportunity to improve meat quality is to maximize intramuscular fat deposition, reducing connective tissue synthesis (Du et al., 2013). The development of adipogenic and fibrogenic lineages within the vascular stroma are initially defined by the expression of the *zinc finger protein 423* (*ZFP423*) and the transforming growth factor (*TGF-β*), respectively (Du et al., 2015). The *ZFP423* expression can favor the intramuscular adipocyte formation through the increase in *PPARG* and *CEBPA* expressions, beyond this capacity of inhibiting partially the *TGF-β1*, and thus fibrogenesis (Huang et al., 2012). Moreover, *TGF-β1* may affect adipogenesis, preventing adipogenic differentiation by *C/EBPA* and *PPARG* inhibition (Rodrigues et al., 2020).

Therefore, strategies able to affect the genes expressions aiming to enhance myogenesis and intramuscular adipogenesis during specific windows of prenatal

development can be a tool to produce animals with high gain potential and with good trails of commercial interest.

2.1.2. Effects of maternal nutrition on skeletal muscle

Maternal nutrition during pregnancy can affect myogenesis, adipogenesis, and fibrogenesis, promoting long-lasting impacts on farm animals' productivity and meat quality. Which response is dependent on factors like the type, window of fetal development, and duration of nutrient restriction or overfeeding during gestation (Hoffman et al., 2017).

Some studies available in the scientific literature involving changes in muscle markers are summarized in **Table 1**. As can be verified, changes may occur at a molecular level, without result in phenotypic changes. In this sense, Jennings et al. (2016) evaluating the effects of energy levels [72, 87 or 146% of net energy for maintenance (NEm) requirements] during early to mid-gestation, did not found effects of maternal nutrition on muscle histology characteristics (fiber area, diameter, and number), despite the effects on mRNA expressions in muscle markers (**Table 1**). In this study, there was an up-regulation of *myogenin* in LOW fetus (72% of NEm) compared with INT (87% of NEm), indicating a potential reduction in myoblasts differentiation, followed by an earlier fusion of these cells in the fetus exposed to sub-nutrition. There was also a greater expression of *PREF-1* in the HIGH fetus (146% of NEm) compared with INT (87% of NEm). These findings indicating a greater number of preadipocytes, as a consequence of a delay in its differentiation, once *PREF-1* inhibited *C/EBPA* and *PPARG* expressions. In contrast, *C/EBPB* presented a greater expression in the LOW fetus, indicating an earlier differentiation (and thus a lower proliferation) of preadipocytes in mature adipocytes. Thus, low energy levels may damage muscle fiber formation and that high energy levels may be used to boost intramuscular adipogenesis.

In tropical breeding systems, it is more likely to find situations of underfeeding of herds. Marquez et al. (2017) evaluated the effects of protein supplementation in pregnant dams grazing low-quality forages. The authors found a greater number of muscle fibers and ribeye area in calves from supplemented dams from mid-to-late gestation, although the bodyweight of the offspring was not affected (**Table 1**).

Table 1. Effects of prenatal nutrition on gene expression and characteristics of skeletal muscle in cattle.

Reference	Treatment	Period of gestation	Altered genes expressions	Genes expressions without alterations	Phenotypic change
Jennings et al. (2016)	LOW = 72% of NEm requirements INT = 87% of NEm requirements HIGH = 146% of NEm requirements	85 to 180 d	*up-regulation for HIGH*: *PREF-1*; *μ-Calpain*; *IGF-II**; *up-regulation for LOW*: *CEBPB*; *FAS**; *MyoG*; *IGF-II**	*PPARG*; *C/EBPA*; *SCD*; *MyoD; MyF5*; *MRF4*; *Myostatin*; *m-Calpain*; *Calpastatin*; *IGF-I*	Fetal weight; Fiber area (μm²); fiber diameter (μm) and fiber number in *Longissimus dorsi* and *Semitendinosus* without effects
Paradis et al. (2017)	HIGH = 140% of ME requirements LOW = 85% of ME requirements	147 to 247 d	*up-regulation for LOW: MyoD*; *MyoG*; *PPARG*; *IGFI*; *IGF1R*; *IGF2R; INSR*	*IGF2*; *MEF2A*; *SRF*	Fetal weight and crown-rump length, without effects
Marquez et al. (2017)	UNS = Unsupplemented with CP MID = Supplemented with CP LATE = Supplemented with CP	MID = 30 to 180 d LATE = 181 to 281 d	*PPARA* = greater for MID, lower for LATE; *FGF2* = greater for MID, lower for UNS and LATE	*TGFβ1*; *COL1A1*; *FGF2R1*; *COL3A3*; *PPARG*; *MCDA*; *UCP3*; *PPKAA2*; *HADH*; *MYH7*; *PDK4*; *PGC1α*; *CPT1*; *ZFP423*; *C/EBPA*	Without effects on the fiber area (μm²); BW at birth and weaning. Greater fat thickness (mm), for LATE. Greater number of muscle fibers for MID and lower for UNS. Greater ribeye area (cm²) for MID and LATE
Gionbelli et al. (2018)[1]	CON = 100% of NRC requirements ON = 190% of NRC requirements	60 to 139, 199, 241 or 268 d	*CTNNB*; *ZFP423* and *PPARG* = greater expression at 139 d for ON; *FN1* = greater for ON	*MyoD*; *MyoG*; *C/EBPA*; *COL1A1*; *COL3A1*; *TGFβ1*	Crude protein content in skeletal muscle (g/kg) greater for ON. Fat content of skeletal muscle; intramuscular collagen deposition (percentage) and the number of myocytes without effects
Rodrigues et al. (2020)	UNS = Unsupplemented with CP SUP = Supplemented with CP	124 to 270 d	*Birth*: *PPARG, WNT10B, CD36, TGFβ1* = greater for SUP; *11 days of age*: *C/EBPA*; *FABP4* = lower for SUP; *Weaning*: *PPARG**, *ZFP423*; *TGFβ1* = greater for SUP	*β-catenin*; *COL3A1*; *FN1*; *MyoD*; *MyoG*; *IGFR1*; *mTOR*; *MyHC1*; *MyHC2a*; *MyHC2x*	Greater BW at birth for SUP. No effects on BW at 120 and weaning.
Costa et al. (2021)	UNS = Unsupplemented with CP SUP = Supplemented with CP	100 to 200 d	*30 d: PAX7** and *MHC2X* = greater for UNS. 450d: without effects. Interactions maternal nutrition × offspring sex: 30d: *ZFP423*; FN*; PDGFRα*; MHC1*; MHC2X*; LOX*. 450 d: *FN**;*TGFβ**;*MMP2**; *MHC1**;*COL1*; *COL3; MHC2X*	*30 d: C/EBPA*; *PPARG*; *TGFβ*; *COL1A1*; *COL3*; *P4Ha1*; *TIMP1*; *TIMP2*; *MHC1*; *MHC2A*; *450 d*: *ZFP423*; *C/EBPA*; *PPARG*; *LOX*; *P4Ha1*; *TIMP1*; *TIMP2*; *PAX7*; *PDGFFRα*; *MHC1*; *MHC2A*; *MHC2X*	Lower muscle fiber number; increase in collagen content in the skeletal muscle and transitory changes in muscle fiber metabolism

ABREVIATIONS: ***18S*** = 18 S ribosomal; ***CD36*** = Cluster of differentiation 36; ***C/EBPA*** = Enhancer-binding protein α; ***C/EBPB*** = Enhancer-binding protein β; ***COL1A1*** =collagen type I, α 1; receptor 1; ***COL3A3*** = collagen type III, α 3; ***CPT1*** = carnitine palmitoyltransferase 1; ***CTNNB1*** = Cadherin-associated protein, beta-1; ***FABP4*** = Adipocyte-type fatty acid-binding protein; **FAS** = fatty acid synthase; ***FGF2*** =fibroblast growth factor 2; ***FGF2R1*** =fibroblast growth factor 2, ***FN1*** = Fibronectin 1; ***HADH*** =hydroxyacyl-CoA dehydrogenase; **IGF1 =** Insulin Like Growth Factor 1; ***IGF1R*** = Insulin Like Growth Factor 1 Receptor; ***IGF2 =*** Insulin Like Growth Factor 2; ***IGF2R =*** Insulin Like Growth Factor 2 Receptor; ***INSR***= Insulin Receptor; ***LOX*** = Lysyl oxidase; ***MCAD*** = medium-chain acyl-CoA dehydrogenase; **MEF2A =** Myocyte Enhancer Factor 2A; ***MMP2*** = Matrix metalloproteinase-2; ***MRF4*** = myogenic regulatory factor-4; ***mTOR*** = Mammalian target of rapamycin; ***MyoD*** = Myogenic Differentiation 1; ***MyoG*** = Myogenin; ***MYH7*** = myosin heavy chain 7; ***MyHC1*** = Myosin heavy chain type I; ***MyHC2a*** = Myosin heavy chain type IIa; ***MyHC2x*** = Myosin heavy chain type IIx; ***P4Ha1*** = Prolyl 4-Hydroxylase Subunit Alpha 1; ***PDK4*** =pyruvate dehydrogenase kinase 4; ***Pax7*** = Paired box 7; ***PDGFRa*** = Platelet-derived growth factor receptor A; ***PGC1α*** =peroxisome proliferator-activated receptor γ coactivator 1 α; ***PPARα*** = Peroxysome proliferator-activated receptor α; ***PPARG*** = Peroxysome proliferator-activated receptor γ; ***PRKAA2*** = protein kinase AMP - activated catalytic subunit α 2; ***PREF-1*** = preadipocyte factor-1; ***SCD*** = stearoyl-CoA desaturase; ***SRF =*** Serum Response Factor; ***TGFβ1*** = Transforming growth factor- β1; ***UCP3*** = uncoupling protein 3; ***TIMP1/2*** = TIMP metallopeptidase inhibitor 1 or 2; ***ZFP423*** = Zinc finger protein 423; ***WNT10B*** = wingless-type MMTV integration site family member 10B. [1]Only the effects of maternal nutrition (N) and of interactions between N × S (fetal sex); N × D (days of gestation); and N × S × D were presented. *Tendency = $P < 0.10$.

These authors also found a greater mRNA expression of *fibroblast growth factor 2* (*FGF2*), which is a marker for skeletal muscle hypertrophy (related with proliferation capacity of satellite cells) in calves born from dams supplemented during mid-gestation, despite the lack of effects on the fiber area between treatments. There were no effects of maternal nutrition plane on the mRNA expression of any adipogenic and fibrogenic marker evaluated. Thus, another important point evidenced in this work regarding fetal programming, is that protein supplementation during late gestation does not cause the same effect of overnutrition on adipogenesis. In this sense, as related by authors, seems that a significant dietary status is necessary to improve the offspring's intramuscular fat.

Rodrigues et al. (2020) investigated the effects of protein supplementation during mid-to-late gestation in grazing beef cows with moderate nutritional restriction on performance and molecular markers in offspring (**Table 1**). The protein supplementation of the dams did not affect the expression of myogenic genes or muscle fiber type. However, a downregulation of C/*EBPA* and *FABP4* genes was observed in 11-day-old calves from supplemented dams. These findings indicate that the offspring from non-supplemented cows showed an early adipogenic differentiation, therefore, this may impair the proliferation of intramuscular adipocytes.

Furthermore, in complementary research of the present study (Costa et al., 2021; **Table 1**), using part of our experimental units and the same treatments, a protein restriction from 100 to 200 days of gestation promoted a lower muscle fiber number in the offspring at the postnatal life. Protein restriction during mid-gestation also increased the collagen content in the skeletal muscle of the offspring, despite the lack of difference in the expression of fibrogenic genes. Thus, these findings demonstrating an adaptive response acquired in the offspring as a consequence of the prenatal sub-nutrition. It was also observed in this same work a tendency to increase the expression of *MHCIIx*, which is a biomarker for type II muscle fibers in 30-day-old calves from non-supplemented cows. However, no differences were found at 445 days of age in these animals, showing the plasticity of skeletal muscle to environmental conditions during postnatal life.

In conclusion, maternal nutrition may change the offspring's developmental trajectory, bringing persistent effects in the long term. Therefore, prenatal nutrition should not be neglected.

2.2. Maternal nutrition and epigenetics effects

Fetal development can be affected by epigenetic changes. Epigenetics refers to mitotically and/or meiotically heritable changes in gene function that are not explained by changes in DNA sequence (Thompson et al., 2020) and epigenetic marks act as a memory of environment exposure (Batistel et al., 2019). There are different mechanisms by which epigenetic can alter gene expression, that including DNA methylation; histone and chromatin modifications; and non-coding RNA (Sinclair et al., 2016).

DNA methylation is the major epigenetic modification in eukaryotes, that consists in the addition of methyl groups to cytosines within the cytosine–phosphorous–guanine dinucleotides islands (CpG - "p" refers to the phosphodiester bond that connects the bases "C" and "G") (Elolimy et al., 2019), which are unequal distributed throughout the genome dinucleotide sequence (Bianchi et al., 2017). DNA methylation occurs through DNA methyltransferase (Dnmt) (Burdge et al., 2007) and is dependent on the availability of methyl groups from *S*-adenosylmethionine (SAM) of one-carbon metabolism (Clare et al., 2019). This condition may alter the gene expression blocking the access of transcription factors and the binding of RNA polymerase II to the promoter's sites, silencing the methylated allele (Oliveira, 2012).

Histones are proteins involved with chromatin condensation that also act modulating the expressions of several genes that participate in different signaling pathways (Bianchi et al., 2017). DNA methylation affects the recruitment of the histone deacetylases (HDACs), proteins that cause chromatin compaction, and repress transcription (Du et al., 2010b). The chromatin condenses because the HDACs remove the acetyl group of lysine residues within histone tails, which are responsible for the neutralization of positive charges, by the chromatin relaxation, and thus by the access facilitation of the transcription factors to target genes (Bianchi et al., 2017).

Histones can also be methylated by the methyl group addiction to its tails (amino acid residues), through the action of histone-methyltransferase, causing transcriptional alteration (Du et al., 2010b). Other histones modifications include phosphorylation, deamination, ubiquitylation, sumoylation, and ADP ribosylation, which can also affect the chromatin structure, promoting alteration in gene expression (Bannister & Kouzarides, 2011).

Also, another epigenetic mechanism consists of about 22 nucleotides non-coding RNA molecules, called MicroRNAs (miRNAs), that can regulate the gene expression at posttranscriptional levels (Penso-Dolfin et al., 2018). They promote gene silencing by

544 binding to the complementary RNA molecules which result in target mRNA degradation
545 or protein translation attenuation (Wang et al., 2018).

546 The epigenetics modifications in the offspring due to maternal nutrition during
547 pregnancy need to better elucidate in farm animals, but there is some evidence in the
548 scientific literature showing its ability to modulate its characteristics. Over-nutrition for
549 example has epigenetics potential. Maternal obesity can cause epigenetic modifications
550 by decreasing the histone modification H3K27me3 leading to a reduction of DNA
551 methylation in *ZFP423* promoter, promoting a greater expression of *ZFP423*, and
552 enhance the adipogenic capacity of progenitor cells in offspring adipose tissue (Yang et
553 al. 2013).

554 Paradis et al. (2017), evaluated the effects of a moderate feed restriction compared
555 with an over nutrition (85% vs. 140% of NRC requirements) during mid-to-late gestation
556 on pregnant beef cows. They verified that animals from dams who faced nutrients insults
557 presented a delay in muscle development and thus on myogenesis to adipogenesis
558 transition, suggesting differences in the methylation level and miRNA expression as
559 responsible by the differences observed in *longissimus dorsi* muscle of offspring. A study
560 with a 50% restriction of nutritional requirements recommended by the NRC in pregnant
561 ewes resulted in global DNA hypomethylation in the liver of male lambs at 10 months of
562 age (Chadio et al., 2017).

563 As DNA methylation depends on methyl donors, the provision of dietetics
564 constituents that participate in the one-carbon metabolism, like methionine, folate, B12,
565 and choline during fetal development may be associated with the epigenome and
566 metabolic phenotype of offspring too (Clare et al., 2019). Research with small ruminants
567 showed that male offspring from ewes under nutrient restriction and supplemented with
568 methionine and B12 vitamin before conception and early gestation lead to epigenetic
569 changes to DNA methylation and modification of their phenotype, as they presented
570 obesity, insulin resistance, and hypertension (Sinclair et al., 2007). Other recent work
571 using rumen-protected methionine supplementation during the last 28 d of pregnancy in
572 dairy cows, indicated that maternal nutrition alters the global DNA methylation in bovine
573 placenta (Batistel et al., 2019). In the same way, other works also demonstrated the methyl
574 donor's capacity in different species to promote epigenetics effects (Jin et al., 2018; Li et
575 al., 2018; Cai et al., 2017; Hou et al., 2018; Burdge et al., 2007).

576 Therefore, it is clear that the organism's development is highly susceptible to
577 different nutritional levels and specifics substances present in the maternal diet, with

epigenetic modifications being the modulator of many productive responses obtained in livestock.

2.3. Maternal nutrition, placental development, and fetal organogenesis

Maternal nutritional deficiency has negative effects on fetal development and has long-term negative impacts on the offspring's performance (Hoffman et al., 2017). During the early phase of fetal development, placental growth, differentiation, and vascularization occur, as well as fetal organogenesis, all of which are critical events for normal conceptus development (Funston et al., 2010).

Most placental growth occurs during early gestation and some in mid-gestation (Redmer et al., 2004). The placenta plays a role in fetal development because it is used for the exchange of metabolites, water, heat, and respiratory gases, being also a site for hormone synthesis and secretion and extensive interconversion of nutrients (NRC, 2000). Moreover, the growth of uteroplacental vascular beds also occurs in the first half of gestation, which is important to support the increase in transplacental exchanges that occur in the last half of gestation (Reynolds & Redmer, 1995).

Conditions of low nutrient availability during gestation may reduce fetal growth and affect placental functions, resulting in an intrauterine growth restriction condition (Zhang et al., 2016). Yet, it is possible that a fetus of well-fed dams present nutrient deprivation when placental size or function is inadequate, and also that fetuses from undernourished dams do not present difficulty to meet its nutrient demand due to the occurrence of compensatory mechanisms in placental systems.

