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ORIENTADOR

Dr. Márcio Machado Ladeira

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APROVADA em 20 de Março de 2018.

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He was a giant
And I was just a kid
I was always trying
To do everything he did
I can still remember every lesson he taught me
Growing up learning how to be like my old man

He was a lion
We were our father's pride
But I was defiant
When he made me walk the line
He knew how to lift me up
And when to let me fall
Looking back, he always had a plan
My old man

My old man
Feel the callous on his hands
And dusty overalls
My old man
Now I finally understand
I have a lot to learn
From my old man

Now I'm a giant
Got a son of my own
He's always trying
To go everywhere I go
Do the best I can to raise him up the right way
Hoping that he someday wants to be
Like his old man

My old man
I know one day we'll meet again
As he's looking down
My old man
I hope he's proud of who I am
I'm trying to fill the boots of my old man

To my old man...

(Zac Brown Band)

BIOGRAPHY

Liziana Maria Rodrigues, daughter of Vanor Rodrigues do Carmo (*in Memoriam*) and Eliza Ines da Silva Rodrigues, was born in Argirita/MG-Brazil on October 24, 1984. She started the undergrad in Animal Science at *Universidade Federal Rural do Rio de Janeiro* in 2004 and became a Bachelor of Science in Animal Science in 2008. At the same year she started the M.S. program with major on Animal Nutrition and Horse Production. In October of 2009 she became a M.S. in Animal Science. From May of 2010 to February of 2012 she worked as a professor at *Instituto Federal de Minas Gerais – Campus Bambuí.* In March of 2014 she started her D.S. program in Animal Science with major on ruminant nutrition and beef cattle production. From July of 2016 to June of 2017 she was a visiting scholar at Purdue University, West Lafayette/IN, USA where part of her research was developed. On March 20th of 2018 Ms. Rodrigues submitted her thesis to the committee to obtain the *Doctor Scientiae* degree in Animal Science.

ABSTRACT

The objective of the present study was to evaluate the effect of maternal nutrition on cow performance as well as progeny growth by gene expression in muscle and intestine from birth until weaning. Nine-two pregnant cows were raised on *Brachiaria brizantha* pasture (8.2% CP and 68.5% NDF) and supplemented with mineral (NS) or a protein supplement (SUPP) (36%CP, 0.2%BW) from middle of gestation until calving. During gestation, cows were weighted and had BCS measured monthly. Cows entered in a FTAI protocol 60 days after calving and had the pregnancy rate measured. At calving, calves were weighed, identified and six calves from each treatment were slaughtered. Body components were measured and samples of muscle and jejunum were collected for histological and gene expression analyses. In addition, 10 calves from each treatment had muscle biopsy samples collected at 11 ± 4 days and at 240± 4. Calves were weighed again at 120 days after birth and at weaning. Least square means of all data were analyzed using the GLM procedure of SAS. Cow BW and BCS did not differ during the study ($P > 0.05$), however, SUPP cows gained more BW (79.2kg vs. 95.3kg, $P = 0.03$) and body condition (0.01 vs. 0.36 units, $P = 0.05$) during gestation. As a result, pregnancy rate in the subsequent breeding season tended (62.1% vs. 78.6%, $P = 0.10$) to be greater for SUPP cows. On the other hand, BW gain during lactation was greater for cows from the NS group (59.1kg vs. 26.4kg, $P = 0.05$). Calves from SUPP cows had greater birth BW compared to NS calves (33.7kg vs. 35.8kg, $P = 0.05$). However no differences in BW at weaning or ADG of calves were found. Body components from slaughtered calves did not differ between treatments ($P > 0.05$), except for smaller intestine length that were greater for NS calves (17.2m vs. 15.9m, $P = 0.05$) with small crypt size (13.1µm vs. 19.5µm $P < 0.01$). Twenty-four hours after birth muscle of calves from SUPP group had greater expression of *WNT10B* ($P = 0.01$), *PPARG* ($P = 0.03$), *CD36* ($P = 0.04$) and

TGFβ1 (P = 0.01) compared to calves from NS cows with no differences in the other genes. This greater expression of *WNT10B*, *PPARG*, *CD36* and *TGFβ1* indicates that 24 hours after birth, calves from SUPP group still had multipotent cells performing differentiation for myogenesis, adipogenesis and fibrogenesis. Muscle of SUPP calves had less expression of *C/EBPA* (P = 0.01) and *FABP4* (P = 0.07) than calves from NS cows 11 days after birth, suggesting greater amount of adipocytes resulting from an early adipogenesis in the NS calves as a metabolic adaptation to increase the body reserves. At weaning, muscle of SUPP calves had a tendency to have greater expression of *PPARG* (P = 0.08), and greater *ZFP423* (P = 0.04) and *TGFβ1* (P = 0.02) than muscle of NS calves. The increase in expression of adipogenic genes in SUPP calves supports the hypothesis that these animals had a late adipogenesis. *β-catenin*, *mTOR COL3A1* and *FN1* genes were not affected by maternal diet (P > 0.05). In conclusion, supplementation of pregnant cows during dry season leads to a positive effects for subsequent breeding season and offspring birth weight. Supplementation had greater effects on myogenesis, and adipogenesis of calves. While calves born from restricted cows presented some gut adaptations for restricted condition.

Keywords: adipogenesis, fetal programming, fibrogenesis, histology, myogenesis.

RESUMO

O objetivo do presente estudo foi avaliar o efeito da nutrição materna sobre o desempenho das vacas, bem como o crescimento da progênie pela expressão gênica no músculo e no intestino desde o nascimento até o desmame. Noventa e duas vacas prenhas de bezerro macho foram criadas em pastagem *Brachiaria brizantha* (8,2% PB e 68,5% FDN) e suplementadas com mineral (NS) ou com suplemento protéico (SUPP) (36% PB, 0,2% PC) a partir do 124º dia até o término da gestação. Durante a gestação, foram realizadas pesagens e avaliação do ECC mensalmente. Após o parto as vacas foram submetidas à um protocolo IATF para verificação da taxa de prenhez subsequente. Os bezerros foram identificados e pesados ao nascimento, e novamente pesados aos 120 dias e ao desmame. Seis bezerros de cada tratamento foram abatidos 24h após o nascimento para aferição dos componentes corporais e coleta de amostras para análises histológicas e da expressão de genes no músculo e jejuno. Aos 11 ± 4 dias e ao desmame, 10 bezerros de cada tratamento foram submetidos à biópsia muscular para análises de expressão gênica. Os dados foram analisados usando o procedimento GLM do SAS. O peso e ECC das vacas não diferiram durante o estudo (P> 0,05), no entanto, as vacas SUPP ganharam mais peso (95,3 kg vs. 79,2 kg, P = 0,030) e condição corporal (0,36 vs. 0,01, P = 0,050) durante a gestação, resultando no aumento na taxa de prenhez na estação de monta subseqüente (78,6 % vs. 62,1%, P = 0,100). O ganho de peso durante a lactação foi maior para as vacas do grupo NS (59.1kg vs. 26.4kg, P = 0.050). Os bezerros das vacas SUPP apresentaram maior peso ao nascimento em relação aos bezerros NS (35,8 kg vs. 33,7 kg, P = 0,050). No entanto, não foram encontradas diferenças no peso ao desmame ou GMD de bezerros durante este período. Os componentes corporais dos bezerros abatidos não diferiram entre os tratamentos (P> 0,05), porém houve aumento no comprimento do intestino delgado (17,2 m vs. 15,9 m, P = 0,05) e diminuição do tamanho de cripta (13,1 μm contra 19,5 μm P <

0,01) nos bezerros de vacas NS. Houve maior expressão de *WNT10B* (*P* = 0,01), *PPARG* (*P* = 0,03), *CD36* (*P* = 0,04) e *TGFβ1* (*P* = 0,01) no músculo de bezerros nascidas de vacas SUPP, 24 horas após o nascimento, indicando maior atividade de células multipotentes que realizavam a diferenciação de miogênese, adipogênese e fibrogênese. O músculo dos bezerros do SUPP apresentou menor expressão de *C/EBPA* (*P* = 0,01) e *FABP4* (*P* = 0,07) do que os bezerros das vacas NS, 11 dias após o nascimento, sugerindo maior quantidade de adipócitos resultantes de uma adipogênese precoce nos bezerros NS como adaptação metabólica para aumentar as reservas corporais. Ao desmame, o músculo dos bezerros SUPP teve maior expressão de *PPARG* (*P* = 0,08) e maior *ZFP423* (*P* = 0,04) e *TGFβ1* (*P* = 0,02) do que o músculo dos bezerros da NS, indicando auemento da adipogênese tardia em relação aos bezerros do grupo NS. Os genes *β-catenina*, *mTOR* *COL3A1* e *FN1* não foram afetados pela dieta materna no bezerro ao nascer. A suplementação afetou a miogênese e a adipogênese dos bezerros, com benefícios para o grupo suplementado. Enquanto bezerros nascidos de vacas restritas apresentavam adaptações intestinais para condições restritas.

Palavra-chave: adipogênese, fibrogênese, histologia, miogênese, programação fetal.

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SUMARY

FIRST CHAPTER

1. INTRODUCTION

The majority beef cattle herd in Brazil is raised in an extensive system, using grass pastures as the main food source. The forage availability and quality change according to the seasons, with low production and quality during drought, which affects entire production system, mainly cow-calf phase. In addition, the most common breed raised in Brazil is Nellore and these animals are rustic and resistant to different hostile environments. However, Nellore cows have low fertility which can negatively affect livestock reproduction and calf production.

In general, the calving and breeding season is planned to happen during the rainy season when pastures are in good conditions and cows have enough nutrients to get pregnant. This is good for fertility, and also for calving season when cows needs good nourishment for milk production and feed for their calves. But in this scenario, mid and late gestation will coincide with drought season. At this moment the lack of forage's quantity and quality can affect the fetal development as well as cow's fertility in the next breeding season. In this condition, protein is the main limiting nutrient in grazing animals because protein limits microbial growth and fiber forage usage as an energy source (SAMPAIO et al., 2009). In addition, the requirement of protein for pregnant cows increases due to the protein deposition for fetal development (GIONBELLI et al., 2015).

The cow-calf operation is greatly affected by seasons and could be impaired by extensive systems. According to HESS et al. (2005), it is important to identify nutritional factors that could potentially affect cows' reproductive performance and calves production. For this reason, increased reproduction within this nutrition system are a challenge.

Nutrition during pregnancy is important for fetal growth and cow fertility in the subsequent breeding season. Failure of cow to become pregnant and the postpartum anestrus interval are the most impairing factors in cow-calf system, and they are strong related with undernutrition (NASEM, 2016). Moreover, maternal diet may affect long-term genetic expression through epigenetic mechanisms. The nutritional stimulus is received and recorded by the genome and will be revealed by gene expression during cell differentiation (FUNSTON; SUMMERS, 2013). Muscle development and adipose tissue begin during fetal phase (DU et al., 2010) and may affect carcass characteristics and beef value for the industry. Therefore, it is important to know the physiological mechanisms, and how dam nutrition interferes with muscle deposition, distribution and regulation of lipid deposition in different fat tissues (HAUSMAN et al., 2014). Nutrients and dietary compounds are able to provide methyl group donors, as well as affect the behavior of enzymes involved in methylation and histones modification, leading to changes in gene expression, cell differentiation and consequently in the composition of the progeny tissues (FUNSTON; SUMMERS, 2013; PARADIS et al., 2017). Although we do not have concrete information on amino acids requirements for pregnant zebu cows, it is known that methionine is one of the first limiting and essential for DNA methylation. This mechanism is an indispensable process for proper embryonic development through transcription regulation, remodeling of chromatin, and so on (CHMURZYNSKA, 2010).