Consistent with this, McCrabb et al. (1992) found that lean pregnancy ewes under moderate restriction during early- to mid-gestation (when placental size are raising), presented reduced placental size without promoting changes in the number of individual placentomes or fetal weight and dimensions. In contrast, Zhang et al. (2016) not found this compensatory mechanism in placental size and function on animals under the poor maternal nutritional plane in early- to mid-gestation, in which both, the fetus development and the functional capacity of the placenta were affected by maternal nutritional plane (100% *vs*. 50% of NRC requirements). Fetus from undernourished ewes had lower weight and crown-rump length tended to have lower total placentome weight and number and had a significantly lower relation fetal weight: placentome weight, which is an index of placental efficiency. Also in undernourished animals, there were lower concentrations of total polyamines putrescine, spermidine, and spermine, (which are critical mediators of

placental growth and angiogenesis and key regulators of cell function and DNA and protein synthesis in the conceptus) in the serum from the uterine artery and fetal umbilical vein, as well as amniotic and allantoic fluids. Although compensatory mechanisms related to placental functioning may occur in conditions of nutritional scarcity during pregnancy in an attempt to amortize the effects on fetal development, there are no guarantees that they will be able to avoid the negative effects on the developing fetus.

Simultaneously with placental development, fetal organogenesis also occurs during the early stages of gestation (Vonnahme, 2007), and several studies had shown that maternal nutrition during gestation can affect the offspring's organ development and function. Zhang et al. (2016) found a low weight of the pancreas, stomach, liver, spleen, kidneys, lungs, and other fetal organs from dams with poor nutritional planes during gestation. However, the effects of maternal nutrition do not appear to be the same for all organs. McMillen et al. (2001) findings indicating that as the brain is considered an extremely vital organ, its mass is preserved in situations of high nutritional stress for the fetus, at the cost of 'less important organs', such as the liver and kidney. This evidence is inserted in the 'thrifty phenotype theory' which proposes that nutrient scarcity during specifics windows of development leads to a nutrient reallocation to favor critical organs for immediate survival at the expense of other organs secondarily necessary, causing failures in the adult life (Hales & Barker, 2001).

Maternal nutrition affects organ function. A study with the ruminants demonstrated that offspring of nutritionally restricted ewes from 50 to 130 days of gestation had a small pancreatic mass, which compromised their enzymatic capacity (Keomanivong et al., 2015). Also, Zhou et al. (2019) found the effects of poor maternal nutrition on the hepatic metabolic profile and the metabolic pathway in ruminant fetuses. The literature has also shown that some adaptive responses on offspring organs can also occur as an attempt to ameliorate the fetal conditions of development. Duarte et al. (2013) found greater weight and length of the small intestine, and intestinal villi in fetuses born from restricted dams, indicating that maternal nutritional restriction can increase the surface of the small intestine, and consequently the absorptive capacity of the offspring.

Thus, there is ample evidence that maternal nutrition throughout gestation has a great potential to modulate the development and functioning of the offspring organs, although compensatory mechanisms may occur in an attempt to mitigate some effects. Therefore, adopting nutritional strategies that fully meet the nutritional requirements of

pregnant females is crucial to not impair the metabolism, physiology, and health of the progeny.

2.4. Maternal nutrition and offspring intake characteristics

Recent researches had shown that the feed intake in postnatal life can be shaped by intrauterine conditions related to maternal nutrition during gestation. In the arcuate nucleus (ARC) of the hypothalamus, where the feed intake control occurs, there are two groups of neurons with opposite roles. Orexigenic neurons are responsible for the secretion of neuropeptide Y (NPY) and agouti protein (AgRP) that stimulate feed intake. In contrast, anorexigenic neurons secrete the cocaine-amphetamine-regulated transcript (CART) and the melanocortin peptide (alpha-MSH) which is derived from proopiomelanocortin (POMC) and relate to the feeling of satiety (Bouret et al., 2015; Bell et al., 2005).

In this context, some studies have shown that modifications in this hypothalamus axis can occur in response to maternal nutrition planes during fetal development. Muhlhausler et al. (2006) verified a greater expression of POMC in the overnourished offspring (160% of metabolizable energy requirements) despite the lack of differences on *CART*, *NPY*, or *AgRP* expression in the ARC nucleus of lambs. Prezotto et al. (2018) investigated the effects of maternal nutrition plane during gestation [100 (CON), 60% (RES), or 60% of NRC requirements plus arginine supplementation (RES+ARG)] on ovine offspring. They verified a lower POMC-containing cell number within the arcuate nucleus of the hypothalamus to RES compared to the RES+ARG group, and also a tendency of lower NPY expression for RES and RES+ARG groups compared to the CON group. Stevens et al. (2010) also show the occurrence of epigenetics changes in the hypothalamus promoted by maternal periconceptional undernutrition (-60 to 30 days around conception). Fetuses from dams in a poor maternal nutrition plane presented hypomethylation of the POMC gene indicating a predisposition of the offspring to deregulation of appetite.

Poor maternal nutrition planes during gestation are also associated with leptin resistance in adult life in the offspring, which promotes a lower density to signals of body mass on neurons, leading to an appetite dysregulation (Delahaye et al., 2008) and a predisposition to obesity (Muhlhausler et al., 2006). Leptin is primarily synthesized by adipose tissue and acts as a hormone regulating feed intake through stimulation of the arcuate nucleus of the hypothalamus (Kowalski et al., 2014). Reduction in feed intake is

due to increases in plasma leptin concentration and, consequently, greater binding to orexigenic neurons, which decreases NPY and AgRP release. On the other hand, leptin stimulates the activity of anorexigenic neurons, which leads to increased release of alpha-MSH and CART (Bell et al., 2005).

Alterations in leptin signaling; changes in the development of hypothalamic appetitive control centers; and leptin resistance in later life, have been associates with alterations in the leptin neonatal peak (Delahaye et al., 2008; Kirk et al., 2009; Vargas et al., 2017). Some authors indicated that there is a typically a peak in circulating leptin concentrations during the first postnatal days, which programs the lifelong activity of the appetitive control centers within the hypothalamus in ruminants (Yura et al., 2005; Bouret et al., 2015). According to Long et al. (2011), this neonatal peak of leptin was not observed in offspring from obese ewes, due to an increase of plasma cortisol concentrations, orchestrating the hyperphagic characteristic in these animals at postnatal life. The same seems to occur for offspring from undernourished dams. In this context, Smith et al. (2018) found that maternal nutrient restriction from early- to mid-gestation was associated with neonatal hypercortisolemia and to a subsequent elimination of the neonatal leptin peak, associate with hyperphagia in the post-natal life.

Although there are not available studies evaluating the effect of maternal nutrition during pregnancy on offspring ingestive behavior in the postnatal phase, there is ample evidence that it can be affected. Thus, further studies are needed to understand these effects.

2.5. Maternal nutrition and endocrine changes in offspring

Insulin and IGF-1 have an important role in metabolism. IGF-1 is important for growth because promotes hypertrophy through stimulation of phosphatidylinositol 3-kinase (PI3K)/ protein kinase B (Akt) / mammalian target of rapamycin (mTOR) pathway. In cattle, IGF-1 not only participates in protein synthesis and degradation but is also involved with muscle cell proliferation (Ge et al., 2013). IGF-1, as well as insulin, has mitogenic potential, acting on cell proliferation through interaction with Src homology and collagen domain protein (Shc) to activate mitogen-activated protein kinase pathways (O'Neill et al., 2015). During prenatal development, IGF-1 has a role in myogenesis promoting mitotic activity (Greenwood & Bell, 2003). In post-natal life, IGF-1 acts as stimuli to activate the mitotic quiescent satellites cells, inducing its proliferation

and leading to hypertrophy (Machida & Booth, 2004). Additionally, IGF-1 is associated with a decrease in glucose levels in the blood (Wang et al., 2011).

Insulin in its turn is a peptide hormone produced by β-cells of pancreatic islets of Langerhans, being secreted packed into secretory granules complexed with zinc, and released in response to high glucose levels (Xavier, 2018). Tissue glucose uptake occurs when insulin binds to its receptor, activating the displacement of GLUT-4 transporters from the intracellular compartments to the plasma membrane through PI3-kinase and MAP-kinase pathways (Huang and Czech, 2007; Khan & Pessin, 2002). In conditions of high substrate availability, insulin also acts favoring fat deposition, stimulating lipogenesis through the expression of transcription factors involved with activation of genes responsible for this process, as the sterol regulatory element-binding proteins (*SREBP1*) (Carvalheira et al., 2002). Additionally, like IGF-1, insulin also stimulates the PI3K/ Akt/ mTOR pathway and inhibiting FOXO, promoting hypertrophy and avoiding protein degradation (Latres et al., 2005).

Maternal nutritional plan during pregnancy may alter IGF-1 and insulin levels in the fetus and its post-natal life. According to Holt (2002), intrauterine growth retardation (IUGR) can modify the GH-IGF-1 axis in the offspring, since the genes encoding IGF-1 and its receptors can be deleted in this condition. This author also related that IUGR promotes hepatic resistance to GH, which is characterized by increases in GH synthesis and decreases in IGF-1 secretion in post-natal life. Consistent with this, results found for Hoffman et al. (2014) show that reduced body weight in ruminants was associated with reduced circulating IGF-I in offspring from restricted-fed ewes.

Poor intrauterine conditions during gestation can cause placental insufficiency, which promotes fetal hypoxemia associated with a reduction in fetal glucose availability, leading to a greater secretion of catecholamine and cortisol by the adrenal gland, which promotes less insulin secretion and consequently leads to lower secretion of IGF-1. This condition decreases the fetal tissue uptake of glucose and amino acids that reflects in a reduction in protein synthesis, mitosis, and fetal cell differentiation, limiting the fetus growth (Greenwood & Bell, 2003). In this sense, a lower concentration of IGF-1 during gestation is one of the main factors that promote fetal growth retardation (Martín-Estal et al., 2016).

Lower expression of IGF-1 through the action of cortisol cause negative effects on the offspring's muscular development (Florini et al., 1996; Liu et al., 1993). As the muscle is the major site of glucose utilization, the effects of maternal nutrition on its mass, type,

743 and growth patterns, will also alter the offspring insulin sensitivity at post-natal life,
744 which may contribute to the onset of diabetes in the progeny (McMillen & Robinson,
745 2005). Moreover, the nutritional disturbance during the gestational period may cause β-
746 cells defects, by a decrease in its proliferation and increase in apoptosis (Gicquel et al.,
747 2008), leading to an irreversible reduction of β-cells mass. This condition causing defects
748 in glucose-stimulated insulin secretion and promoting lower insulin secretion in offspring
749 later in life (Jones & Ozanne, 2009).

750 Protein restriction during prenatal life in animal models using rats also demonstrated
751 the potential to modulate key hepatic enzymes of glucose metabolism in offspring liver.
752 May occur an increase in PEPCK activity, a glucose-producing enzyme, and also a
753 decrease in the activity of hepatic glucokinase, involved with glucose use (McMillen &
754 Robinson, 2005). According to Burdge et al. (2007), the increased expression of PEPCK
755 and consequently the gluconeogenic capacity in the liver due to protein restriction during
756 prenatal life is linked to the hypomethylation of the glucocorticoid receptor promoter,
757 promoting a greater effect on PEPCK activity.

758 As presented here, there is ample evidence in the literature showing the potential of
759 maternal nutrition planes to cause endocrine changes in the offspring which could restrain
760 the growth and lead to metabolic disturbances.

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SECOND SECTION – ARTICLE

ARTICLE 1 - Effect of maternal nutritional plane of Zebu beef cows on growth, metabolism and performance of male or female offspring

Article formatted according to Livestock Science guidelines

Highlights

- The effect of prenatal nutrition on the offspring are dependents of sex.
- Protein restriction cause chronic growth retardation in the offpsring.
- Maternal protein supplementation in mid gestation improve the of offspring size.
- Intramuscular lipogenesis is enhanced in females from supplemented dams.
- Dams protein supplementation enhance offspring hypertrophy.

ARTICLE 1 - Effect of maternal nutritional plane of Zebu beef cows on growth, metabolism and performance of male or female offspring

ABSTRACT

This study aimed to evaluate the performance, metabolism, and expression of muscle markers of offspring from dams that were supplemented or not with protein during mid-gestation. At 100 days of gestation 43 purebred Tabapuã beef cows pregnant of females (n = 20) and males (n = 23) were randomly assigned into 2 groups: Control [(**CON**) - supply of basal diet achieving 5.5% of crude protein (CP); n = 24]; or Supplement [(**SUP**) - basal diet plus a supplement with 45% of CP provided at the level of 3.5 g/kg of body weight; n = 19]. Offspring were evaluated from birth to 445d of age. Differences were declared at $P < 0.10$. The CON offspring were lighter 3.8 kg at birth, 16.5 kg at weaning, and 30.8 kg at 445 days of age ($P \leq 0.049$). At birth and weaning, SUP calves had greater ($P < 0.10$) morphometric measurements. Overall, the insulin ($P = 0.076$) and IGF-1 ($P = 0.002$) levels were greater foir CON-females at weaning. Maternal nutrition (MN) and offspring sex (OS) interaction were observed on plasma insulin and IGF1 concentrations from offspring at weaning ($P \leq 0.076$), but not at 445d ($P \geq 0.378$). There were no effects of treatments on the *Longissimus* muscle area (LMA; $P > 0.10$) and rump muscle length ($P \geq 0.60$). The SUP offspring had a greater muscle fiber area at 30 days of age than the CON group ($P = 0.007$). There was an up-regulation of *mTOR* mRNA expression in *Longissimus* for SUP ($P = 0.056$) at 7d. *MyoD* was highly expressed in CON-females ($P = 0.022$). *MyoG* expression did not differ among treatments ($P > 0.10$). There was no effect of MN, OS, or MN × OS interaction on the adipogenic transcription factors expressions ($P > 0.10$). Up-regulation of *CPT2* was observed in CON offspring at 445d ($P = 0.037$), indicating a greater fiber type I proportion for this group. There was an up-regulation of the *ACACA* ($P = 0.072$) and *LPL* ($P = 0.090$) genes in the SUP offspring at 445d. *FABP-4* ($P < 0.001$), *PPARG* ($P < 0.001$) and *SCD1* ($P < 0.001$) were up-regulated in SUP-female. The lack of prenatal supplementation reduces the offspring performance by the impairment of mesenchymal cells commitement; by metabolic changes; reduction in hypertrophy capacity; and by alteration related to how skeletal muscle behaves concerning energy partitioning. ***Keywords*:** fetal programming, gene expression, lipogenesis, morphometric measurements, sexual dimorphism

1. Introduction

It is widely accepted that in a mammal, both, sub-nutrition and over-feeding can remodel the offspring development trajectory through fetal programming effects (Hales and Barker, 2001; Hoffman et al., 2017; Sartori et al., 2020). In this sense, some management practices applied during prenatal development can be more effective rather than in postnatal life. For example, there is no net increase in muscle fiber number after birth in cattle (Picard *et al*., 2002). Around 95% of the muscle fibers population is formed during secondary myogenesis (Zhou *et al*., 2021) concentrated during mid-gestation (Du *et al*., 2010). During late gestation, there is a greater development of intramuscular adipocytes and likewise the proliferation of fibroblasts, which are both competitive processes (Du et al., 2013). Furthermore, studies with ruminants (Costa et al., 2021) had been demonstrated that prenatal nutrition may change the muscle fiber metabolism. These changes may occur for example by modification in the GLUT-4 transporters availability, as well by changes in the proteins and enzymes expressions involved with the energy substrates use in muscle (Zhu et al., 2006). In this sense, this type of modification may could lead to persistents effects on the muscle growth efficiency and on the intramuscular fat content of the offspring. Herein, once many processes involved with the offspring performance and with a quality grade of meats are sensitive to the maternal supply of nutrients, understanding the consequence of prenatal nutritional strategies is a promising method to produce animals with good trails of commercial interest.

Despite this importance, in many production systems, maternal nutrition during pregnancy is still neglected (Gionbelli et al., 2018). In extensive pasture systems, which operate in tropical regions, like happens with the majority of beef cattle production in Brazil, pregnant beef cows face nutritional restriction in part of gestation. In such systems, there is a recurrent productive seasonality over the year, affecting the net forage production and its nutritive value (Reis *et al.*, 2013). The better quality and availability of grasses are used to favors reproduction and lactation, being the breeding season in general programmed to align with the rainy period (Lemos *et al*., 2012; Paulino and Duarte, 2014). With this practice, mid to late gestation overlaps with the dry season, making that pregnant beef cows experience poor nutritional conditions (Gionbelli *et al*., 2018). Among multiples nutrients deficiencies that occur during this phase, considerable emphasis is necessary around a striking reduction in the total content of nitrogenous compounds of forage (Detmann *et al*., 2009). This poor ruminal nitrogen environment impairs microbial growth (Leng et al., 1990), and consequently the degradation of fibrous

carbohydrates in the forage, reducing the intake (Lazzarini et al., 2009). Thus, protein supplementation can be used as a strategy to increase the nutrients flow to the dam and fetus (Marquez et al., 2017).

Furthermore, there is evidence that maternal nutrition may have different effects associated with the offspring sex (Micke et al., 2010; Gionbelli et al., 2018; Costa et al., 2021), probably as a consequence of steroid exposure produced from the early stages of fetal development (Dominguez et al., 1998). Therefore, this study aimed to evaluate the effects of protein supplementation during mid-gestation in nutritionally restricted beef cows on the performance, metabolism, and skeletal muscle development of the offspring. In addition, it aimed to assess the sex-specific responses of offspring to maternal nutritional management. We hypothesize that strategic supplementation will boost skeletal muscle development and the offspring gain potential in the postnatal stage and that these differences will be more pronounced in males.

2. Materials and methods

The experiment was conducted at the Beef Cattle Facilities of the *Universidade Federal de Lavras* (UFLA), Lavras, Minas Gerais, Brazil. This study was divided into two repetitions of 2 and half years each, with the identical experimental procedure. Every stage covered the phases of insemination, gestation, parturition, lactation (cow-calf phase), and the post-weaning period (background, growing 1, and growing 2 phases). All experimental procedures were previously approved by the UFLA Ethics Committee on Animal Use (CEUA) Protocol No. 015/17.

2.1.Experimental management

Pre-parturition. Forty-three purebred Tabapuã (*Bos taurus indicus*) multiparous cows [average age = 6.3 ± 0.6 years; initial BW = 490.5 ± 17.8; initial body condition score (BSC) = 5.6 ± 0.5] were used, being utilized 24 and 19 cows in the first and second experiment repetitions, respectively. In the second repetition, part of the cows used in the first was re-used, with the designation of treatments for these animals performed randomly. Cows were inseminated using semen from different males and at 60 days of gestation, fetal sexing was performed to have homogeneous control over treatments. In the mid-gestation (from 102 ± 5 days of conception), cows were transferred from a pasture area of *Brachiaria brizantha* cv. Marandu to a feedlot with individual pens. Then, they were randomly divided into 2 groups, with different feeding levels: **Control** (**CON**) **-**

supply of basal diet [corn silage + sugarcane bagasse, achieving 5.5% crude protein (CP) plus a mineral mixture] (*n* = 27) or **Supplement** (**SUP**) **-** basal diet plus protein supplementation [45% CP at the level of 3.5 g / kg body weight (BW)] (*n* = 19). The percentage composition of roughage sources was 75% of corn silage [dry matter (DM) = 35.8 %; CP = 6 %, and neutral detergent fiber (NDF) = 57.3%] and 25% of sugarcane bagasse (DM = 82%; CP = 2%, and NDF = 77.2%) and a mineral mixture was provided *ad libitum*. The protein supplement consisted of a 50:50 mixture of soybean meal and a commercial supplement (Probeef Proteinado Sprint®, Cargill Nutrição Animal, Itapira, SP, Brazil) (**Table 1**). The basal diet was adopted to represent CP levels similar to those found for pregnant beef cows raised on pasture systems during the dry season in the central west and southeastern Brazil. The mid-gestation was chosen as the supplementation period considering the theoretical window of skeletal muscle development, proposed by Du et al. (2010). The amount of diet provided was adjusted for DM content weekly, based on DM content of corn silage and sugarcane bagasse. For made adjustments in the supplement supply, once a month the cow's body weight was recorded after 12 hours of fasting. The average crude protein restriction was equivalent to 70% of protein and 50% of energy requirements for CON cows [calculated according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho *et al*., 2016)] at the mid-gestation, being the average supplement intake equivalent to 1.50 kg/ day during mid-gestation. From 208 ± 6 days of gestation until the parturition, all cows were fed *ad libitum* and received only corn silage and mineral mixture (**Table 1**). In both periods of gestation, animals were fed twice a day (at 0700 and 1300 h) and had free access to clean water. Close to scheduled calving, cows are allocated in large individual pens, for a better ambiance during parturition.

Post-natal managements. The offspring was characterized by 20 females (*n* = 10 SUP; *n* = 10 CON) and 23 males (*n* = 9 SUP; *n* = 14 CON). Seven days after calving, cows and their sons were allocated together in a *Brachiaria decumbens* cv. Marandu pasture area (DM = 29.7 %; CP = 13.0 %; NDF = 62.6%). This area was the same used in both periods of the experiment, been used a continuous stocking system with a variable stocking rate, in intensive grazing management. Cows received a mineral mixture, and calves a protein-energy supplementation (5 to 7 g/ kg of BW per day) through a commercial supplement (Probeef maxima creep®, Cargill Nutrição Animal, Itapira, SP, Brazil) at the level of 5 to 7 g per kg of BW, by the creep-feeding technique. The assurance level per kilogram of product were: 200 g crude protein (min); 20 g Ca (max); 30 g Ca

(min); 3 mg Co (min); 51 mg Cu (min); 1 mg (min) Cr; 10.4 g dextrose; 3000 mg S (min); 0.42 ethoxyquin (min); 2000 mg F (max); 6000 mg P (min); 3 mg I (min); 700 mg mananas (min); 108 mg Mn (min); 60 mg monensin; 0.90 mg Se; 10 g Na (min); 12000 UI vitamin A (min); 15000 UI vitamin D3 (min); 50 UI vitamin E (min); 180 mg Zn (min). At 210 days, calves were weaned. Nevertheless, as the birth data were similar but not equal among the offspring after weaning, animals were maintained in a pasture system and received a supplementation, to wait for more calves to be weaned to form uniform groups to be confined. Thus, at 255 ± 29 days of average age, heifers and steers were housed in a feedlot with individual pens, being confined during the background (from 255 to 320 days of average age); growing 1 (from 321 to 381 days of average age) and growing 2 phases (from 382 until 445 days of average age). In the confinement, animals had free access to water and were fed twice daily (0700 and 0100 h). Males and females received different diets with the same ratio roughage: concentrate in the background and growing 1 phase, but in growing 2 phase the diets were equal for both sexes. The ingredients used and the chemical composition of feedstuffs used in each phase of both experimental stages are described in **Table 2**. From the 43 progeny used in this experiment, 2 were born extremely weak and died until 7 days of age, being both CON males. Moreover, after weaning, one female from CON group died due to an external factor. Thus, the data presented at the cow-calf phase are from results obtained from 41 animals (excepted during the neonatal period), while data presented from weaning to end of growing 2 phase are from 40 animals.

2.2. Measurements

Vigor score, body weight gain, and biometric measures. The same person observed calves during the neo-natal period to vigor score evaluation. The vigor score was attributed using a number on a scale of 1 to 4, in which: 1 = calf that died in the neonatal period due to extreme debility; 2 = calf that was born weak and apathetic; who presented difficulty and a delay to standing up after birth to ingested the colostrum; who needed frequent human intervention to help in the milk intake from dams; or/and who was born with some apparent imperfection (such as curved limbs); 3 = Calves considered normal, without trails of debility; which did not demand special care; 4 = robust and astute calves, who stand up in few minutes after birth and which present higher vitality over the neonatal period. The morphometric measurements evaluated at birth, 60 and 210 days of age were: (**1**) the height at withers; (**2**) rib depth; (**3**) rump depth; (**4**) rump height; (**5**) ischial bones

distance; (**6**) ileus bone distance; (**7**) abdomen width, (**8**) girth circumference, and (**9**) body length. All measurements were done by the same person using one hipometer (Walmur, Porto Alegre, Brazil), with exception of girth circumference, which was obtained with a flexible tape. Animals were positioned with their heads up and with the four limbs perpendicular to the ground. The body length was taken in a straight line as the distance between the neck and the tail and the thorax width was taken at the maximum point of the body. The remaining measurements were taken according to Fernandes et al. (2010). At birth, calves were isolated from the dam before the colostrum intake to be weighted and morphometric measurements are taken. In the other points of development in the cow-calf phase, these procedures were done with the calf in fasting, after 12 hours of isolation from its dam. At the feedlot, the animals were weighed before the morning feeding.

Carcass Ultrasound. The ultrasound scans were done at 100 and 210 days of age at the cow-calf phase and also at 350 and 445 days of age, at the confinement period. Carcass images were taken to evaluate the longissimus muscle area (LMA, cm^2); LMA subcutaneous fat thickness (SFT, mm); rump muscle length (RML, cm), and rump fat thickness (cm). Animals were ultrasonically scanned by the right side using an Aloka 500-V machine (Corometrics Medical Systems, Wallingford, CT), equipped with a 3.5-MHz, 17.2-cm linear array transducer. The LMA and SFT images were done between 12^{th} and 13^{th} ribs, $^3/_4$ the length ventrally over the longissimus muscle. The RML and rump fat were taken at the junction of the biceps femoris and gluteus medius between the ischium and ileus and parallel to the vertebral column. The images analysis was performed using the BioSoft Toolbox® II for Beef software (Biotronics Inc., Ames, IA, USA).

Blood hormone and metabolites. Blood samples were collected at 210 days (weaning) and 445 days of age (end of experimental period), in the morning (0700 h) before the feed supply. Samples were collected by coccygeal venipuncture in vacutainer tubes (First Lab, São José dos Pinhais, PR, Brasil), being the serum harvested by centrifugation (1.500 × *g* for 15 min at +4ºC) and stored at −20°C until analysis. Commercial kits were used for serum analysis of glucose (133, Labtest, Lagoa Santa, MG, Brazil), insulin (2425300, Monobind, Lake Forest, USA), and insulin-like growth factor 1 (IGF-1; SEA050BO, Cloud-Clone Copr, Houston, Texas, USA).

198 *Histological analyses.* At 30 days, bipsies of *Longissimus thoracis* muscle were
199 done between the 9th and 10th rib to obtain one cubic centimeter of muscle tissue for
200 morphological analysis of muscle fiber. Samples were stored embedded in fresh 10%
201 (w/v) formalin in phosphate buffer (pH 7.4) for 48 hours and then embedded in alcohol
202 70 until analyses. Samples were submitted to dehydration with ethanol, diafanized in
203 xylol, and included in histological paraffin. Subsequently, a minimum of 3 histological
204 sections was obtained from each sample at a thickness of 5.0 μm using a Lupetec MRP09
205 microtome, being the sections stained with Haematoxylin-Eosin (HE) (Pluske et al.,
206 1996). Histological images were captured (40×) using a light microscope OLYMPUS
207 CX31 (Olympus Corp., Tokyo, Japan). The morphometric analysis of muscle fiber was
208 done using the ImageJ® analyzer software (National Institutes of Health, Bethesda,
209 Maryland, USA). The muscle fiber area was calculated considering 120 fibers from
210 different photomicrographs of each animal (Carani et al., 2006).

211 *Gene expression analysis.* Muscle biopsies of *Longissimus thoracis* muscle to gene
212 expression analyses were done in the offspring at 7 and 445 days of age, as described by
213 Arrigoni et al. (2004). All tissue samples obtained were immediately stored in sterile
214 cryotubes, frozen in liquid nitrogen, and stored at -80ºC until analysis. The total RNA
215 was extracted from 50 mg of muscle sample using QIAzol (QIAGEN, Valencia, CA).
216 The isolated RNA was treated with DNA-free DNAse (Ambion, Austin, TX) according
217 to the manufacturer's instructions. The total RNA was electrophoresed in a 1.0% (m/v)
218 agarose gel stained with GelRed nucleic acid gel (Biotium, Hayward, CA). Its 28S and
219 18S RNA bands were analyzed using a UVItec FireReader XS D-77Ls-20M (UVItec,
220 Cambrige, UK), and its optical density was quantified by Image StudioTM Lite (LI-COR
221 Biosciences) to check a possible degradation. A cDNA was produced from the RNA
222 template for gene expression analyses, using the high-capacity cDNA Reverse
223 tTranscription Kit (Applied Biosystems, Foster City, CA, USA). Reverse-transcription
224 quantitative PCR (RT- qPCR) was performed on an Eppendorf Realplex system
225 (Eppendorf, Hamburg, Germany) using SYBR Green detection system (Applied
226 Biosystems, Foster City, CA, USA) set as follows: 50°C for 2 min, 95°C for 10 min, 40
227 cycles of 95°C for 15 s, 60°C for 1 min, and 95°C for 15 s. For each reaction, 1.0 μl
228 cDNA (10 ng/μl), 0.3 μl of each primer (1.5 μM; forward and reverse), and 5.0 μl SYBR
229 Green Master Mix were combined in a 10.0-μl/sample final volume in a 96-well
230 MicroAmp Optical plate (Applied Biosystems). The results of RT-qPCR were normalized

231 by the threshold cycle (CT) method for the expression of the reference genes β-actin and
232 Cancer susceptibility candidate 3 (CASC3). The relative expression levels were
233 calculated according to the method described by Pfaffl (2001), based on Ct values that
234 are corrected for the amplification efficiency for each primer pair. To primers design, the
235 sequences published in a database of Biotechnology Information platform (GenBank)
236 were used and the target genes were analyzed and their respective primers sets are shown
237 in **Table 3**.

238 *2.3 Statistical analysis.*

239 Descriptive statistics were obtained through the mixed model's methodology
240 (procedure MIXED) of SAS 9.2 (SAS Inst. Inc., Cary, NC). The maternal dietary
241 treatment, offspring sex, and the interaction between MN × OS were considered as fixed-
242 effect. The period in which the experiment was done (repetitions) and the dam's index of
243 genetic merit expected for growth traits (GEN) was considered as a random effect. GEN
244 was calculated using the data available on Tabapuã Genetical Enhancement Program
245 using the information of expected progeny difference (EPD) about the cow's parents for
246 weight at weaning, 2 and 18 months of age, since all animals were registered in this
247 database. The use of GEN as a random effect allowed more applicable results, being the
248 responses not restricted to the use of a single bull. When pertinent ($P < 0.05$), the dam's
249 numbers of parturitions; empty weight at 100 days of gestation (beginning of the
250 treatment application); size; body corporal condition at 100 days of pregnancy; gestation
251 length; as well the offspring age and/or its body weight at the respective evaluation were
252 used as a covariate. When not pertinent ($P > 0.05$), they were taken out from the model.
253 The following statistic model was used:

254 $$Yijkl = \mu + Di + Sj + (DS)ij + Tk + Gl + \varepsilon ijkl$$

255 Where: *Yijk* is the observed measurement; μ is the overall mean; *Di* is the fixed-
256 effect of the i^{th} level of maternal dietary treatment; *Sj* is the fixed effect of the j^{th} level of
257 offspring sex; *DSij* is the interaction between D and S; *Tk* is the random effect of
258 the k^{th} period; *Gl* is the random effect of the l^{th} index of dam's genetic merit expected for
259 growth traits and $\varepsilon ijkl$ is the random error associated with *Yijkl*, with $eijkl \sim N(0, \sigma e2)$.

260 Before the final analyses, studentized residuals were removed when not within ± 3
261 standard deviations, and normality ($P > 0.05$) was assessed using Shapiro-Wilk's test.

Some mRNA expression data (*7 days* = CEBPA, FABP4, ZFP423, IGFR1, mTOR, MYOG, TGFβ1, FN1; *445 days* = ACACA, FABP4, PPARG, SCD1) did not follow a normal distribution (Shapiro-Wilk test) and had to be normalized. The ultrasound data were analyzed as repeated measures over time within each period.

Least-squares means were separated using Fisher's least significant difference test. When the interaction between the fixed effects was significant, the least square means were compared using Tukey's method. The comparison between the means of the groups was performed using $\alpha = 10\%$ of probability for type I error for all tests performed, once in this type of study there is a greater incidence of type-II error.

3. Results

3.1. *Vigor score and performance measurements*

Table 4 presents the results of vigor score and performance in the offspring. There was an interaction between MN plane and OS for vigor score ($P = 0.086$). Although the maternal supplementation did not improve the vigor score for males, it was able to improve for females. Performance measures such as BW and ADG were affected by the maternal nutritional plane and some others by offspring sex (**Table 4**, and **Figures 1** and **2**). SUP calves were ~3.8 kg heavier at birth ($P = 0.049$) and presented an additional gain of ~16.5 kg at weaning ($P = 0.019$) compared to CON offspring. After weaning, SUP calves were ~7.8% heavier at all weights recorded during the background, growing 1, and growing 2 phases. At the end of this trial, the additional gain difference was ~30.8 kg ($P = 0.016$) for SUP calves. There was a difference in weaning weight affected by offspring sex. In this case, male calves were ~16.8 kg heavier compared to females ($P = 0.018$) and this difference persisted over experimental period with ~17% additional BW at 445 days of age ($P < 0.001$).

The ADG was greater ($P = 0.050$) for SUP calves during the cow-calf phase. Nevertheless, the ADG course differed over this phase. From birth to 120 days of age, the ADG was 9% superior for the SUP group ($P = 0.039$), but from 120 days of age to the 210 days, there were no differences in the offspring ADG for maternal nutrition plan during pregnancy ($P > 0.10$) (**Figure 1**). In the confinement period, there were differences for MN treatment on the ADG only at the background phase ($P = 0.064$), not been detected effects for ADG at growing 1 and growing 2 phases ($P > 0.10$) (**Figure 1**). There were also no effects for ADG comprising the entire confinement period (255 - 445 days

294 of age; $P = 0.376$). However, the total ADG from birth to the end of the growing 2 phase
295 was ~8% higher in offspring from supplemented dams ($P = 0.016$).
296 The average daily gain was influenced by offspring sex, in this case, male calves
297 gained ~8% additional weight per day compared to female offspring ($P = 0.025$) until
298 weaning. During the confinement period, the additional weight gains for males was
299 ~0.184 kg/day ($P < 0.001$; **Table 4**).

300 3.2. *Morphometric measurements*

301 The morphometric data are shown in **Table 5**. The SUP calves had ~8.3%, ~14.9%,
302 and ~19.0% greater measures of rump height, ischial bones distance, and abdomen width
303 compared to the CON group at birth ($P \leq 0.053$), respectively. Also, it was observed at
304 birth that males presented ~16.4% greater ischial bones distance, but ~12.3% smaller
305 thorax width compared to female offspring ($P \leq 0.036$). At 210 days of age, SUP calves
306 had an average of height at withers ~4 cm ($P = 0.082$) and rump deep ~2 cm ($P = 0.054$)
307 superior to CON calves. Other morphometric measures evaluated during birth, at 60 days
308 of age, and weaning were not affected by MN, OS, or MN × OS interaction ($P >$
309 0.10; **Table 5**).

310 3.3. *Blood parameters*

311 There was no effect of MN, OS, or MN × OS interaction on blood glucose
312 concentrations of offspring at weaning and 445 days of age ($P >$ 0.10; **Table 6**).
313 Meanwhile, insulin and IGF-1 concentrations in weaning calves showed an MN × OS
314 interaction with higher values for both metabolites in CON-females ($P \leq$ 0.076).
315 Nevertheless, no MN × OS interaction on insulin and glucose levels was found at 445
316 days. At 445 days of age, insulin and IGF-1 concentrations in blood were higher in males
317 regardless of maternal nutritional management ($P \leq 0.029$; **Table 6**).

318 3.4. *Muscle morphology*

319 **Figure 4** shows the muscle fiber area (μm²) of the *Longissimus thoracis* of 30 days-
320 old offspring. Calves from SUP dams presented an area of muscle fiber 26.4% greater
321 than calves of CON group ($P = 0.007$). There was also an effect of sex on muscle fiber
322 size, where females had ~20.5% greater fiber area than male calves ($P = 0.031$). MN ×
323 OS interaction was not observed for this parameter ($P = 0.187$).
324

325 3.5. *Ultrasound carcass measurements*

326 At the cow-calf phase, only MN × OS interaction with ~18.9% more SFT in male
327 calves from SUP dams was observed (P = 0.083; **Table 7**). In the confinement period,
328 there was only an effect of offspring sex on LMA with ~5.7 cm^2 greater area in males
329 than females (P = 0.002). Concerning to other carcass variables evaluated in both phases,
330 they were not affected by MN, OS, or MN × OS interaction (P > 0.10; **Table 7**).

331 *3.6. Skeletal muscle genes expressions*

332 Gene expression in offspring skeletal muscle is shown in **table 8**, and the MN × OS
333 interactions in **Figure 4**. The mRNA expression of the IGFR1 was not affected by fixed
334 effects (P > 0.10). Calves from SUP dams showed up-regulation of *mTOR* at seven days
335 of age (P = 0.056). Moreover, a sex effect was observed with up-regulation of *FABP4*
336 and TGF-β1 genes in skeletal muscle of females at seven days of age ($P \geq 0.031$; **Table**
337 **8**). An MN × OS interaction with up-regulation of the *MyoD* gene in CON-females (P =
338 0.022; Figure 4A), and down-regulation of *FN1* in CON-male calves (P = 0.057; **Figure**
339 **4B**) were verified at seven days. Other myogenic and fibro-adipogenic genes evaluated
340 in the offspring at this period were not affected by MN, OS, or MN × OS interaction (P
341 > 0.10; **Table 8**).