On the other hand, manipulation of progeny tissues composition may be interesting for zebu cattle, which is generally leaner and tougher than taurine. Hence, the knowledge of epigenetic and nutrigenomic mechanisms that affect the prenatal development can be used to improve meat quality. However, the reason for tougher and leaner beef in zebu are not completely clear and there is a need for studies to clarify if this effect is an

exclusively genetic feature or if it is possible to manipulate it through nutritional plane during fetal development, which usually happen during the drought.

It has been demonstrated that maternal nutrition can affect fetus development in different species, but how Nellore cows respond to this effect is still not clear. On the other hand, pregnant cows can mobilize body reserves to compensate for undernourishment, and it will delay reproductive cycle. Thus, it is important to understand the mechanisms of interference on muscle growth to improve calves performance. Likewise, understanding the nutrition requirement of pregnant cows is possible to promote calving recover soon and maintain cow fertility. Also, fetal programming or dam nutrition effect on the offspring is a mechanism that should be better understood and can help to improve beef cattle production (NASEM, 2016).

The hypothesis of this study is that feeding protein supplement to pregnant cows during dry season would increase cow nutrition resulting in better reproduction performance in the next breeding season, as well as greater weaning weight and myogenesis of the progeny. Therefore, this study had the objectives to evaluate the effects of dietary crude protein (CP) supplementation during mid to late gestation on cow performance, calves growth, myogenesis, lipogenesis and fibrogenesis of the progeny.

2. LITERATURE REVIEW

2.1. Nutritional requirements of pregnant cows

It is important to know the proper nutritional requirements for pregnant cows due to the importance of cow-calf operations on beef cattle production. However, there is not enough information about nutrient requirements of pregnant zebu beef cows. Once the cow gets pregnant, she adapts her metabolism to support fetus development. This

adaptation starts with hormone signals sent from the fetus. Moreover, for the proper recommendation, it is necessary to consider the requirements for fetal development, for the maintenance of gestational tissues and for udder development, especially when there is an increase in parenchyma production and preparation for milk production (GIONBELLI et al., 2016). Consideration should also be given to the parity, in primiparous cows the requirements for growth should be considered. According to GIONBELLI et al. (2016) beef cattle production system in Brazil, has a potential to increase around 30 to 40% with improving the cow-calf operations in nutrition, genetics and reproduction characteristics.

Most nutritional requirements systems consider calf weight at birth and the factors that affect this weight as a reference to calculate pregnant cow requirements (NRC, 2000). In other words, factors such as the breed of the parents, heterosis effect, parity, number of fetuses, progeny gender, temperature and maternal nutrition are factors that can alter birth weight and consequently the requirements (FERRELL, 1991).

The main source of energy for fetal development is glucose, amino acids, and lactate. In this case, pregnant cow change her nutrients metabolism to supply the requirement. In addition, nutritional cost may reach 75% higher in relation to non-pregnant cows of the same body weight (BAUMAN; CURRIE, 1980).

As pointed out by BELL & EHRHARDT (2000), 35 to 40% of energy requirements for fetus development comes from placental glucose and lactate, 55% comes from amino acids and just 5 to 10% from acetate. Besides, amino acids are the main source of nitrogen for the fetus. For this reason, with gestation advancement, cows mobilize amino acids from their bodies and increase hepatic gluconeogenesis to support fetus necessity. Because the fetus cannot use non-esterified fatty acids (NEFA) as an energy source, maternal lipid metabolism needs to change when mobilization occurs. With this, released

NEFA are used primarily by maternal tissues as a way to save glucose and amino acids for the fetus (BELL & EHRHARDT, 2000).

Gestational maintenance is a priority in energy partitioning. When the cow is undernourished, she mobilizes nutrients for the fetus and uses alternatives metabolic pathways for her own maintenance. In such case, the cow should be in a good nutritional status. A body condition score (BCS) of around 5 (scale from 1 to 9), allows her to support a moderate nutritional restriction with modest weight loss after calving and thus allows her to return to estrus faster (NRC, 2000). As a result, a satisfactory interval between calving and subsequent conception (60 to 90 days) can be achieved. On the other hand, in a poor nutritional status and low BCS, the energy balance can be impaired and increase postpartum anestrous, and embryonic mortality, and decrease gametogenesis, conception rate, and fetal development (NRC, 2000). Table 1 shows equations used by the main requirements systems to predict the energy and protein requirement of pregnant cows.

Table 1. Prediction equations of energy and protein requirements for pregnant cows.

System	Equation
	Energy
NRC (2000)	$EM\ (Mcal/d) = CBW\ (0{,}4504 - 0{,}000766t)\ e^{(0{,}03233 - 0{,}0000275t)t}$
BR-Corte (2010)[1]	$ELg\ (Mcal/d) = BW \times 97{,}84\ e^{0{,}0024\ .\ CEM}$; or $ELg\ (Mcal/d) = 97{,}84\ kcal/EBW^{0{,}75}$ with CEM=0
BR-Corte (2016)[2]	$EM_t = EM_m + EM_{gest} + EM_g$ where $EMm = 120 \times EBW^{0.75}$; $EL_{gest} = CBW \times 0.000000793 \times DG^{3.17} /1000$; $EM_{gest} = EL_{gest}/0.12$ $EL_g = 3.82 \times EBG_{np}^{1.07} \times BCS^{0.35}$; and $EM_g = El_g / 0.53$