342 At 445 days, male offspring showed down-regulation of the *SREBF1*, *ACACA*,
343 and *LPL* genes ($P \leq$ 0.020). SUP offspring showed higher expression
344 of *ACACA* and *LPL* ($P \leq$ 0.090). Calves from CON cows had an up-regulation
345 of *CPT2* (P = 0.037; **Table 8**). An MN × OS interaction with up-regulation
346 of *FABP4*, *PPARG*, and *SDC1* genes in SUP-female offspring was observed (P <
347 0.001; **Figure 4C**, **4D,** and **4E**).

348 **Discussion**

349 Neonatal losses demonstrate that nutrient deficiency during pregnancy negatively
350 affects the offspring's survivability, generating a great economic impact on beef cattle
351 production. Thus, this found reinforcing the importance of nutritional corrections in
352 conditions of low nutrient availability during mid-gestation for pregnant beef cows. The
353 interaction MN × OS for the vigor score indicates that protein supplementation in cows
354 has a greater positive effect on female (**Table 4**). Moreover, we report the death at birth
355 of two male calves of the CON treatment. These found suggesting that during the bovine
356 fetal growth trajectory in utero, the male bovine fetuses are more susceptible to the effect

of fetal programming than the female fetuses, and that nutritional restriction during pregnancy affects more intensely the viability of males.

The phenotypic differences observed among sons from dams supplemented with protein or not in the middle third of gestation, were evident even at birth, in which CON calves were lighter. This result is consequence of the improvements provided by protein supplementation on the nutritional status of cows, which consequently promoted a greater nutrient delivery to the fetus. Moreover, as nutrients are necessary at the same time to the oxidative metabolism and to fetal anabolism (Bauman, 1980), the nutrient scarcity promoted by CON treatment probably makes that CON fetuses had to catabolized more amino acids as an energy source, reducing nitrogen and carbon for growth and resulting in lower birthweight.

There was chronic growth retardation in the CON group even after nutritional rehabilitation in a long term at the postnatal life. The serial ADG analyses at different points of post-natal development showed differences influenced by maternal nutrition between birth and 120 days of age, and later during the background phase. This fact is because these periods are included at the stage of the higher rate of lean muscle tissue deposition in cattle (Berg and Butterfield, 1979). Additionally, in the transition of animals from pasture to a feedlot, that is, in the background phase, an expressive ADG for SUP animals was evident, probably as a consequence of greater availability of dietary nutrients, and also of the consequence of the greater potential for growth in these animals.

The higher gain potential for SUP offspring was in agreement with the greater *Longissimus thoracis* fiber area in these animals, and may be related to the upregulation of *mTOR* expression for this group. The *mTOR* gene encoded the mTOR protein, which joins with other proteins of mTOR complex 1 and 2 (Takahara et al., 2020). This signaling factor is involved with several processes, including protein synthesis and also the prevention of protein degradation (Latres et al., 2005). Nevertheless, we highlight that due to the limitation of the study in evaluating only the abundance of the transcript for mTOR, we cannot infer about the effects on the mTOR pathway activation over protein synthesis.

The mTOR may be stimulated by amino acids, such as leucine (Li et al., 2011), as well for hormones like insulin and IGF-1 (O'Neill et al., 2015). Despite the insulin and IGF-1 levels have not been evaluated at 7 days, when the highest mRNA expression of *mTOR* was verified for SUP group, was expected that the gene that encodes the IGF-1 receptor (*IGF-1R*) was up-regulated. This receptor has structural and functional

391 similarities with the insulin receptor (Duan et al., 2010), therefore, it can bind to both
392 hormones and exert the same effect on the activation of the mTOR to promote protein
393 synthesis. Nevertheless, the lack of statistical effects on *IGF-1R* mRNA expression could
394 indirectly indicate that the greater mTOR expression was not induced by insulin or IGF1.
395 However, the mTOR can also be activated by energy status and amino acids (Liu and
396 Sabatini, 2020). Therefore, the upregulation of *mTOR* in the muscle of seven-day-old
397 SUP calves was probably related to a higher intake of amino acids from higher milk
398 production in cows that received an adequate nutritional plan during pregnancy verified
399 in our complementary study (unpublished data). SUP cows produced two liters of milk
400 more compared to CON cows at seven days postpartum (P = 0.02; SUP = 8.68 vs. CON
401 = 6.60 kg), supporting this hypothesis.
402 Exposure to low protein diets during mid-gestation promoted sex-specific effects
403 on insulin and IGF-1 levels at 210 days, but not at 445 days. Thus, the effects observed
404 on the offspring's metabolism were not persistent. Both insulin and IGF-1 control the
405 energy homeostasis, making that GLUT-4 glucose transports, migrate from the
406 intracellular compartments to the plasma membrane (Huang and Czech, 2007; Wang et
407 al., 2012; Siddle, 2011). The greater insulin and IGF-1 levels for CON-females at 210
408 days may indicate a failure in the recognition of these hormones by receptors, and a
409 possible resistance. Thus, due to a possible reduction of insulin and IGF-1 receptors, the
410 body of female-CON adapted to maximize these hormones production, as an attempt to
411 promote the same glicose amount uptake by cells. This compensatory mechanism
412 demonstrated to be effective, once no fluctuations in glucose levels were verified at 210
413 days. Following our findings, Micke et al. (2011) also verified sex-specific changes in
414 IGF-1 levels due to the maternal protein levels during pregnancy.
415 Protein restriction during pregnancy was not only a potential modulator of offspring
416 BW but also of offspring shape at birth. Nevertheless, these differences were transitory,
417 once they ceased to be evident at 60 days of age. This response shows that CON animals
418 requiring at a maximum of 60 postnatal days to reach the same size as their
419 contemporaries, being lighter but with equal size of SUP at this point. In accordance,
420 Hoffman et al. (2014) using a sheep model, also verified that morphometric changes
421 present in offspring at one day of age between sons of dams fed either 100% or 60% of
422 National Research Council requirements, disappeared at 3 months of age. However, in
423 the current study although the CON group showed adaptive advantages over
424 morphological development at 60 days, at 210 days the SUP animals also showed discrete

425 superiority on morphological traits, suggesting that morphological superiority for SUP
426 animals may be persistent in the long term.

427 Since the number of muscle fibers is defined during the middle third of pregnancy,
428 and myogenesis is sensitive to the effects of maternal nutrition (Du et al., 2010), we
429 previously hypothesized that skeletal muscle development in SUP animals would be
430 greater, due to a greater commitment of mesenchymal progenitor cells with the myogenic
431 lineage. Nevertheless, despite the offspring from supplemented dams had greater gain
432 potential in the postnatal phase, these results were not supported by a greater longissimus
433 muscle area or by a greater rump muscle length, which was similar between treatments.

434 Exposure to low protein diets during mid-gestation promoted sex-specific effects
435 on *MyoD*, which was up-regulated in CON-females, while *MyoG* expressions were
436 similar between groups. Thus, despite the lack of statistical effects on muscle
437 measurements performed by ultrasound scans, these molecular findings suggest that there
438 was a smaller window for increasing the pool of myoblasts in CON-females, due to an
439 earlier fusion and differentiation of these cells in animals exposed to protein restriction.
440 The lack of MN effects on adipogenic and fibrogenic markers, demonstrated that protein
441 restriction during mid-gestation did not affect the intramuscular adipocyte or fibroblasts
442 hyperplasia, probably because they predominantly occur during late gestation (Du et al.,
443 2010) when maternal treatment was not applied.

444 Some studies with beef cattle (Costa et al., 2021) suggest that collagen
445 accumulation in the offspring from dams exposed to nutritional insults may improve as a
446 compensatory mechanism promoted by the inadequate nutritional environment. Even so,
447 in the present study, there are evidence toward that collagen content was not affected by
448 maternal nutrition, since the *COL3A1* mRNA expression, which encodes type III
449 collagen, one of the main types of collagen present in the extracellular matrix (Light et
450 al., 1998), was similar between treatments. Moreover, the *FN1* mRNA expression was
451 down-regulated in CON-males, suggesting a possible lower fibronectin content in the
452 extracellular matrix of this group.

453 Zebu animals have a lower degree of intramuscular fat compared to taurine animals,
454 due to a lower abundance of fibro-adipogenic progenitor cells in these animals (Martins
455 et al., 2010). Even if, there was a greater expression of lipogenic markers for SUP animals
456 at 445 days, indicating that maternal protein supplementation during mid-gestation had
457 the potential to increases the intramuscular fat content. The intramuscular fat content is a
458 reflex of balance between triglycerides uptake from the blood, by its synthesis and its

degradation (Jurie et al., 2007). In animals from the SUP group, there was an increase in *ACACA* mNRA expression, which encodes the enzyme Acetyl-CoA carboxylase, responsible for the carboxylation of Acetyl-CoA to form malonyl-CoA in the first steps of *de novo* synthesis of fatty acids (Ladeira et al., 2016). Moreover, the enzyme lipoprotein lipase hydrolyzes the circulating triglycerides present in lipoproteins to release the fatty acids (Park et al., 2018), which depends on the fatty acid-binding proteins to performed its intracellular trafficking (Jurie et al., 2007). Thus, not only the *de novo* synthesis of fatty acids may be impaired by the dam's protein restriction, but also the uptake of fatty acids from the blood, once *LPL* and *FABP-4* genes were down-regulated in CON offspring.

There was also an indication toward a greater fatty acids oxidation by animals exposed to protein restriction during mid-gestation, thought modification in the *CPT2* mRNA expression, which encodes the enzyme carnitine palmitoyltransferase 2 (Teixeira et al., 2017). In mitochondria, CPT2 a peripheral inner-mitochondrial-membrane protein; dissociates acyl CoA from carnitine to be used in β-oxidation (Houten et al., 2016) indicating that more fatty acid may be utilized as an energy source in CON offspring. This pattern indicates a possible modification toward the muscle fiber type, suggesting greater proportion of fiber Type I in CON offspring at 445 days.

Type I fibers present oxidative metabolism, using fatty acids as the predominant energy source, while Type II fiber present glycolytic metabolism, using glucose as primary energy fuel (Schiaffino and Reggiani, 2010). Such response may have occurred due to possible insulin resistance in females and due to the lower concentrations of this hormone in males of CON group, verified at weaning. In this sense, the desrregulation in insulin levels may be impared the glucose use, leading to a switch from carbohydrate to lipid oxidation, conducing to a greater fiber type I proportion in CON animals in a long term. This is consistent with previously reports with rats, in which protein restriction during prenatal period changed the source for ATP synthesis, favoring the fatty acids use by the skeletal muscle (Aragão et al., 2014). Therefore, the skeletal muscle of CON animals was less efficient to fat storage and more efficient in the fat oxidation, leading to a potential decrease in intramuscular fat content for this group.

Furthermore, this pattern also contributes to explaining the lower performance verified for CON group. The muscle is the main site of energy use (Zhu et al., 2006). Thus, even if there was available glucose from dietary metabolism, the fibers type I preferred to use fatty acids as an energy source (Aragão et al., 2014). This probably could

affect the CON homeostasis as a whole, changing the energy partitioning from fat stores, to provide more substrates for these fibers. Despite the lack of statistical difference verified for SFT in the post-weaning phase, we hypothesize that the source of fatty acid for these fibers came from the other fat stores, such as visceral deposits. Therefore, lower CON performance was an associated consequence of the impairment of mesenchymal cells, in the hypertrophy capacity, and also of how skeletal muscle behaves concerning energy partitioning.

Furthermore, females from dams with protein supplementation during the second third of gestation demonstrated a greater *FABP-4* expression at 445 days, gene significantly associated with intramuscular fat in bovines (Michael et al., 2006). This pattern happened accompanied by an increase in *PPARG* expression, which has a central role in fatty acid storage, binding with important genes of lipogenesis (Ladeira et al., 2016). This response was consistent, once the FABP-4/fatty acid complex interacting with *PPARG*, promoting its activation, which in turn orchestrate the *FABP-4* transcription (Damcott et al., 2004). Moreover, there was an increase in *SCD1* mRNA expression, which encodes stearoyl-CoA desaturase (Ladeira et al., 2016), involved with biosynthesis of monounsaturated fatty acids (MUFAs) in SUP-females (Taniguchi et al., 2004). Therefore, strategic protein supplementation during mid-gestation has the potential to be an effective management to improve intramuscular fat, especially in in females.

Regardless of isolated sex effects on animal's BW, this work confirmed the consolidated superiority for males in livestock. Yet, the absence of sex effects on body weight at birth, 7, 30, 60, and 120 days of age, and on serial ADG until 120 days of age, showed, that phenotypic changes between males and females seem to become expressive from this point in Tabapuã beef cattle, in accordance with Govoni et al. (2003). This fact may be related to the rapid increase in the steroid hormones levels, in the bloodstream of calves around 5 months of age (Baatar and Hwang, 2020), accentuating the differences between males and females. Although at 30 days the BW had been similar between the sexes, females had greater *Longissimus thoracis* fiber area. This found may be a reflex of physiological differences related to the dynamic of fat and muscle deposition between males and females. In this sense, as females reach maturity earlier (Bonilha et al., 2015), they probably increased muscle hypertrophy precocious than males. Moreover, the greater *MyoD* expression for 7 days-old calves, also supports the greatest precocious hypertrophy in females, because *MyoD* is involved with quiescent satellite cells activation and proliferation to increase muscle fiber size (Mohammadabadi et al., 2021).

Isolated effects of sex were found on *TGF-β1* mRNA expressions, which was upregulated in females, indicating greater fibrogenesis in the early days of post-natal life for this group. Moreover, there was a greater *FABP-4* expression for females, which is a marker of adipocyte differentiation (delPino et al., 2020), suggesting less intense adipocyte hypertrophy in males in the early days of post-natal life. Furthermore, isolated effects of sex were found on *ACACA* and *LPL* mRNA expression, with greater mRNA expression in females. This found shows reduced fat storage in males compared with females at 445 days, as previously described above. Moreover, there was a greater *SREBF1* mRNA expression in females, while the *SREBP-2* expression was similar between the sexes. Sterol regulatory element-binding proteins are a group of transcription factors which together regulate lipid homeostasis, composed of SREBP-1a and SREBP-1c isoforms, which are encoded by the *SREBF1* gene; and also for *SREBP-2*, encoded by sterol regulatory element-binding transcription factor 1 (*SREBF2*) gene (Eberlé et al., 2004). SREBP-1 isoforms are more specific to activate genes involved with fatty acid biosynthetic genes, while SRBP-2 is relatively cholesterolemia gene expression (Amemiya-kudo et al., 2002). Therefore, although the control of the expression of enzymes required for cholesterol synthesis had not been affected by sex, females demonstrated to be more efficient in the control of the expression of enzymes required for fatty acid synthesis than males.

Conclusions

In summary, our results showed evidence that the prenatal nutrition effects are dependents on offspring sex. Strategic protein supplementation is effective to prevent a chronic growth retardation and to enhance muscle hypertrophy in the offspring. In practical terms, this condition represents economic advantages related to the time necessary for the offspring to achieve the market weight requirements. Furthermore, maternal protein supplementation during mid-gestation probably lead to a greater intramuscular fat content in the offspring, especially in females, improving the quality grade of beef.

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Declaration of interest

The authors declared that they don't have conflict of interest.

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Tables

Table 1. Medium chemical composition of the feedstuffs used in different phases of gestation for pregnant cows.

	Roughage Sources[1]			Supplement (mid-gestation)[2]
	Early	Mid	Late	
Chemical composition, g/kg of DM				
Dry matter	284.90	418.26	330.3	881.10
Organic matter	916.70	951.05	941.4	957.80
Crude protein	154.33	53.31	72.20	400.53
Ash and protein-free neutral detergent fiber	568.00	631.48	549.20	213.03
Non-fibrous carbohydrates	112.57	242.13	290.70	342.10
Ether extract	25.50	24.12	29.20	41.20

[1]Roughage sources: Early gestation = tropical grass pasture (*Brachiaria decumbens* cv. Marandu); Mid gestation = corn silage + sugarcane bagasse; Late gestation = corn silage

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

Table 2. Ingredients and chemical composition of the feedstuffs used in the confinement period.

	Background[1]		Growing 1[2]		Growing 2[3]
	Male	Female	Male	Female	
Ingredients, g/kg of DM					
Corn Silage	717.50	717.50	650.00	650.00	309.00
Ground Corn	129.00	152.50	204.50	232.50	577.00
Soybean meal	116.00	92.50	106.50	77.00	100.8
Urea	6.75	6.75	8.10	8.10	2.88
Ammonium Sulfate	0.75	0.75	0.90	0.90	0.32
Mineral Nucleus[4]	30.00	30.00	30.00	30.00	10.00
Chemical composition of experimental diet, g/kg of DM					
Concentrate					
Dry matter	910.46	900.55	900.01	865.98	917.97
Organic matter	828.60	865.92	849.68	890.47	930.41
Crude protein	401.08	401.08	315.03	242.96	219.61
Ash and protein-free neutral detergent fiber	144.30	180.58	151.08	231.53	253.31
Non-fibrous carbohydrate	266.80	262.95	363.02	398.13	426.58
Ether extract	16.42	21.29	20.57	17.88	30.90
Corn Silage					
Dry matter	321.60		347.28		329.38
Organic matter	948.84		883.29		905.32
Crude protein	92.66		72.05		80.29
Ash and protein-free neutral detergent fiber	558.34		547.03		553.76
Non-fibrous carbohydrates	272.45		244.18		246.39
Ether extract	25.39		20.03		24.88

[1] Background phase = 255 – 320 days

[2] Growing 1 phase = 321 – 381 days

[3] Growing 2 = 382 – 445 days

[4] Nutronbeef Maxima Marathon® (Cargill Animal Nutrition, Itapira, SP, Brazil). Assurance levels per kilogram of product were: 220 g Ca (max); 200 g Ca (min); 10 mg Co (min); 500 mg Cu (min); 6.60 mg Cr (min); 24 g S (min); 333 mg Fe (min); 18 g P (min); 17 mg I (min); 1500 mg Mn (min); 835 mg monensin; 6.60 mg Se (min); 50 g Na (min); 100000 UI vitamin A; 13300 UI vitamin D3; 233 UI vitamin E; 2333 MG Zn (min).

Table 3. Sequences (5′ to 3′) of primers the used in quantitative real-time.