	Protein
NRC (2000)	PBN x (0,001669-0,00000211 x t) x $e^{((0,0278-0,0000176 \times t) \times t)}$ x6,25/0,65
BR-Corte (2010)[1]	$PM_m = 4,0 \times BW^{0,75}$
BR-Corte (2016)[2]	$PM_t = PM_m + PM_{gest} + PM_g$; where $PM_m = 3.9x\ EBW^{0.75}$; $PL_{gest} = CBWx0.0000001773 \times DG^{2.945}$; $PM_{gest} = PL_{gest} /0.27$; $PL_g = 307 \times EBWG - 34 \times EL_g$; $PM_g = PL_g/0.27$

where: EM= metabolizable energy; CBW = calves birth weight; t= gestation time; BW = body weight; CEM = metabolizable energy intake; EBW = empty body weight; ELg = net energy for gestation; DG = days of gestation; PMm = metabolizable protein for maintenance; ELNg = net protein requirement for pregnancy, EBWG = non-pregnant empty body weight gain; EM_t = total metabolizable energy; EM_m = metabolizable energy for maintenance; EM_{gest} = metabolizable energy for gestation; EM_g = metabolizable energy for gain; ; PM_t = total metabolizable protein; PM_m = metabolizable protein for maintenance; PM_{gest} = metabolizable protein for gestation; PM_g = metabolizable protein for gain. [1] VALADARES et. al., (2010); [2] GIONBELLI et. al., (2016).

The BR-Corte system (GIONBELLI et al., 2016) was developed to address the lack of information on nutritional requirements of Zebu cattle. And in its last edition, nutrient predictions for pregnant cows are presented considering maintenance, gestation and gain separately. Considering requirements described by GIONBELLI et al. (2016), at final third of gestation, the nutritional requirement in CP of a pregnant Nellore cow is around 1,036 g/day and this value is not reached in national conditions with low quality pastures.

Considering different levels of dry matter intake according to the BW, and different levels of CP in the pasture, it is observed that under low-quality forage conditions it is difficult to achieve the requirements for gestation without protein supplementation. In addition, the low ingestion of CP impairs bacterial growth in the rumen and consequently the digestion of NDF and organic matter of the pasture (SAMPAIO et al., 2009). Therefore, under brazilian conditions, the cow will not only have the nutritional restriction of protein, but also energy. According to GIONBELLI et al. (2015), dry matter intake at this stage is 1.4% BW and is negatively influenced by fetal growth. Thus, in addition to the low pasture quality, limiting the intake of food is another factor that increases the nutritional restriction of pregnant cows.

There are many factors that can affect reproductive performance of the cow. ARNOTT et al. (2012) described some sources of stress that could change the hypothalamic-pituitary-adrenal (HPA) axis and affect the reproduction rate in cows. For example, the handling, weight, transportation, weather, and nutrition of pregnant cows. In beef cattle production, mainly in Brazil, the most common factor that causes stress is lack of of satisfactory nutrient intake. Because drought, the quality and quantity of roughage is limited and it could affect the recovery post calving, increasing time between gestations and represent a loss for the production system.

Maintaining pregnancy is also an important factor for fertility. According to CROUSE et al. (2016), almost 80% of embryonic losses occur within 40 days after AI. For this reason nutrient supply during this period should be adequate to maintain gestation and fetus development. Protein supplement could improve nutrition and provide essential amino acids important for angiogenesis that makes nutrients more available to the fetus, keeping the pregnancy and proper development.

According to HESS et al. (2005), reproduction and nutrition still are the most important factors affecting financial viability of a cow-calf system. And the milestone to achieve optimal reproduction is a resumption of estrus within a relatively short timeframe following parturition. Poor nutrition can affect the Hypothalamus-hypophysis-gonadal axis and undernourished cows are more sensitive to a negative feedback effect of estradiol and may remain acyclic for a long time due to decreased amplitude and frequency of LH secretion (HESS et al., 2005). Nellore cows have a longer anestrus period after parturition compared to other breeds, so it is important to feed them properly to avoid financial and production impairment to the cow-calf system.

Nutrition before calving is more important to control the anestrus period. Furthermore, the low energy on diet during pregnancy has a more negative effect even though there may be adequate energy during lactation (HESS et al., 2005). Body condition score around 5 can save body reserves for reproduction. But if the undernourishment continues during the lactation the impairment of reproduction can cause a longer anestrus.

2.2. Maternal nutrition and fetal programming

Beef cattle are raised to produce meat, in the other words, they need to produce a good amount of muscle to be used for human nourishment. Muscle development occurs in priority at the fetal phase, after birth, the number of muscle fibers does not change, they just increase the fiber size and muscle mass. In addition, to the genetic background, proper muscle development in utero is important to improve the development and performance of the animals after birth (DU et al., 2015).

It is well known that undernourished cows produce calves susceptible to death, diseases and impaired growth (WU et al., 2006). But the way that the dams nutrition

affects the offspring is complex and have different patterns. The dam can influence the phenotype of the offspring by genetic and associated epigenetic markings, through somatic epigenetic reprogramming, via the ooplasmic contribution to the fetus and via the provision of the intrauterine environment (AIKEN; OZANNE, 2014). BONASIO et al. (2010) reported that the mechanism of fetal programming can occur during cell division in response to recent stimulus and transferred to other cells. For example, a poor maternal nutrition can cause adaptation in the offspring leading to metabolic changes for saving energy resulting in an increase of fat deposition and decrease muscle mass on the progeny body (BLAIR, 2013).

2.2.1. Epigenetic effects

Epigenetic is the study of how genes and environment interaction affect phenotype. The concept of epigenetics is about changes in gene functions related to parental inheritance without altering the base sequences of the DNA and can act as a key mechanism that allows phenotypic plasticity regarding fixed genotype (FUNSTON; SUMMERS, 2013; HEARD; MARTIENSSEN, 2014). Changes in chromatin structure by DNA methylation, histone modification, and noncoding microRNAs are the most common mechanisms in epigenetics and they regulate timing and intensity of gene expression, allowing those changes to pass through generations (GOLDBERG et al., 2007; LINK et al., 2010).