Gene Name	*Gene Abbreviation*	Primer	Access code
Insulin-like growth factor 1 receptor	IGF1R	**F:** GAGTGGACAACAAGGAGAGAAC **R:** CTTCTCAGCCTCATGGTTACAG	NM_001244512.1
Mechanistic target of rapamycin kinase	mTOR	**F:** GTCATGGAGGACACGGATTAG **R:** GGACCAGTGAGGTAATGAGATG	XM_015475105.1
Myogenic differentiation 1	MyoD	**F** CGACGGCATGATGGACTAC **R** CGCCTCGCTGTAGTAAGTGC	NM_001040478.2
Myogenin	MyoG	**F:** CCTACAGACGCCCACAATCT **R:** TATGGTTTCATCTGGGAAGG	NM_001111325.1
Zinc finger protein 423	ZFP423	**F:** AGACAGGAACAGCGTGACAA **R:** CTGACAGTGATCGCAGGTGT	NM_001101893.1
Enhacer Biding protein alpha constitutive α	C/EBPA	**F:** CACGGTGCGTCTAAGATGAG **R:** TCCAAGGCACAGGGTTATTC	XM_027515988.1
Peroxisome proliferator-activated receptor γ	PPARG	**F** CGACCAACTGAACCCAGAGT **R** TCAGCGGGAAGGACTTTATG	NM_001098905.1
Transforming growth factor β1	TGF-β1	**F:** CTGGGCTGGAAGTGGATTC **R:** TCCAGGCTCCAGATGTAAGG	NM_001166068.1
Fibronectin 1	FN1	**F:** GGGGGCAGTCCTACAAGATT **R:** TTTGCCATTACCCAGACACA	NM_001163778.1
Collagen type III	COL3A1	**F:** AACCAGAACCGTGCCAAATA **R:** TGGGGCAGTCTAATTCTTGG	NM_001076831.1
Sterol regulatory element-binding factor 1	SREBF1	**F:** GAGCCACACACTTCAACGAA **R:** TGTCTTCTATGTCGGTCAGCA	NM_001113302.1
Fatty acid synthase	FAS	**F:** ATCAACTCTGAGGGGCTGAA **R:** CAACAAAACTGGTGCTCACG	U34794.1
Sterol regulatory element-binding protein 2	SREBP2	**F:** CGACGGCATGATGGACTAC **R:** CGCCTCGCTGTAGTAAGTGC	NM_001040478.2
Carnitine O-palmitoyltransferase 2	CPT2	**F:** CATGACTGTCTCTGCCATCC **R:** ATCACTTTTGGCAGGGTTCA	BC105423.1
Acetyl-CoA carboxylase 1	ACACA	**F:** TGAAGAAGCAATGGATGAACACA **R:** TTCAGACACGGAGCCAATAA	NM_174224.2
Adipocyte-type fatty acid-binding protein 4	FABP-4	**F:** GGATGATAAGATGGTGCTGGA **R:** ATCCCTTGGCTTATGCTCTCT	NM_174314.2
Stearoyl-CoA desaturase 1	SCD1	**F:** TTATTCCGTTATGCCCTTGG **R:** TTGTCATAAGGGCGGTATCC	NM_173959.4
Lipoprotein lipase.	LPL	**F:** CTCAGGACTCCCGAAGACAC **R:** GTTTTGCTGCTGTGGTTGAA	NM_001075120.1

Table 4. Effects of MN planes during mid-gestation and of OS on the offspring vigor score, body weight and average daily gain.

Item	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
Vigor Score, *arbitrary units*	2.52	3.39	2.94	2.96	0.208	<0.001	0.906	0.086
Female	2.33[c]	3.55[a]						
Male	2.70[bc]	3.22[ab]						
BW, *kg*								
Birth	27.3	31.1	29.2	29.2	3.957	0.049	0.976	0.565
7 days	31.7	36.7	34.7	33.7	4.205	0.014	0.623	0.483
30 days	51.2	59.4	55.4	55.3	3.322	0.003	0.990	0.628
60 days	78.9	92.6	84.4	87.1	4.484	0.001	0.474	0.543
120 days	126.4	138.1	131.1	133.5	5.730	0.037	0.674	0.345
210 days[1]	197.2	213.6	197.0	213.8	7.100	0.019	0.018	0.224
255 days[2]	212.6	229.2	209.6	232.2	15.000	0.030	0.005	0.381
320 days[3]	283.3	305.3	273.0	315.6	12.300	0.016	<0.001	0.303
381 days[4]	347.1	372.9	333.1	386.9	17.187	0.015	<0.001	0.280
445 days[5]	398.4	429.2	380.8	445.7	19.532	0.016	<0.001	0.441
Total ADG, *kg/d*								
Cow-calf phase[6]	0.818	0.875	0.812	0.881	0.304	0.050	0.025	0.239
Confinement[7]	0.947	0.984	0.874	1.058	0.047	0.376	<0.001	0.891
Total[8]	0.812	0.877	0.765	0.924	0.028	0.016	<0.001	0.767

[1] 210 days = weaning; [2] 255 days = begging of confinement phase; [3] 320 days = end of background phase; [4] 381 days = end of growing phase 1; [5] 445 days = end of growing 2 phase; [6] Cow-calf phase = birth from 210 days; [7] Confinement phase = 255 from 445 days; [8] Total ADG = 0 from 445 days.

[a-c] Different sub-scripts represents different means ($P < 0.10$).

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

Table 5. Effects of MN planes during mid-gestation and of OS on the offspring morphometric measurements (cm) at birth, 60 and 210 days.

Item	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
Birth								
Height at withers	67.2	71.5	67.8	70.9	3.467	0.146	0.291	0.933
Rump height	71.6	77.6	73.7	75.5	3.710	0.034	0.499	0.452
Rib deep	23.8	25.1	23.9	25.0	0.925	0.118	0.187	0.652
Rump deep	22.4	23.7	23.2	23.0	1.525	0.296	0.879	0.512
Body length	27.0	24.1	25.0	26.2	13.600	0.221	0.599	0.867
Ileus bones distance	12.1	13.3	13.0	13.0	1.395	0.259	0.987	0.204
Ischial bones distance	12.8	14.7	12.7	14.8	1.140	0.053	0.036	0.302
Abdomen width	14.8	17.6	16.9	15.5	1.454	0.015	0.179	0.321
Thorax width	16.8	17.1	17.9	15.9	1.097	0.733	0.015	0.588
Girth circumference	69.6	71.8	70.2	71.2	2.995	0.412	0.699	0.578
60 days								
Height at withers	84.2	84.5	85.0	83.7	2.604	0.906	0.573	0.832
Rump height	92.2	94.3	94.0	92.5	1.739	0.264	0.406	0.406
Rib deep	31.4	30.4	29.8	32.0	5.249	0.754	0.519	0.439
Rump deep	30.5	30.3	30.5	30.3	1.914	0.808	0.816	0.554
Body length	79.9	78.8	80.1	78.6	3.269	0.714	0.602	0.336
Ileus bones distance	16.2	16.9	15.8	17.3	1.899	0.425	0.145	0.144
Ischial bones distance	16.1	17.5	17.3	16.3	2.194	0.115	0.256	0.747
Abdomen width	20.5	20.8	18.9	22.4	4.539	0.92	0.251	0.937
Thorax width	24.5	24.4	23.9	24.9	0.776	0.915	0.160	0.663
Girth circumference	102.2	100.4	102.5	100.0	3.646	0.622	0.466	0.889
210 days								
Height at withers	114.9	119.3	117.6	116.6	5.058	0.082	0.699	0.651
Rump height	118.2	120.3	118.3	120.2	1.637	0.164	0.216	0.101
Rib deep	50.7	51.8	49.9	52.5	3.131	0.596	0.206	0.421
Rump deep	40.5	42.6	41.8	41.3	3.43	0.054	0.662	0.699
Body length	94.2	97.1	97.3	94.0	4.110	0.292	0.249	0.852
Ileus bones distance	26.8	28.3	27.9	27.2	1.677	0.214	0.520	0.716
Ischial bones distance	16.0	16.2	16.4	15.8	1.792	0.845	0.544	0.470
Abdomen width	36.2	35.4	34.5	37.2	3.771	0.729	0.264	0.295
Thorax width	68.9	68.1	64.5	72.5	20.368	0.955	0.589	0.334
Girth circumference	135.1	138.4	134.8	138.7	4.420	0.225	0.162	0.222

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

Table 6. Effects of MN planes during mid-gestation and of OS on the offspring blood concentration of insulin, glucose and IFG-I levels at 210 and 445 days.

Item	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
210 days								
Glucose, *mg/dL*	86.93	89.86	87.74	89.04	4.024	0.353	0.668	0.737
Insulin, *µUI/mL*	21.33	15.15	23.42	13.07	7.445	0.420	0.181	0.076
Female	33.46[a]	13.38[bc]						
Male	9.20[c]	16.92[abc]						
IGF-1, *ng/mL*	279.73	218.73	269.36	229.10	55.832	0.199	0.391	0.002
Female	381.00[ab]	157.71[c]						
Male	178.45[c]	279.75[bc]						
445 days								
Glucose, *mg/dL*	90.10	83.39	87.51	85.98	5.423	0.142	0.732	0.263
Insulin, *µUI/mL*	54.76	55.08	47.05	62.79	9.305	0.963	0.029	0.966
IGF-1, *ng/mL*	331.44	338.63	230.86	439.21	35.866	0.727	<0.001	0.378

[a-c] Different sub-scripts represents different means ($P < 0.10$).

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

Table 7. Effects of MN planes during mid-gestation and of OS on the offspring muscle development and fat thickness at cow-calf and confinement phases.

Item	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
Cow-calf phase[1]								
LMA, *cm²*	36.6	34.6	34.0	37.2	3.647	0.474	0.286	0.550
SFT, *mm*	2.30	2.47	2.39	2.38	0.189	0.219	0.908	0.083
Female	2.43[ab]	2.36 [ab]						
Male	2.17[b]	2.58[a]						
Confinement phase[2]								
LMA, *cm²*	51.2	51.7	48.6	54.3	6.169	0.750	0.002	0.767
SFT, *mm*	4.43	4.50	4.62	4.30	2.097	0.908	0.610	0.663
RML, *cm*	8.15	7.98	7.75	8.38	0.419	0.645	0.103	0.953
Rump fat, *cm*	5.02	5.37	5.35	5.04	1.033	0.701	0.730	0.382

[1]Cow-calf phase = 0 – 210 days

[2]Confinement phase = 255 – 445 days

[a-b] Different sub-scripts represents different means ($P < 0.10$).

Abbreviations: MN = Maternal nutrition; OS = offspring sex; CON (control) = offspring from CON cows; SUP (supplemented) = offspring from supplemented cows from 102 ± 5 to 208 ± 6 days of gestation; LMA = longissimus muscle area; SFT = subcutaneous fat thickness; RML = rump muscle lengh

Table 8. Least square means for mRNA expression at 7 and 445 days of age on the offspring *longissimus thoracis* according to MN and OS.

Item	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
7 days								
IGFR1	0.86	0.85	0.78	0.94	0.145	0.927	0.350	0.664
mTOR	0.88	1.02	0.97	0.93	0.086	0.056	0.825	0.525
MyoD	1.00	0.72	1.05	0.67	0.256	0.100	0.034	0.022
MyoG	0.94	0.90	1.10	0.73	0.29	0.879	0.170	0.343
ZFP423	0.80	0.76	0.82	0.73	0.224	0.384	0.578	0.607
C/EBPA	0.94	0.78	0.94	0.78	0.177	0.491	0.289	0.974
PPARG	0.97	0.95	1.01	0.91	0.166	0.878	0.535	0.822
FABP4	0.97	0.92	1.47	0.42	0.510	0.872	0.096	0.950
TGF-β1	0.99	1.01	1.12	0.89	0.234	0.778	0.031	0.460
FN1	1.02	1.09	1.18	0.92	0.343	0.423	0.054	0.057
COL3A1	1.04	1.19	1.08	1.16	0.125	0.203	0.506	0.222
445 days								
SRBF1	1.10	1.15	1.29	0.96	0.115	0.699	0.013	0.994
FAS	1.01	1.18	1.02	1.16	0.137	0.445	0.408	0.670
SREBP2	0.99	1.03	1.00	1.02	0.100	0.759	0.843	0.747
CPT2	1.01	0.82	0.94	0.89	0.065	0.037	0.528	0.859
ACACA	1.04	1.31	1.35	1.00	0.083	0.072	0.004	0.265
FABP4	1.05	1.84	1.62	1.27	0.395	0.009	0.866	<0.001
PPARG	1.04	1.99	2.08	0.94	0.362	0.121	<0.001	<0.001
SCD1	1.04	0.94	1.25	0.73	0.319	0.200	0.008	<0.001
LPL	1.08	1.45	1.53	1.00	0.163	0.090	0.020	0.263

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

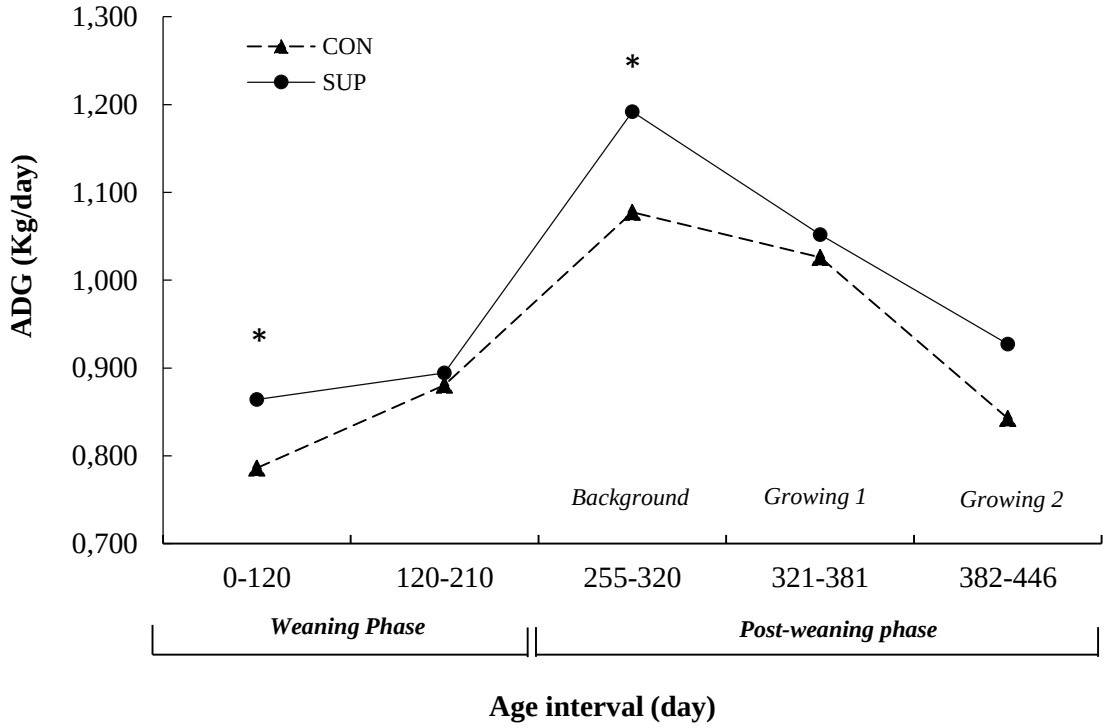

Figure 1. Effects of maternal nutrition plan during mid-gestation on offspring average daily weight gain. The symbols represent the average data from SUP (●) and CON (▲) groups. * $P \leq 0.10$; ** $P \leq 0.01$ and *** $P \leq 0.001$.

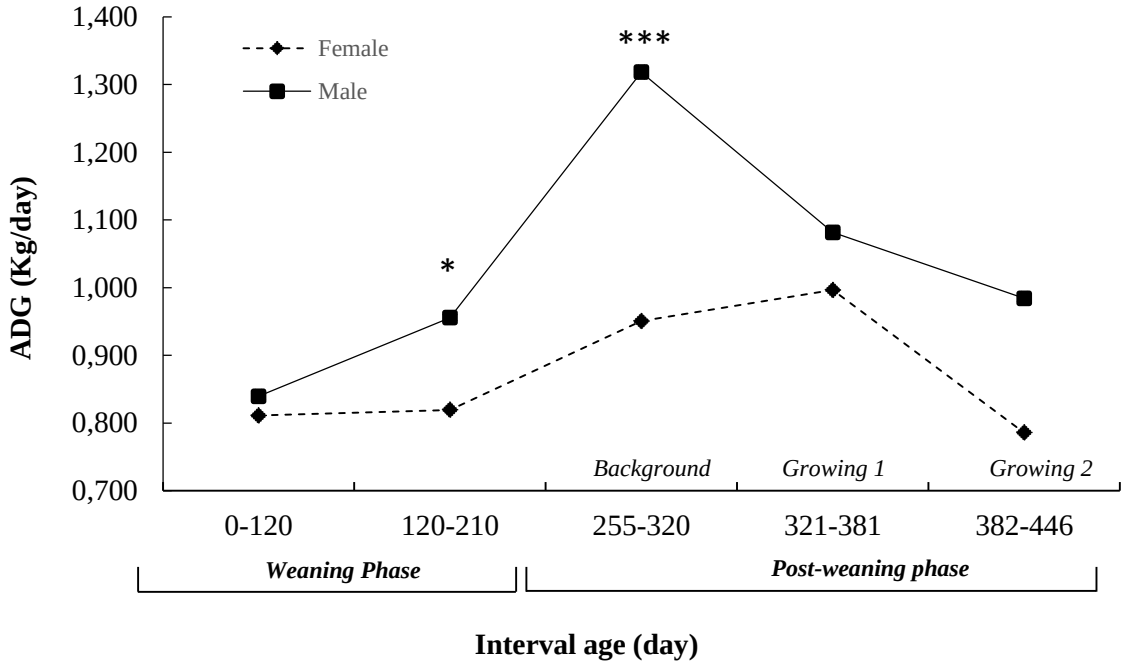

Figure 2. Effects of offspring sex on its average daily weight gain. The symbols represent the average data from Male (■) and Female (●) groups. * $P \leq 0.10$; ** $P \leq 0.01$ and *** $P \leq 0.001$.