DNA methylation is a common process, many regions rich in CpG dinucleotides in the 5'-3' direction contain methylation. However, the CpG islands in promoter regions are usually unmethylated, and methyl group adhesion in these regions may interfere with gene expression (HOLIDAY et al., 1993). Methylation patterns vary according to the tissue and process occurs by the activation of DNA methyltransferase (DNMT) using S-

adenosylmethionine (SAM) as a methyl group donor (JONES, LIANG, 2009). In this case, the diet is able to alter this process, regulating the availability of the methyl group or by altering the activity of enzymes involved in the methylation process, more specifically the DNMT. The main responsible for changes in progeny phenotype is Dmnt1, thus nutritional factors such as folic acid (positively) and homocysteine (negatively), affects the methylation of this enzyme and are responsible for changes in progeny metabolism (BURDGE et al., 2007).

According to BURDGE et al. (2007), protein-restricted diets during gestation may affect lipid metabolism by a hypomethylation of glucocorticoid receptor (GR) promoter resulting in an increase in GR expression. The increased activity of GR in the liver up regulates phosphoenolpyruvate carboxykinase expression and activity, and so increases capacity for gluconeogenesis and may contribute to the insulin resistance. Figure 1 shows the effect of protein-restricted nutrition during gestation on DNMT activity and DNA methylation. The Dnmt (3a and 3b) silence gene expression in the early embryo. On the other hand, 1-carbon metabolism is affected in protein-restricted offspring by increased glucocorticoid exposure or as a direct consequence of the restricted diet, which down regulates Dnmt1. This down regulation impairs capacity to methylate DNA during mitosis and decrease promoter expression. After sequential mitotic cycles expression, with impaired promoter expression, the genetic expression is induced in cells that were previously not expressed. In addition, increased gene expression is facilitated by lower binding and expression of methyl CpG binding protein (MeCP)-2 and reduced recruitment of histone deacetylase (HDAC)–histone methyltransferase (HMT) complex, resulting in higher levels of histone modifications which permit transcription (BURDGE et al., 2007).

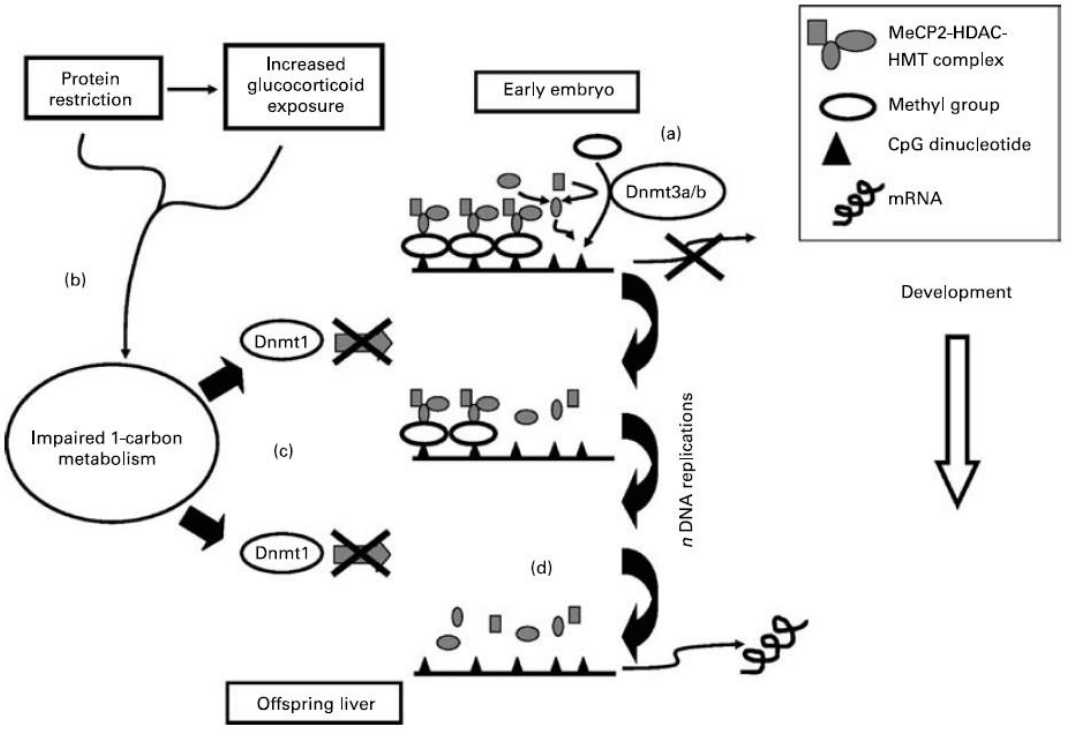

Figure1. Epigenetic pathway for regulation of genes expression in the offspring fed a protein-restricted (PR) diet during pregnancy (BURDGE et al., 2007).

Within the nucleus, DNA is wrapped in a globular octet envelope with two copies of each nucleus of histones (H3, H4, H2A, H2B). The histone tail that emerges from this globular heart fold is susceptible to great variation in the form of post-transcriptional modifications of specific residues of amino acids (Kouzarides, 2007). There are more than hundreds different histone transcriptional modifications, such as acetylation, ubiquitination, phosphorylation, and so on (FUNSTON; SUMMERS, 2013; MCKAY; MATHERS, 2011).

Due to the variety of possible histone modifications, there are several ways when diet can affect it and its interaction with DNA. In general, according to McKAY and MATHERS (2011), there are two main ways to be affected by diet: by altering amount and/or enzymatic response efficiency to the modification, or by altering substrate

availability for the enzymes. Because histone modifications and DNA methylation play a role in transcriptional regulation, nutritional factors that alter one process have the ability to change the other as well.