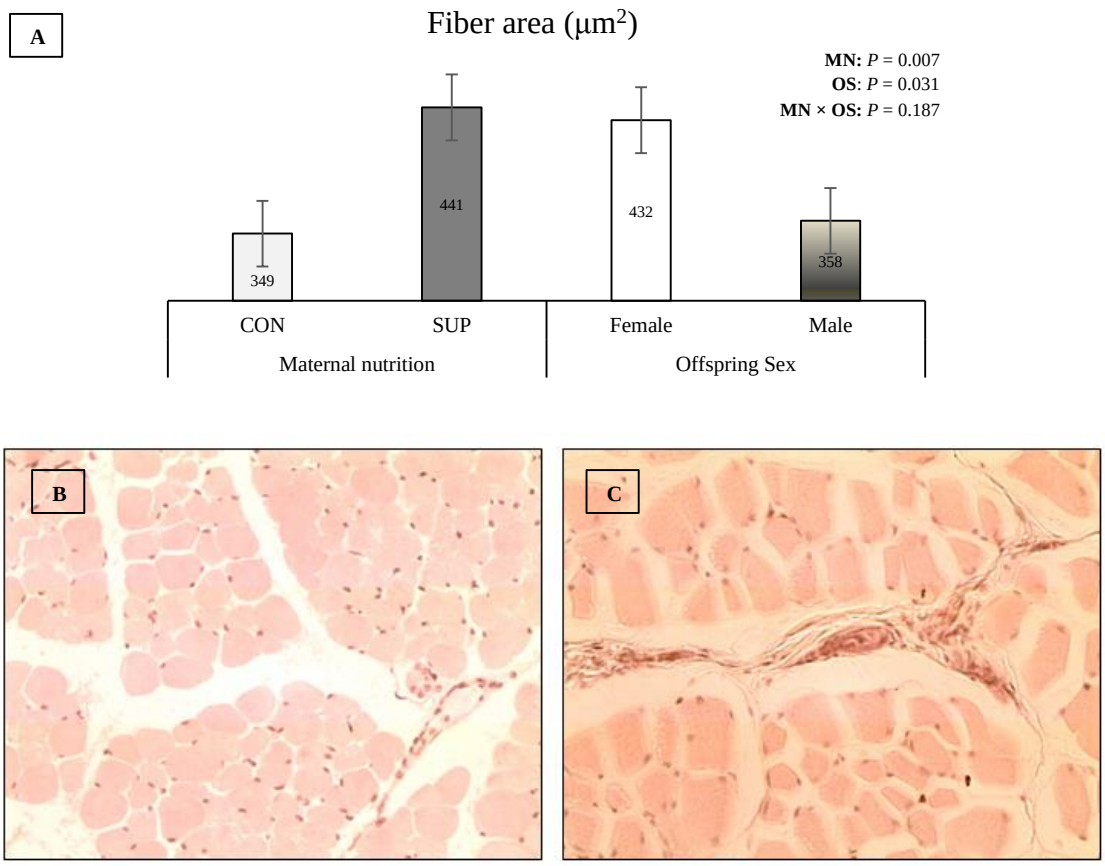

Figure 3. (**A**) Effects of MN (*n* = 24 CON; 19 SUP) and of OS (*n* = 20 females; 23 males) on fiber area of *longissimus thoracis* muscle collected though biopsies at 30 days of age Average of fiber size were accounted on 120 fibers per animal (µm²) captured at 40×. Representative image from CON (**B**) and SUP (**C**) groups. Bars represent means ± SEM. CON (offspring from CON cows); SUP (offspring from supplemented cows from 102 ± 5 to 208 ± 6 days of gestation).

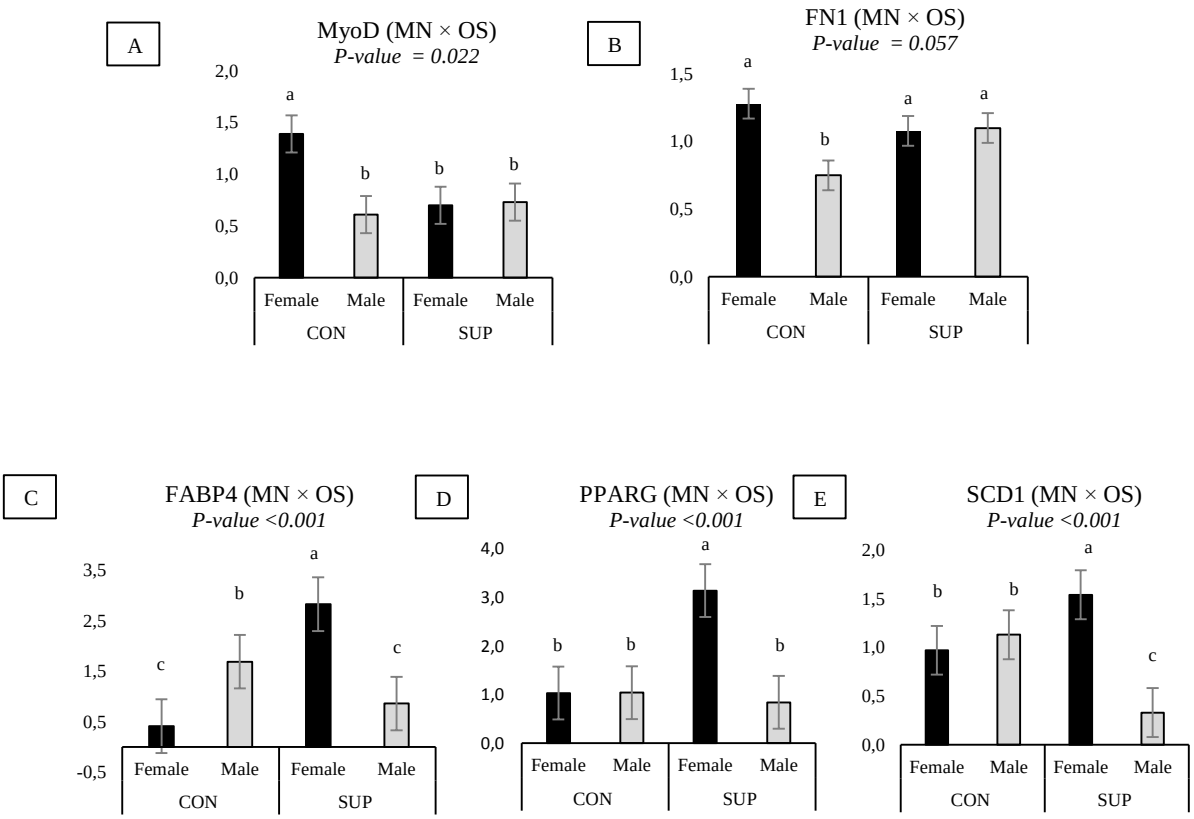

Figure 4. Interaction effect between nutrition treatment (CON *vs*. SUP) and offspring's sex (Female vs. Male) on the relative gene expression of *MyoD* (**A**), *FN1* (**B**), *FABP-4* (**C**) at 7 days and of *PPARG* (**D**), and *SCD1* (**E**) at 445 days. Different sub-scripts represent different means ($P < 0.10$).

SECOND SECTION – ARTICLE

ARTICLE 2 - Effect of maternal nutritional plane and calf sex on intake, digestibility and ingestive behavior of the offspring

Article formatted according to Livestock Science guidelines

Highlights

- Prenatal nutrition effects on the offspring are dependents of the sex.
- Prenatal nutrition programs the offspring intake and its ingestive behavior.
- Dam protein restriction promotes compensatory digestibility in the offspring.
- Females were less susceptible to variations on digestibility by maternal nutrition.
- Dam protein restriction does not change the offspring's feed efficiency.

ARTICLE 2 - Effect of maternal nutritional plane and calf sex on intake, digestibility and ingestive behavior of the offspring

ABSTRACT

This study aimed to assess the effects of maternal protein restriction during mid-gestation and of the offspring sex (OS) on the progeny intake, ingestive pattern, digestibility, feed efficiency for gain (FE_{gain}), and liver size in a long term. A completely randomized 2 × 2 factorial design, referring the maternal nutrition (MN) and OS was used. Forty-three Tabapuã cows from 102± 5 to 208 ± 6 days of gestation were randomly assigned in two nutritional plans: the control group [**CON**; basal diet with 5.5% crude protein (CP); *n* = 24] or supplemented [**SUP**; 40% CP at the level of 3.5 g/kg body weight (BW); *n* = 19]. Offspring were evaluated from birth to 445 days. The cow-calf phase was performed in a pasture system. Post-weaning (background, growing 1, and growing 2) phases were performed in feedlot. Statistic differences were declared at $P < 0.10$. The dry matter intake (DMI) was higher in SUP offspring at weaning ($P = 0.065$), growing 1 ($P = 0.044$) and in total confinement period ($P = 0.060$). The feed efficiency for gain (FE_{gain}) was higher for males in the background ($P = 0.024$). At weaning CON-males presented lower organic matter (OM) digestibility compared to CON-females (MN × OS: $P = 0.070$). At same age, females presented greater CP digestibility greater than males ($P = 0.050$), while SUP offpring presented greater non detergent fiber (NDF) digestibility than CON. In the background the DM digestibility (MN × OS: $P = 0.042$) was worsened in CON-males compared to SUP-males. A MN × OS interaction was detected for total tract digestibility of all diets components in the growing 2 phase ($P \leq 0.089$). Overall, lower digestibility coefficients were found for SUP-males compared to CON-males during this phase. The 100-days-old offspring of SUP group spent more time eating supplement ($P = 0.050$). CON-females spent lower time in rumination at 100 days of age (MN × OS: $P = 0.071$) and at confinement period (MN × OS: $P = 0.039$). At weaning, CON group spent more time in idleness ($P = 0.004$), but SUP group spent more time in others activities ($P = 0.019$). No effects of MN, OS, or MN × OS interaction were observed for liver size or liver size adjusted for BW ($P > 0.10$). In conclusion, adequate maternal nutrition increases the offspring's feed intake in the long term and reduces the digestibility of some nutrients as a function of sex.

***Keywords*:** appetite control, fetal programming, organogenesis, sexual dimorphism, zebu beef cows

1. Introduction

In tropical livestock systems such as Brazil, grasses are the main source of feed for herds (Poppi et al., 2018). In these feeding systems, seasonality affects forage production and nutritional value (Reis et al., 2013), being the drop in protein content the most striking factor (Paulino et al., 2002). Among the consequences of dietary protein restriction for ruminants are the impaired ruminal microbial growth, reduced dietary fiber degradation, altered passage rate, and a ruminal filling effect which reduce the intake (Lazzarini et al., 2009). Thus, it is expected not only a nutritional restriction of protein but also a reduction in energy supply for cattle managed under rangeland conditions at dry season. In the tropics, the breeding and calving seasons align with the rainy period (Gionbelli *et al*., 2018), as a strategy to attend to the greater nutrient requirements for lactation and also to promote an earlier reproductive return. However, with this practice, pregnancy cows raised under extensive systems experience this extremely unfavorable nutritional scenario at mid-to-late gestation (Rodrigues *et al*., 2020).

According to the fetal programming hypothesis, alterations in the prenatal environment orchestrate the future productivity of the offspring (Greenwood et al., 2010). Nevertheless, few efforts had been done to investigate if, in ruminant animals, changes observed in the offspring performance are related to modifications in the animal's intake characteristics and nutrients used. There is evidence that maternal nutrition can program the intake (Stevens et al., 2010; Long et al., 2011; Smith et al., 2018), affect the mass of several organs (Zhang et al., 2016; Duarte et al., 2013), the membrane transporters in the small intestine (Cruz et al., 2019), the enzyme activity (Keomanivong et al., 2015) and metabolic pathways (Zhou et al., 2019) in the offspring. However, although the presence of studies indicating that prenatal nutritional planes may modify the offspring intake and its capacity to use nutrients, changes in characteristics as intake, ingestive behavior, and digestibility need to be father investigating in beef cattle.

In this sense, this study aimed to evaluate the effects of protein supplementation during the mid-gestation in Zebu beef cows and the sex-dependent interaction on intake parameters, ingestive behavior, digestibility, feed efficiency, and liver size in the offspring. Our hypothesis is that protein restriction during the mid-third of gestation can alter behavior and ingestion parameters, and promote compensatory responses on nutrient digestibility and feed efficiency, differently in male and female offspring. As far as we

know, this is the first study to assess the effect of the maternal nutritional plane and the interaction with offspring sex on intake and digestion parameters in Zebu beef calves.

2. Materials and methods

This study was carried out in the Beef Cattle Facility at the *Universidade Federal de Lavras* (UFLA - Lavras, Minas Gerais, Brazil) and was done in two stages of 2 and half years each with the same experimental procedures. Each period included the accompaniment of beef cow's gestation and the offspring evaluation from birth to 445 days. Animal welfare and all procedures were previously approved by the Brazilian Ethics Committee on Animal Use of UFLA (Protocol No. 015/17).

2.1. Animals, housing and feeding

Animal handling procedures have been previously reported in Nascimento et al., (2021). Briefly, forty-three purebred Tabapuã (*Bos taurus indicus*) multiparous cows (490.5 ± 17.8 kg of initial BW) previously inseminated using semen from different bulls and pregnant from females (n = 20) and males (n = 23) were confinement in individual pens at mid-gestation. At 102± 5 days of gestation, they were randomly divided into two groups: **Control (CON) -** supply of basal diet [corn silage + sugarcane bagasse, achieving 5.5% crude protein (CP) plus a mineral mixture] (n = 24) or **Supplemented (SUP) -** basal diet plus protein supplementation [40% CP at the level of 3.5 g/kg body weight (BW)] (n = 19). The experimental diet (DM basis) provided from 102± 5 to 208 ± 6 days of gestation was based on 75% of corn silage [DM = 35.8 %; CP = 6 %, and neutral detergent fiber (NDF) = 57.3%], by 25% of sugar bagasse (DM = 82%; CP = 2%, and NDF = 77.2%) and by a macro and micro mineral mixture provided *ad libitum.* For cows from SUP group, the protein supplement consisting of a 50:50 mixture of soybean meal with a commercial supplement (Probeef Proteinado Sprint®, Cargill Nutrição Animal, Itapira, SP, Brazil). The average crude protein restriction was equivalent to 70% of protein and 50% of energy requirements for CON cows [calculated according to the Nutrient Requirements of Zebu and Crossbred Cattle - BR-CORTE 3.0 (Valadares Filho et al., 2016)] at the mid-gestation. From 208 ± 6 days of gestation until the parturition all cows were fed *ad libitum* with corn silage (DM = 35.2%; CP = 7.2%, and NDF = 54.9%) and a mineral mixture. In both periods of gestation, animals were fed twice a day (at 0700 and 1300 h). After parturitions, cows and their calves were allocated in a *Brachiaria decumbens* cv. Marandu pasture area (DM = 29.7 %; CP = 13.0 %; NDF = 62.6%) and

raised in an intensive grazing management. Cows received a mineral mixture, and calves received supplementation (3.5 g/kg of BW) through a commercial supplement (Probeef maxima creep®, Cargill Nutrição Animal, Itapira, SP, Brazil) provided by the creep-feeding technique at the level of 5 to 7 g per kg of BW. The assurance level per kilogram of product were: 200 g crude protein (min); 20 g Ca (max); 30 g Ca (min); 3 mg Co (min); 51 mg Cu (min); 1 mg (min) Cr; 10.4 g dextrose; 3000 mg S (min); 0.42 ethoxyquin (min); 2000 mg F (max); 6000 mg P (min); 3 mg I (min); 700 mg mananas (min); 108 mg Mn (min); 60 mg monensin; 0.90 mg Se; 10 g Na (min); 12000 UI vitamin A (min); 15000 UI vitamin D3 (min); 50 UI vitamin E (min); 180 mg Zn (min).

Calves were weaned at 210 days but remained in a pasture system until 255 days when they were transferred for a feedlot. Animals were confined during 190 days, being this period divided into three phases with different diets - the background (255 to 320 days of average age); growing 1 (321 to 381 days of average age), and growing 2 (382 until 445 days of average age) phases. Males and females were fed *ad libitum* and received diets with the same ratio roughage: concentrate in the background (72:28), growing 1 (65:35), and growing 2 phases (30:70). Nevertheless, the concentrate formulation on the background and growing 1 phases were different for males and females, aiming to fully met the specific nutritional requirements for each sex. The diets provided during the confinement period were provided as total mixed rations (TMR). The ingredients used and the chemical composition of feedstuffs used in each phase of both experimental stages are described in **Table 1**. Samples of corn silage were collected 3 times per week; orts samples were collect ever when the quantity was considered significant (orts over 20% of the total mixed ration provided), and representative concentrated samples were collected after every ration mixture confection in the feed factory. All samples were stored at -20°C until analyses. Heifers and steers had free access to water and were fed twice daily (0700 and 1300 h) at the feedlot.

From the 43 progeny used in this experiment, 2 were born extremely weak and died until 7 days of age, being both CON males. Moreover, after weaning, one female from the CON group died due to an external factor. Thus, the data presented at the cow-calf phase are from results obtained from 41 animals (excepted during the neonatal period), while data presented from weaning to end of growing 2 phase are from 40 animals.

2.2. Measurements

Intake, digestibility trials, and feed efficiency. In the cow-calf phase, calves were subject to two digestibility trials at 120 and 210 days of age. For estimation of forage and supplement intake and fecal production quantification, the indigestible neutral detergent fiber (NDF_i) (Valente *et al*., 2011), chromium oxide (CrO_2) (Kimura *et al*., 1957), and the titanium dioxide (TiO_2) (Myers *et al*., 2004) were used as indicators, respectively. The titanium dioxide was provided one time per day in the morning (0700 h) wrapped in paper cartridges in doses of 10 g of TiO_2 per animal administered using an esophagus probe. The chromium oxide was provided mixed in the calves' supplement in a concentration of 0.5% of the supplement consumed during the digestibility trials. Both indicators were provided during 10 consecutive days, being the fecal sample collected by spot technique in the morning (0700 h) and afternoon (1830h) of the last four days of the indicators supply. Pasture samples were obtained on days 6, 7, 8, and 9 of the digestibility period through the manual grazing simulation technique. For pasture collection, the total area was subdivided into parts, which were individually sampled, to ensure the obtaining of a final representative sample.

The milk intake was obtained as the product between the daily milk production and milk DM content. The fecal production, the supplement dry matter intake (DMI_{Sup}), and the roughage dry matter intake ($DMI_{Forrage}$) were estimated according to **Equations 1**, **2**, and **3**, respectively:

$$FP(kg/day) = \frac{I_{suplied}}{I_{feces}} \quad \text{Eq. (1)}$$

Where: FP = fecal production; $I_{Supplied}$ = concentration of indicator supplied to the animal (kg/d) and I_{Feces} = concentration of indicator in the feces (kg/kg) (Myers *et al*., 2004).

$$DMI_{sup}(kg/day) = \frac{(FP \times I_{feces})}{I_{sup}} \quad \text{Eq. (2)}$$

Where: FP = fecal production (kg/day), I_{Fe} = indicator concentration in the feces (kg/kg) and I_{Sup} = indicator concentration in the supplement (kg/kg) (Kimura *et al*., 1957).

$$DMI_{forage}(kg/day) = \frac{(FP \times I_{feces})}{NDFi_{forage}} \quad \text{Eq. (3)}$$

Where: FP = fecal production (kg/day), NDF*i* $_{feces}$ = concentration of NDF*i* in the feces (kg/kg) and NDF*i* $_{Forrage}$ = concentration of NDF in roughage (kg/kg) (Valente *et al*., 2011).