Another epigenetic toll, microRNAs (miRNAs) are vital components in gene control, they are involved in several important biological events such as cell proliferation and differentiation, development, regulation of the nervous system and so on. Furthermore, targets of microRNAs (miRNAs) include transcription factors, secreted factors, receptors, transporters and signaling proteins (BARRETT; FLETCHER; WILTON, 2013). Several dietary and nutritional factors can change miRNAs expression. However, due to the complexity and variation of these structures, the way this occurs is still poorly known. The miRNAs are small non-coding RNA and its action occurs by binding to 3' UTR regions of the target mRNA by regulating expression and affecting stability and/or leading to degradation (MCKAY; MATHERS, 2011).

Therefore, a combination of these 3 epigenetic mechanisms: DNA methylation, histone modifications, and miRNAs are responsible for controlling genetic expression, maintaining a robust combination that allows this regulation to be passed from one generation to another. The way that epigenetics works with nutritional stimulus was simplified and described by MATHERS (2016) and it is called as 4Rs of nutritional epigenomes. First, the animal RECEIVED nutritional stimuli and it's RECORDED by the genome. Then these exposures are REMEMBERED by following cell generations and finally is REVEALED in changed gene expression, cell function, and overall health.

Embryogenesis, gestation, and puberty are key-time-points for epigenetics changes (NEIBERGS; JOHNSON, 2012) and the knowledge of physiology, metabolism and gene expression, their interactions and environment stressors during development and growth could help to better understand and improve beef cattle production system (ANDERSEN

et al., 2005). Once, during fetal life it is possible to manipulate the diet more effectively, because the nutrients supplied to the mother are readily available to fetus.

Nutrients and dietary compounds such as folic acid, vitamin B12, choline, and betaine are able to provide methyl group donors, as well as, affect activity of enzymes involved in methylation and histones modification, leading to changes in gene expression, cell differentiation and consequently in the composition of the progeny tissues (FUNSTON; SUMMERS, 2013; PARADIS et al., 2017). Maternal diet can affect fetus development by an epigenetic modification that remains in a long-term life (WU et al., 2006). Changes in methionine-homocysteine and folate cycles associated with low methionine supply during the growth stages may lead to hypomethylation of DNA affecting gene expression and metabolism of progeny (BACH, 2012).

Although the way maternal nutrition affects gene expression and fetal development is not totally clear, it is well known that maternal nutrition and metabolism affect nutrient supply in the fetus and it may alter their metabolism by reducing availability of methyl donors and specific amino acids groups involved in DNA methylation and histone modifications (FUNSTON et al., 2013; PARADIS et al., 2017). In the Figure 2 is possible to see how maternal nutrition and epigenetic effects regulate adipogenesis.

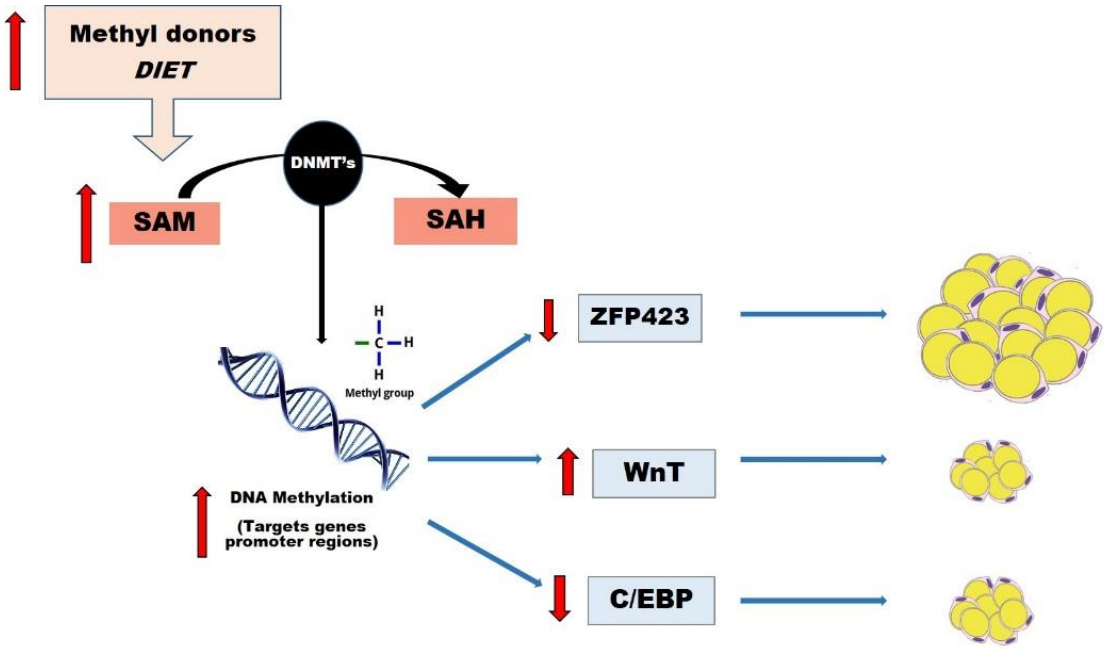

Figure 2. Effect of maternal diet on the epigenetic regulation of adipogenesis.

The interaction among genes and ligands that occurs due to a nutritional stimulus during fetal development is important for adaptation of chromatin structures and gene expression in long-term. Often these changes occur even before the need to use the gene, creating an adaptation of the progeny to the future environmental conditions. If there is a nutritional restriction, increasing sensitivity to a particular gene will result in better metabolism performance. Likewise, if there is a great availability of nutrients, it is stored in the form of adipose tissue avoiding the effect of lipotoxicity. Hence the reason nutritional restrictions during pregnancy cause obese animals when they reach adult life (REES; MCNEIL; MALONEY, 2008).