Cows were milked to the determination of milk intake by calves, being calves isolated from their dams for approximately 12 hours before each procedure. Cows were manually milked using 2 ml of oxytocin (Ocitocina Forte UCB, Uzinas Chimicas Brasileiras S/A, Jaboticabal, Brasil) in the morning (0600 h). Then, the milk was weighed and milk samples were collected in sterile vials containing a bronopol tablet (D & F Control Systems Inc., San Ramon, CA). The tubes were kept at 4° C until analysis. The daily milk yield (MY) was calculated as:

$$MY(kg/day) = \frac{MY_{morning}(kg/day)}{(Time_1+1) - Time_2} \quad \text{Eq. (4)}$$

Where: MY = milk yield; $Time_1$ = hour that the milk procedure end; $Time_2$ = hour of calf isolation of dams (Galvão, 2018).

In the post-weaning phase, there was one digestibility trial per phase during the confinement period. Trials were performed at 310, 370, and 425 days of age in the background, growing 1 and growing 2 phases, respectively. Fecal samples were collected by spot technique directly by the rectum, for 5 consecutive days at different times at each day (day 1 = 0600 h; day 2 = 0900 h; day 3 = 1200 h; day 4 = 1500 h and day 5 =1800 h). The orts were recorded daily before the morning feeding and DMI was measured for each animal. Daily, during the trials, samples of corn silage and orts were collected, being also collected representative samples of concentrates used in respective phases. Samples were stored at -20°C until analyses. The NDF_i was used as an indicator to measure the fecal production, which was estimated through **Equation 1**.

At cow-calf phase and confinement phases, the apparent total tract digestibility of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), and total digestible nutrients (TDN) expressed in g/kg of DM were determined by the difference between intake and the content in feces divided by intake.

To calculate the intake related to body weight, weighing data were obtained from performance tests were, however, these data will not be presented here. Offspring performance response may verify in detail at Nascimento et al. (2021). The feed

efficiency for gain was obtained as the ratio between the average daily gain (ADG) and the DMI, considering the average of ADG and DMI during the entire period of each phase.

Chemical analyses. For chemicals analysis, all samples (feed, orts, and fecal samples) were individually dried in a forced dry oven (65°C) for 72 h and ground (Wiley mill; A. H. Thomas, Philadelphia, PA) in 1 and 2 mm bolters. Samples were chemically analyzed following AOAC (1990) methods, (CP, 984.13; Ash, 119 942.05; EE, 920.39; Moisture, 934.01). The neutral detergent fiber (NDF) content was analyzed according to Van Soest *et al*. (1991) using heat-stable α-amylase. Non-fibrous carbohydrates (NFC) were calculated according to Detmann and Valadares Filho (2010). The quantification of Cr_2O and TiO_2 was done through atomic absorption spectrophotometry and thought colorimetric determination, according to Kimura *et al*. (1957) and Myers *et al.* (2004), respectively. The NDF_i quantification was performed according to Valente *et al.* (2011), thought samples in situ incubation by 288 h. Milk samples were analyzed for composition determination in a commercial laboratory using an infrared analyzer (Bentley2000, Bently Instruments).

Liver ultrasound scans. At the background phase (276 days of average age), young bulls and heifers were submitted to liver ultrasound scans of model A6v (Sonoscape®, Henzhen, China) using a multi-frequency convex transducer (3.0 to 5 MHz). The liver was located between the 5^{th} and 12^{th} intercostal space at the right side of the animal. This area was previously trichotomized before ultrasound scans to facilitate image capture. Scans were performed with constant topical application of 70% liquid alcohol and done according to the method proposed by Braun (2009) and Haudum et al. (2011). The ultrasonography measurements necessary for liver size determination were: (**1**) the distance from the dorsal midline to dorsal liver margin; (**2**) distance from the dorsal midline to the ventral liver margin; (**3**) distance from the lateral wall of the liver to the portal vein; and (**4**) the distance from the lateral wall of the liver to the caudal vena cava. The liver size was determined as the difference of distance 2 and 1.

Ingestive Behavior. The ingestive behavior was monitored by human observation for 48 hours uninterrupted at 100 and 210 days of age at the cow-calf phase and in the middle of the confinement period (360 days of age), at growing 1 phase. Calves were monitored for frequency and time of milk and supplement intake continuously. The

activities of grazing, rumination, idleness or other activities (locomotion or water intake) were evaluated every 10 min in the weaning phase. In the confinement period, young bulls and heifers were monitored for the time of rumination, consumption, idleness, or other activities (locomotion or water intake) also at intervals of 10 min.

2.3 Statistical analysis.

All data analyses were performed using SAS 9.2 (Statistical Analysis System Institute, Inc., Cary, NC, USA). The dietary treatment (2 levels) and offspring sex (2 levels) were considered as fixed-effect. The period in which the experiment was done (two stages of 2 and a half years each) and the parents' index of genetic merit expected for growth traits (GEN) was considered as a random effect. GEN was calculated using the data available on the Tabapuã Genetical Enhancement Program using the information of expected progeny difference (EPD) about the cow's parents. The EPD parents' data consisted of weight at weaning, weight at 12 months, and weight at 18 months. Since the concentrate formulation was different between females and males in the background and growing 1 phases, for these data the diet was also considered as a random effect.

When pertinent ($P < 0.05$), the dam's numbers of parturitions; empty weight at 100 days of gestation; cow size; BSC with 100 days of pregnancy; gestation length, the offspring age at evaluation period, and/or its body weight at evaluation period were used as a covariate. When not pertinent, they were taken from the model. The following statistic model was used:

$$Yijkl = \mu + Di + Sj + (DS)ij + Tk + Gl + Dm + \varepsilon ijklm$$

Where: $Yijk$ is the observed measurement; μ is the overall mean; Di is the fixed-effect of the i^{th} level of maternal dietary treatment; Sj is the fixed effect of the j^{th} level of offspring sex; $DSij$ is the interaction between D and S; Tk is the random effect of the k^{th} period; Gl is the random effect of the l^{th} index of dam's genetic merit expected for growth traits; Dm is the random effect of the m^{th} dietetic factor and $\varepsilon ijkl$ is the random error associated with $Yijkl$, with $eijklm \sim N(0,\sigma e2)$.

Before the final analyses, studentized residuals were removed when not within ± 3 standard deviations, and normality ($P > 0.05$) was assessed using Shapiro-Wilk's test. Least-squares means were separated using Fisher's least significant difference test. When

the interaction between the fixed effects was significant, the least square means were compared using Tukey's method. Results were deemed significant when $P \leq 0.10$.

3. Results

3.1. *DMI, DMI/BW and feed efficiency for gain*

Table 2 presents the responses of intake and intake in relation to the offspring's BW. Male calves had an ~0.6 kg additional DMI at 120 days of age ($P = 0.020$) than females, without discriminated differences for milk, pasture, and supplement intake ($P > 0.10$). On the other hand, at 210 days of age the only differences were observed in pasture intake with ~13.3% additional for male calves ($P = 0.091$). At weaning, 210-days-old SUP offspring had ~11.3% higher total DMI ($P = 0.065$). Overall, the DMI during entire confinement period was ~7.9% higher in SUP ($P = 0.060$) offspring, mainly influenced by intake during growing 1 phase ($P = 0.044$, 6.92 *vs*. 7.56). In the background and in growing 2 phases, no effects of MN, OS, or MN × OS interaction were observed ($P > 0.10$; **Table 2**) on the feed intake.

The DMI/ BW was influenced by offspring sex, with an additional intake of ~25.1% per kg of BW in 120-days-old males ($P = 0.012$). At weaning, the SUP calves consumed ~2.1 g of additional feed per kg of BW compared to the CON group ($P = 0.081$). During confinement period, only one MN × OS interaction was observed in growing 1 phase with higher DMI per kg of body weight in SUP-females compared to SUP-males ($P = 0.013$; **Table 2**).

The feed efficiency for gain data that are shown in **Figures 1** and **2**. The prenatal diet did not affect the offspring feed efficiency in any point of their development ($P \geq 0.10$). Only an effect of offspring sex was observed during the background phase on this parameter, where males gained ~36 additional g per kg of feed intake compared to females ($P = 0.024$).

3.2. *Components of diet intake and apparent total-tract digestibility*

Tables 3 and **4** present the results of nutrient intake and apparent total tract digestibility of the offspring, respectively. The 120-days-old males had additional intake of ~70 g/day of CP ($P = 0.046$), while at weaning they present an additional intake of OM ~590 g/day compared to females ($P = 0.015$). At weaning, an MN effect was observed for OM and CP intake, with additional intake of ~410 and ~90 g/day for SUP animals

($P \leq 0.083$; **Table 3**), respectively. During the growing 1 phase, no effects of MN or OS were verified on the components of diet intake ($P \geq 0.10$). In growing 2 phase, males presented an ~7.6% additional intake of OM than females ($P = 0.047$). A MN × OS interaction was observed for TDN intake, where CON females and SUP males had the lowest intake per day ($P \leq 0.001$).

Regarding apparent digestibility parameters, females had ~2.9% greater CP digestibility compared to males at 210 days of age ($P = 0.051$; **Table 4**). At the same age, the SUP offspring had ~3.5% greater NDF digestibility ($P = 0.011$). In this period, a MN × OS interaction was detected ($P = 0.070$) on OM digestibility (**Figure 3**). This parameter was worsed in CON-males compared to CON-females. In the background phase, an MN × OS interaction was observed for DM and NDF digestibility ($P \leq 0.086$). The DM digestibility was worsened for CON-males compared to SUP-males. By the other hand, the FDN digestibility was improved for CON-females. During growth phase 1, no effects of fixed effects were found on digestibily parameters ($P \geq 0.10$). MN × OS interaction was verified for all nutrients digestibility in the growing 2 phase ($P \leq 0.089$; **Figure 3**). Overall, lower coefficients were found for SUP-male compared to CON-males.

3.3. *Ingestive Behaviour*

Data of ingestive behavior are presented in **Table 5**. 100-day-old male calves spent ~6.7 and ~8.4 additional minutes per day suckling and eating the supplement ($P \leq 0.099$), respectively. The time spent in other activities and idleness were also affected by the OS in this period. Males spent ~41 additional minutes per day in other activities, but ~60.5 minutes less per day in idleness than females ($P \leq 0.043$; **Table 5**). In this same evaluation period, calves from SUP cows spent ~8.7 additional minutes per day eating the supplement ($P = 0.050$). There was MN × OS interaction for rumination time at 100 days. CON-female spent less time per day in this activity ($P = 0.071$).

At weaning, the offspring from CON dams spent ~1:45 additional hours per day in idleness and ~1:10 hours less per day in other activities ($P \leq 0.019$; Table 5). At 360 days of age, at the confinement, males spent ~ 2 additional hours per day for idleness than females ($P = 0.041$). There was also an interaction between MN × OS for rumination time, with lower values verified for CON-females compared to SUP-females.

3.4. *Liver size*

There were no effects of MN (P = 0.908; CON = 24.36 *vs*. SUP = 24.48; EPM = 1.34) and of OS on the offspring liver size (P = 0.118; females = 23.53 *vs*. males = 25.31; SEM = 1.49). The liver size adjusted for BW was also not influenced by MN plane (P = 0.483) or by OS (P = 0.911). No interactions between the fixed effects were found for liver size (P = 0.588) and liver size adjusted for BW (P = 0.352). MN × OS interaction for liver size (P = 0.588) and liver size adjusted for BW (P = 0.352) were not observed.

4. Discussion

To our knowledge, this is the first study in beef cattle that investigates the effect of maternal protein supplementation on intake characteristics and nutrient use in offspring throughout productive life. In this way, our study demonstrated that strategic supplementation could program the offspring intake pattern in a long term. The greater intake for SUP offspring detected during some points of its development suggests the programming of the appetite control *in utero*. Researches had been shown that the hypothalamic circuit which controls the offspring feed intake may be shaped by dams' nutritional status during gestation (Muhlhausler *et al*., 2006; Prezotto *et al*., 2018; Stevens *et al*., 2010), indicating a predisposition of the offspring to deregulation of appetite. In the arcuate nucleus (ARC) of the hypothalamus, where the feed intake control occurs, there are two groups of neurons with opposite roles, denominated orexigenic and anorexigenic neurons. The orexigenic neurons secrete neuropeptide Y (NPY) and agouti-related protein, stimulating feed intake. In contrast, anorexigenic agents promote feeling satiety by secretion of cocaine-amphetamine-regulated transcript and the melanocortin peptide, which is derived from proopiomelanocortin (POMC) (Bouret *et al*., 2015; Bell *et al*., 2005). Studies suggested that maternal malnutrition during gestation increases NPY expression and decreases POMC gene promoter methylation in the offspring (Warnes et al., 1998; Begum et al., 2012). Both genes are involved in ingestion control and in ingestive behavior (Ginane et al., 2015). Others studies also showed that maternal nutrition plane during gestation is associated with leptin resistance (Delahaye *et al*., 2008). Since leptin normally decreases feed intake through inhibition of orexigenic neurons and stimulation of anorexigenic neurons (Bell et al., 2005), this suggests a predisposition to hyperphagia and obesity in offspring (Muhlhausler et al., 2006).

Moreover, according to the theory of the thriftiness genotype proposed by Hales and Barker (1991), sons of mothers who faced nutritional insults during pregnancy are more efficient in the nutrients acquisition and stock. Consistent with this hypothesis, some

works indicates that offspring from dams in such conditions may present hyperphagia characteristics in later life (Greenwood *et al*. 1998; Ginane 2015; Smith *et al*., 2018). Based on this, a study using ewes with nutritional restriction of energy and protein during early- to mid-gestation resulted in hyperphagic ram lambs, with greater feed efficiency (George et al., 2014). However, in contrast with these findings, our responses showed a different pattern from those reported in scientific literature. Thus, the highest intake was verified in animals from well-nourished dams, without differences detected for feed efficiency in the present study. Such response may be related to the greater BW and gain potential of these animals, which possibly increases their nutritional requirements and the intake level, explained the behavior verified. Nevertheless, although changes in the hypothalamic axis and leptin levels were not measured in our study, it is probably that these differences in ingestive patterns of offspring from dams with different nutritional planes during gestation may be also related with alterations on these caractetristics.

This work also revealed new information inherent to the effect of maternal protein supplementation on the offspring digestibility, being these effects sex-dependents. Interestingly our results demonstrated that the effects of maternal nutrition on digestibility did not follow a single pattern under different stages of offspring development and under different diet types. In general, the nutrients digestibility of young males from CON dams was reduced at weaning and background phase. On the other hand, when males from supplemented dams received high-concentrate diets in the growing 2 phase, the digestibility of all nutrients was reduced. This pattern suggested the presence of a compensatory mechanism in the digestion and absorption of high-energy diets developed for CON-males in a long term.

Consistent with our findings, there is recent evidence for ruminant animals, showing that small intestine mass per kg of BW, its length, villus length, and permeability increase in offspring from dams who suffered nutritional restriction during gestation (Duarte *et al*., 2013; Zhang et al., 2018). Thus, these reports shown that there is a greater absorptive capacity in animals form undernourished dams. Farther, Cruz *et al*. (2019) demonstrated that changes promoted by prenatal diet involving the small intestine persist in adult life. These authors showed that males from restricted Zebu beef cows without protein supplementation from mid-gestation had a greater intestine length, and also a greater expression of key genes related to glucose and fatty acids absorption in the small intestine. Although key genes related nutrients absorption were not measured objectively, our subjective observations agree with these findings. Nevertheless, its important to

highlight that the comprensatory digestibility for CON-males in the growing 2 phase did not reflected in a greater performance. In this sense, nourish properly pregnant beef cows is essential for producing higher productive animals.

Furthermore, the effects of protein supplementation of the dams during mid-gestation on dietary digestibility in females were slight. Therefore, our findings suggesting that females seem to be less susceptible to changes in digestibility than males.

When milk production of the dam is not sufficient to meet the demand of the offspring, calves try to compensate for this condition by spending more time grazing (Vargas *et al*., 2010). In this study, milk production and composition did not change between CON and SUP cows (unpublished data). Thus, this point agrees with the absence of maternal nutrition effects on suckling and grazing times by calves during the cow-calf phase. At 100 days of age, SUP calves spent more time eating supplements than CON calves. Yet, from a practical point of view, this change was small, which corroborates with the lack of maternal nutrition effects on supplement intake verified in the digestibility trial performed at 120 days of age. Moreover, at 100 days, there was an interaction between MN × OS for ruminating time, showing that females from supplemented dams during mid-gestation spent more time ruminating than females from unsupplemented dams. These effects on rumination time were persistents in a long term, being also detected during the ingestive behavior trial performed at 360 days. With rumination the feed is regurgitated and remasticated, reducing the particle size and stimulating salivation, to lubricate the bollus. In this sense, saliva maintains the optimum rumen pH, acting as a buffer (Beauchemin, 2018), contributing to prevent ruminal acidosis. Thus, CON-female may probable be more susceptible to this disturbance ocurrence. Moreover, the greater time in idleness for CON group and in other activities for SUP group at weaning showing that offspring from supplemented dams during mid-gestation exhibit an ingestive pattern more compatible to achieve the productive goals. Thus, maternal nutrition during pregnancy is able to affected the offspring behavior during the long life.

Liver biometric measurements were performed aiming to investigate possible effects of maternal nutrition on offspring organ development in a long term. Several studies had shown that maternal nutrition during pregnancy can affect the offspring's organ development and physiological functions. Zhang *et al*. (2016) found low weight in the pancreas, stomach, liver, spleen, kidneys, lung, among other organs in fetuses from dams with poor nutritional planes during gestation. Nevertheless, the effects of maternal

nutrition plane seem to be not equal to all organs. McMillen *et al.* (2001) found an inverse relationship between relative brain mass and fetal body weight, indicating that, since the brain is considered a vital organ, its mass is preserved in situations of high stress for the fetus. Moreover, the 'thrifty phenotype theory proposes that nutrient scarcity during specifics windows of development leads to a nutrient reallocation to favor critical organs for immediate survival at the expense of other organs secondarily necessary, causing failures in the adult life (Hales and Barker, 2001). In this sense, we previously hypothesized that MN level could lead to a lower liver size. As animals used in this study were part of the permanent research herd and could not be slaughtered, ultrasound scans were used for this purpose. However, the results verified in this study did not demonstrate differences inherent to the effect of protein supplementation on the liver size of the adult offspring, demonstrating the plastic nature of the liver.

The main objective of this work was to investigate the differences in the ingestive parameters of offspring affected by gestational maternal nutrition and a possible interaction of sexual dimorphism. However, research with Zebu animals studying the same variables in our trial is scarce. It is well established that males and females have different nutritional requirements and that they have physiological differences related to the dynamic of fat and muscle deposition, which brings as consequence discrepancies on the DMI between the sexes (NRC, 1996). Dry matter intake decrease as fat deposition increases. Consequently, as females reach a degree of finish earlier than males, they have a lower feed intake at certain points of development (Bonilha *et al*., 2015). This explains the higher DMI for males compared to females. Furthermore, the lower feed efficiency for gain in heifers compared to steers in the confinement may be occurred due to the higher energy demand for fat accumulation than for muscle deposition, making the gain process more expensive for females (Lage *et al*., 2011).