2.2.2. Skeletal muscle development and fiber type

The structural characteristics of skeletal muscle are relevant for meat productions and meat quality, and factors that affect those need to be better understood. The most important moment in muscle development is during fetal phase. After birth, the cells

committed with muscle tissue are all defined and there is no increase in muscle fiber number (hyperplasia), just increase in muscle fiber size (hypertrophy) (DU et al., 2011). Muscle mass is the result of the relation between size and number of muscle fiber. Connective tissues have low participation in mass composition, however, the high proportion of this type of tissue can reduce meat tenderness (WU et al., 2006). In terms of meat production, a high number of muscle fibers is an advantage to optimize the percentage of lean meat and growth efficiency (REHFELDT et al., 2004). Thus, studies demonstrate that the best way to increase meat production without impairing its quality is the selection of animals with a greater number of muscle fibers of moderate diameter (LEE; JOO; RYU, 2010; REHFELDT et al., 2004). In this sense, DU et al. (2010) observed that nutrient deficiency during pregnancy can reduce the number of muscle fibers, muscle mass and consequently affect the performance and meat quality of progeny.

The skeletal muscle express different isoforms of Myosin heavy chain (*MyHC*) gene, which defines the type of muscle fiber. The most common isoforms are *MyHC-I*, *MyHC-IIa*, *MyHC-IIb*, and *MyHC-IIx*. Those are translated and differentiate according to animal age and muscle location (ZHANG et al., 2014). Those isoforms differ in metabolic properties and produce fibers with slow twitch oxidative type I, fast twitch oxidative glycolytic type IIa, fast twitch glycolytic IIb and fast twitch glycolytic IIx, respectively (SCHIAFFINO; REGGIANI, 2011). Stem cells are able to differentiate and auto-renew producing different types of muscle fiber. This variation depends on intrinsic factors as a signaling pathway, or/and extrinsic factors such as exercise and maternal nutrition (BI; KUANG, 2012). Animals with high potential for growth have more number of muscle fibers and a higher proportion of fast glycolytic fibers and a low proportion of slow twitch oxidative fibers (BERNARD et al., 2009).

Some studies have shown that maternal diet can change the relationship between the oxidative and glycolytic type of fiber (VESTERGAARD et al., 2000). According to ZHANG et al. (2014), young bulls have more type IIa muscle fiber, and it becomes more flexible metabolic properties for growth, once this kind of fiber has an intermediate fast-oxidative-glycolytic metabolism. On the other hand, these researchers reported that bovine muscle could be adapted to slow-oxidative conditions because they didn't find Type IIb fiber in the *longissimus dorsi*, *semitendinosus*, and *soleus* muscle and this type of fiber is the most glycolytic among the *MyHCs*.

Muscle fiber type is also related to the process of cooling and beef quality (ZHANG et al., 2014). The primary muscle fibers have a fixed genetic effect and are not affected by the uterine condition since the secondary fibers are altered by the uterine condition (BEE; BEE, 2004). In pigs, lighter born animals have a greater quantity of oxidative glycolytic fibers numerically and in relation to the measured area, the fast glycolytic fibers are more numerous. On the other hand, muscle fiber type may affect *post mortem* metabolism and consequently the quality of the meat. Fibers of type IIx uses only glycolytic pathway to produce energy for contraction, and after the slaughter of the animals the excess lactate produced results in a rapid decrease pH impairing water holding capacity and color (LEE; JOO; RYU, 2010; REHFELDT et al., 2000).

2.2.3. Nutrigenomics in development and growth

Nutrition is one of the most important factors in animal production. The interaction among nutrients and genome express the potential of each individual dynamically, and varies according to the key stages of the animal life (NEIBERGS; JOHNSON, 2012). Nutrigenomics or functional genomics in Animal Science is a science that studies animal nutrition and metabolic gene expression interaction that affect livestock production

(LADEIRA et al., 2016). During the fetal and neonatal phase, progeny metabolism are intense and can adapt to an environment and influence long-term animal life. Understanding gene expression of metabolic genes at this stage may help in dietary manipulation to improve animal production and meat quality.

According to DU et al., (2010) WNT signaling induces proliferation, differentiation or maintenance of cellular precursors in an autocrine or paracrine manner (Figure 3). Maternal nutrition may affect *WNT* expression in an epigenetic manner. According to TONG et al. (2009) and YAN et al. (2013), maternal overnutrition during pregnancy impairs myogenesis and elevates adipogenesis, which is partially explained by down-regulation of the *WNT* signaling pathway. Likewise, the maternal restriction may increase visceral adipogenesis (WANG et al., 2016). Which is in agreement with DU et al., (2010) that reported an increase of myogenesis promotion from upregulation of *WNT* signaling, whereas downregulation promotes adipogenesis.

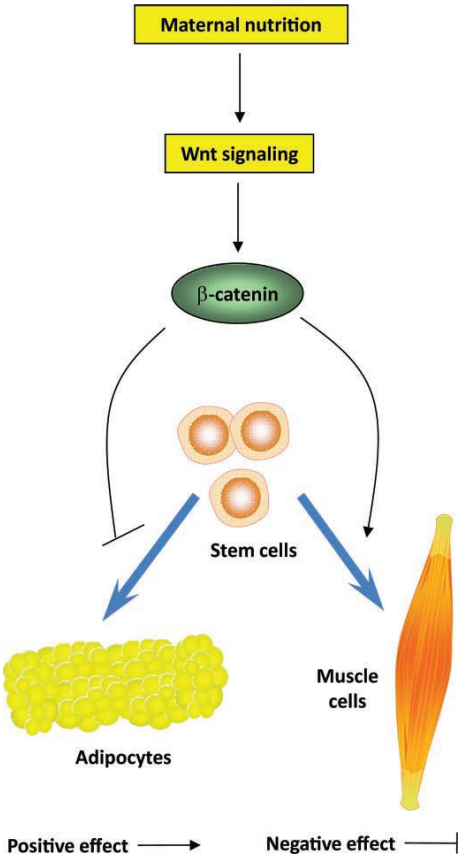

Figure 3. Wingless and Int (Wnt) signaling and fetal skeletal muscle development (DU et al., 2010).