Regarding sexual dimorphism effects on ingestive behavior, this work showed that young calves exhibit sex-specific differences. Although males and female dams did not differ about their daily milk production at the points evaluated (unpublish data), 100-days-old males spent more time per day sucking than females. Similar behavior was verified by Costa *et al*. (2006), which reported that this response occurs due to the higher demand for milk for males than for females, once they are heavier. It is possible, that the greater time eating supplement verified for males and the greater time in idleness for females at 100 days, was also a reflection of the higher growth potential of males.

The lack of significant differences in the behavior trial at 210 days, demonstrated that with advancing age, with reduction of dam's dependence (Silva *et al*., 2016), the differences in the ingestive pattern between sexes became unexpressive. Consistent with this in the confinement phase, there was only an isolated OS effects for time spent in idleness between males and females.

5. Conclusions

In summary our results shown evidences that the prenatal nutrition effects are dependents of the offspring sex. Together, our results demonstrated that protein supplementation during the mid-gestation has positive effects on the offspring intake and in its ingestive behavior in a long-term. Although protein restriction may cause a compensatory response in the offspring nutrients digestibility, this response is not associate with an increase in the its performance. Therefore, protein restriction during prenatal period is not productively beneficial to the offspring in a long term.

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Conflicts of interest

The authors declare no conflict of interest.

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633 **Tables**

634 **Table 1.** Ingredients and chemical composition of the feedstuffs used in the confinement.

	Background[1]		Growing 1[2]		Growing 2[3]
	Male	Female	Male	Female	
Ingredients, g/kg of DM					
Corn Silage	717.50	717.50	650.00	650.00	309.00
Ground Corn	129.00	152.50	204.50	232.50	577.00
Soybean meal	116.00	92.50	106.50	77.00	100.8
Urea	6.75	6.75	8.10	8.10	2.88
Ammonium Sulfate	0.75	0.75	0.90	0.90	0.32
Mineral Nucleus[4]	30.00	30.00	30.00	30.00	10.00
Chemical composition of experimental diet, g/kg of DM					
Concentrate					
Dry matter	910.46	900.55	900.01	865.98	917.97
Organic matter	828.60	865.92	849.68	890.47	930.41
Crude protein	401.08	401.08	315.03	242.96	219.61
Ash and protein-free neutral detergent fiber	144.30	180.58	151.08	231.53	253.31
Non-fibrous carbohydrate	266.80	262.95	363.02	398.13	426.58
Ether extract	16.42	21.29	20.57	17.88	30.90
Corn Silage					
Dry matter	321.60		347.28		329.38
Organic matter	948.84		883.29		905.32
Crude protein	92.66		72.05		80.29
Ash and protein-free neutral detergent fiber	558.34		547.03		553.76
Non-fibrous carbohydrates	272.45		244.18		246.39
Ether extract	25.39		20.03		24.88

[1] Background phase = 255 – 320 days

[2]Growing 1 phase = 321 – 381 days

[3]Growing 2 = 382 – 445 days

[4]Nutronbeef Maxima Marathon® (Cargill Animal Nutrition, Itapira, SP, Brazil). Assurance levels per kilogram of product were: 220 g Ca (max); 200 g Ca (min); 10 mg Co (min); 500 mg Cu (min); 6.60 mg Cr (min); 24 g S (min); 333 mg Fe (min); 18 g P (min); 17 mg I (min); 1500 mg Mn (min); 835 mg monensin; 6.60 mg Se (min); 50 g Na (min); 100000 UI vitamin A; 13300 UI vitamin D3; 233 UI vitamin E; 2333 MG Zn (min).

Table 2. Effects of MN and OS on the intake and DMI/kg of BW.

Item	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
Intake, *kg of DM/day*								
120 days								
Total DMI	2.09	1.97	1.73	2.33	0.347	0.451	0.020	0.354
Milk	1.32	1.22	1.21	1.32	0.108	0.325	0.253	0.178
Pasture	0.76	0.83	0.67	0.91	0.155	0.609	0.107	0.583
Supplement	0.15	0.18	0.16	0.16	0.071	0.645	0.964	0.107
210 days								
Total DMI	3.97	4.42	3.99	4.40	0.384	0.065	0.104	0.766
Milk	0.87	0.81	0.90	0.78	0.146	0.528	0.241	0.426
Pasture	2.64	2.97	2.63	2.98	0.377	0.103	0.091	0.488
Supplement	0.41	0.57	0.42	0.56	0.112	0.145	0.220	0.188
Background	5.31	5.76	5.05	6.03	2.161	0.132	0.350	0.654
Growing 1	6.92	7.56	6.71	7.77	4.525	0.044	0.627	0.668
Growing 2	8.14	8.33	7.99	8.48	0.412	0.528	0.165	0.795
Total Confinement	6.56	7.08	6.296	7.348	2.487	0.060	0.384	0.877
DMI/BW, *g of DM/kg of BW*								
120 days	17.40	16.82	15.20	19.02	1.337	0.586	0.012	0.297
210 days	18.88	20.98	19.18	20.68	2.022	0.081	0.220	0.638
Background	22.45	22.71	22.54	22.63	8.61	0.759	0.983	0.510
Growing 1	23.00	23.36	23.64	22.72	12.78	0.433	0.880	0.013
Female	22.86^{ab}	24.43^{a}						
Male	23.14^{ab}	22.30^{b}						
Growing 2	22.65	23.33	22.48	23.50	1.045	0.344	0.241	0.923
Total Confinement	22.396	22.415	22.654	22.158	7.763	0.974	0.894	0.120

[1] 255 days = begging of confinement period; [2] 320 days = end of background phase; [3] 381 days = end of growing 1 phase; [4] 445 days = end of growing 2 phase. [a-d] Different sub-scripts represents different means ($P < 0.10$). Abreviations: MN = Maternal nutrition; OS = offspring sex; CON (control) = offspring from unssuplemented cows; SUP (supplemented) = offspring from supplemented cows from 102± 5 to 208 ± 6 days of gestation.

Table 3. Effects of MN and OS on the diets components intake during the digestibility trials, expressed in kg/day.

	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
120 days								
OM	1.00	1.15	1.00	1.16	0.173	0.338	0.423	0.906
CP	0.50	0.49	0.46	0.53	0.033	0.630	0.046	0.893
NDF	0.60	0.65	0.55	0.70	0.127	0.676	0.244	0.678
210 days								
OM	2.34	2.75	2.25	2.84	0.248	0.083	0.015	0.954
CP	0.66	0.75	0.68	0.73	0.046	0.038	0.177	0.851
NDF	2.14	2.42	2.14	2.41	0.372	0.142	0.162	0.665
310 days – Background Phase								
DM	6.13	6.08	6.01	6.20	1.648	0.888	0.920	0.788
OM	5.79	5.886	5.78	5.89	1.508	0.744	0.946	0.685
CP	1.19	1.20	1.17	1.22	0.526	0.974	0.942	0.244
NDF	3.08	3.06	3.07	3.07	0.299	0.877	0.993	0.680
TDN	4.28	4.16	4.28	4.16	1.344	0.661	0.940	0.659
370 days – Growing 1 Phase								
DM	7.52	7.79	7.70	7.62	2.119	0.241	0.980	0.681
OM	7.14	7.43	7.42	7.16	2.823	0.205	0.936	0.360
CP	1.11	1.13	1.03	1.21	0.347	0.402	0.730	0.639
NDF	3.70	3.77	3.88	3.59	1.053	0.597	0.580	0.912
TDN	4.36	4.66	4.60	4.42	2.733	0.303	0.957	0.694
425 days – Growing 2 Phase								
DM	9.32	9.56	9.28	9.59	0.800	0.625	0.621	0.653
OM	8.94	8.83	8.56	9.21	0.553	0.720	0.047	0.793
CP	1.76	1.79	1.71	1.84	0.138	0.714	0.160	0.833
EE	0.28	0.29	0.29	0.29	0.019	0.974	0.552	0.885
NDF	3.22	3.34	3.16	3.39	0.279	0.382	0.119	0.848
TDN	5.06	4.96	5.06	4.96	0.479	0.726	0.818	<0.001
Female	4.54[b]	5.57[a]						
Male	5.58[a]	4.35[b]						

[a-b] Different sub-scripts represents different means ($P < 0.10$). Abreviations: MN = Maternal nutrition; OS = offspring sex; CON (control) = offspring from unssuplemented cows; SUP (supplemented) = offspring from supplemented cows from 102 ± 5 to 208 ± 6 days of gestation.

Table 4. Effects of MN and OS on the offspring apparent total-tract digestibility, expressed in g/kg of nutrient.

	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
120 days								
DM	879.32	872.96	874.66	877.63	11.567	0.511	0.758	0.363
OM	826.72	819.59	824.44	821.87	19.848	0.662	0.875	0.466
CP	913.04	904.79	911.34	906.50	13.231	0.457	0.661	0.851
NDF	735.11	755.20	743.35	746.96	30.700	0.408	0.907	0.423
210 days								
DM	814.02	814.04	818.72	809.34	20.101	0.998	0.387	0.391
OM	809.20	805.97	811.41	803.76	17.167	0.790	0.519	0.070
CP	868.38	858.89	875.91	851.36	25.518	0.427	0.051	0.303
NDF	794.24	822.19	799.50	816.93	19.948	0.011	0.110	0.243
310 days - Background Phase								
DM	717.91	726.13	719.67	724.38	26.899	0.432	0.693	0.042
OM	723.98	731.55	730.78	724.75	32.091	0.549	0.868	0.113
CP	756.10	726.29	751.15	731.24	61.693	0.112	0.528	0.619
NDF	626.65	615.89	635.61	606.93	14.374	0.406	0.032	0.086
TDN	720.68	710.28	723.18	707.78	33.252	0.412	0.300	0.130
370 days - Growing 1 Phase								
DM	643.39	636.11	663.74	615.76	109.25	0.739	0.627	0.921
OM	645.02	639.77	672.35	612.44	97.567	0.800	0.499	0.754
CP	581.21	568.83	559.18	590.85	103.49	0.674	0.735	0.835
NDF	595.77	535.67	597.28	534.17	53.07	0.227	0.205	0.938
TDN	744.00	768.00	797.78	714.22	222.05	0.553	0.677	0.732
423 days - Growing 2 Phase								
DM	612.36	599.81	610.23	601.94	35.778	0.664	0.780	0.006
OM	692.79	668.58	691.36	670.01	33.681	0.396	0.469	0.018
CP	645.01	596.64	622.56	619.10	58.682	0.139	0.918	0.089
NDF	627.60	614.30	625.91	615.99	49.017	0.674	0.760	0.024
TDN	698.84	684.13	680.61	702.35	36.127	0.549	0.404	0.002

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

Table 5. Effects of MS and of OS on the offspring ingestive behavior.

	MN		OS		SEM	*P-value*		
	CON	SUP	Female	Male		MN	OS	MN × OS
100 days, *min/day*								
Suckling	19.88	24.64	18.90	25.62	6.573	0.200	0.099	0.479
Eating supplement	16.54	25.20	16.67	25.08	4.208	0.050	0.056	0.830
Grazing	247.24	233.50	238.42	242.33	28.070	0.341	0.793	0.847
Ruminating	213.59	230.84	219.43	225.00	32.569	0.283	0.747	0.071
Female	196.07[b]	242.79[a]						
Male	231.11[ab]	218.90[ab]						
Idleness	817.43	798.59	838.27	777.75	58.265	0.453	0.032	0.261
Others activities	125.12	136.04	110.00	151.16	29.643	0.576	0.043	0.806
210 days, *min/day*								
Suckling,	25.85	24.31	25.14	25.01	4.365	0.682	0.973	0.744
Eating supplement	33.40	34.54	28.59	39.35	6.806	0.870	0.127	0.628
Grazing	384.49	420.28	427.40	377.37	57.345	0.289	0.156	0.593
Ruminating	212.71	236.21	212.54	236.38	22.968	0.215	0.214	0.146
Idleness	723.22	618.51	669.85	671.88	45.511	0.004	0.953	0.133
Others activities	125.73	195.34	167.42	153.65	54.589	0.019	0.651	0.215
360 days								
Eating	186.99	200.45	187.95	199.49	54.625	0.425	0.698	0.262
Ruminating	340.77	428.86	353.95	415.69	41.99	0.003	0.057	0.039
Female	279.79[b]	428.10[a]						
Male	401.75[a]	429.62[a]						
Idleness	695.42	760.22	667.95	787.69	53.19	0.155	0.041	0.518
Others activities	90.39	87.28	81.93	95.73	173.36	0.675	0.868	0.967

[a-b] Different sub-scripts represents different means ($P < 0.10$). Abreviations: MN = Maternal nutrition; OS = offspring sex; CON (control) = offspring from unssuplemented cows; SUP (supplemented) = offspring from supplemented cows from 102± 5 to 208 ± 6 days of gestation.

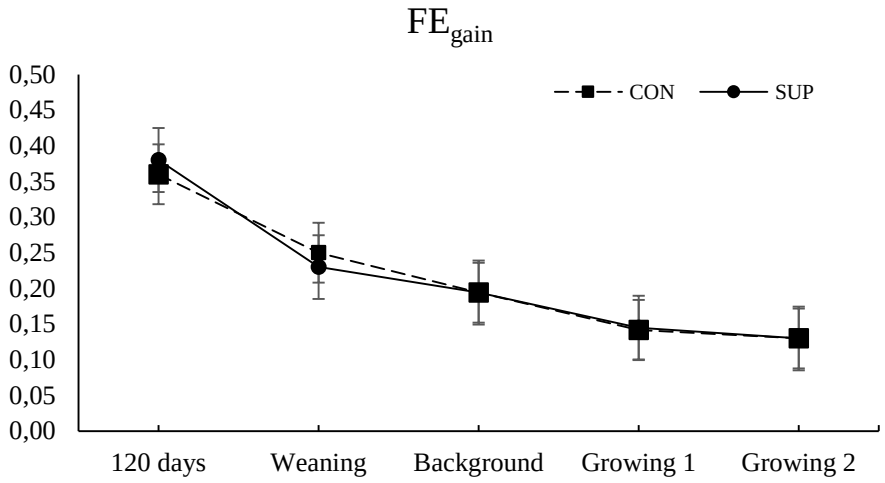

Figure 2. Effects of MN on offspring feed efficiency for gain [FE_{gain} (g/day of BW per kg of DMI/day)]. CON (offspring from unsupplemented dams at mid-gestation, *n* = 24); SUP (offspring from dams supplemented with protein at mid-gestation *n* =19). The symbols represent the average data from SUP (●) and CON (■) groups.

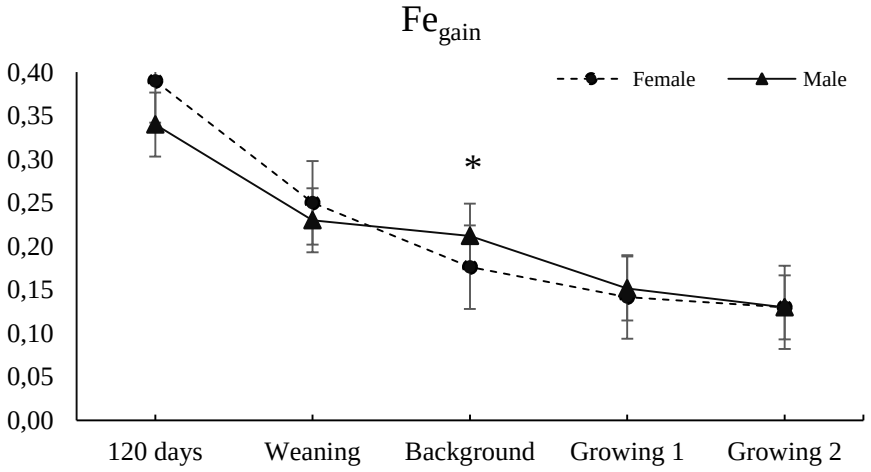

Figure 3. Effects of OS (female *n* = 20; male *n* = 23) on offspring feed efficiency for gain [FE_{gain} (g/day of BW per kg of DMI/day)]. The symbols represent the average data from Male (▲) and Female (●) groups. * $P \leq 0.10$.

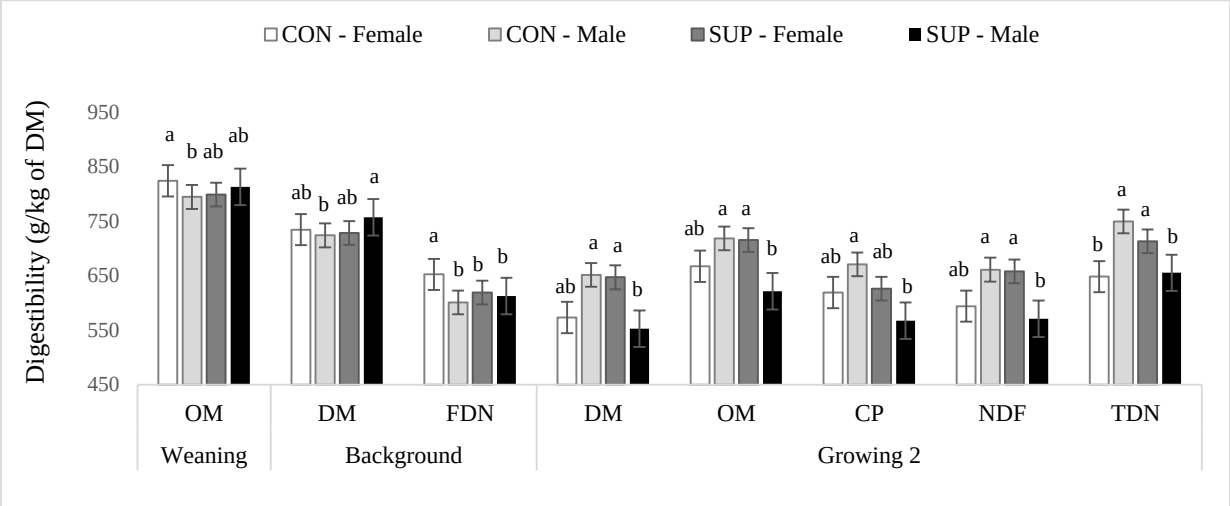

Figure 3. Interactions between MN × OS on the dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), non-fibrous carbohydrate (NFC) and on total digestive nutrients (TDN) apparent total-tract digestibility (complementary data from **Table 4**). Bars represent means ± SEM. [a,b] Significant differences between the groups ($P < 0.10$).