VONNAHME et al. (2007) observed that when cows had a protein supplementation at 190 days of gestation there was an increase in uterine blood flow, resulting in an improvement of calves' performance due to increased transfer of nutrients through the placenta. The maternal nutrition during the beginning and middle of gestation can affect the glucose metabolism, and glucose exchange, causing *IGF1* sensitivity (DU et al., 2015). Thus, nutrient restriction, in the beginning of pregnancy, can induce an *IGF1* sensitivity, however, once the nutrients intake is restored this animal start producing more adiposity.

PPARG is a primary regulator of lipid metabolism and insulin sensitivity (BIONAZ et al., 2013), it acting in the maintenance of metabolic homeostasis according to diet

changes (REES; MCNEIL; MALONEY, 2008). *PPARG* is important for meat production due to its relation with intra-muscular fat and its effect on marbling (BIONAZ et al., 2013) and can be used as a cell marker of muscle and adipose tissue growth (DU et al., 2015). *PPARG* and *C/EBPA* act together on adipocytes differentiation as pleiotropic transcriptional activators of a large group of genes that produce the adipocyte phenotype (DEL PINO et al., 2017). Increased expression of *C/EBPA* in the final phase of differentiation is followed by an increase in *FABP4* expression, leptin and other markers (ZAMANI; BROWN, 2011). *FABP4* is a lipid transporter and has a low expression at the beginning of adipogenesis and increases with the differentiation process of pre-adipocytes (HAUSMAN et al., 2014). The higher *FABP4* expression may be an indicator of the number of adipocytes within the muscle tissue in young calves, which implies a greater number of adipocytes, although smaller ones (DEL PINO et al., 2017).

In the process of corporal mobilization, dams changes their metabolism to send amino acids to the fetus and use fatty acids as energy source (BELL; EHRHARDT, 2000). Despite fatty acids are not able to pass through the placenta, it is possible that these changes in lipid metabolism in cow can be transmitted to the fetus in an epigenetic way (REES; MCNEIL; MALONEY, 2008). As it was observed by PARADIS et al. (2017) where increased *PPARG* gene expression in fetuses whose dams received restricted diets compared to animals whose dams received adequate nutrition. These authors explain this unexpected effect as a consequence of the epigenetic effect related to changes in maternal metabolism.

CD36 is also involved in lipid metabolism. It is a membrane protein transporter that facilitates the delivery of fatty acids to the cell. This transporter is present in different tissues such as muscle and intestine. In the intestine, *CD36* is located in the luminal

surface of enterocytes and has broad specificity. In cases of reduced expression of *CD36*, there is no compensation with other transporters (NASSIR et al., 2007).

TGFβ1 superfamily have a varied effect on adipogenesis. They act through cooperation with the *WNT10B* pathway, suppressing *C/EBPA* activity, expression of *PPARG* and *C/EBPA*, decreasing the accumulation of lipid, prevents differentiation of mature adipocytes, stimulates the proliferation of pre-adipocytes (ZAMANI; BROWN, 2011). *TGFβ1* also increases the proliferation of preadipocyte-specific cells by promoting an increase in the progenitor population while inhibiting their differentiation (DERYNCK et al., 2007). However, the mechanism that defines adipogenic determination or fibrogenic differentiation is not yet completely defined (DU; HUANG; DAS, 2013).

On the other hand, *MyF5* is a myogenic transcription factor responsible to differentiate stem cells in myoblast. Despite *MyF5* is specific, there is a group of transcription factors that can interact in this process regulating *MyF5* action, such as *ZFP423* and *WNT*, among others (CHOY; DERYNCK, 2003).

Another gene involved in the regulation process of muscle cell hypertrophy is *mTOR* signaling pathway. Transcription factors, energy status, oxygen availability and amino acids are factors that activate the *mTOR* signaling pathway regulating cell growth. *mTOR* regulates cell growth and proliferation by promoting anabolic processes such as protein synthesis, lipids, and organelles, limiting intracellular autophagy and promoting protein turnover (LAPLANTE; DAVID, 2009).

2.2.4. Fetal programming and progeny performance

During the first three months of gestation, the embryonic growth is slow but important for adequate fetal development. At this point, establishment of functional and uteroplacental fetal circulation, and organogenesis and myogenesis occurs (DU et al.,

2010). The adequate cow nutrition and condition at this moment are critical to support the fetus. Midgestation is when secondary myogenesis and adipogenesis start, and maternal nutrition can affect muscle fiber number and muscle mass of the offspring (DU et al., 2010). However, the higher nutritional demand for fetal growth occurs in late gestation, when approximately 75% of fetus growth occurs (FUNSTON; LARSON; VONNAHME, 2010). In several countries, mid and late gestation coincide with winter and/or the dry season, when pastures are impaired and there are not enough nutrients to support an adequate pregnancy. Thus a poor uterine environment can affect calf birth weight with a great possibility of affecting health and performance in the long term offspring.

According to ZHU et al. (2006), the energy for muscle development is not a priority. During the fetal phase, the energy partition prioritizes the development of vital organs, and if the mother is not properly nourished the fetus can direct energy use for heart and liver rather than muscle, and it could affect their performance for all their life.

The characteristic of the muscle fiber type like myofiber number, size and type can affect the yield and quality of the meat (WU et al., 2006; ZHANG et al., 2014). And this caractheristic depends on myogenic differentiation that is regulated in parallel for intrinsic (signaling way) and extrinsic (exercises and maternal nutrition) signaling pathways (BI; KUANG, 2012).

Nutrient restriction or excess during fetal and neonatal development can have long-term consequences on beef production and quality, particularly if they occur during critical periods of muscle and adipose tissue development. Primary muscle fibers form in the fetal stage starting at 2 months of gestation and secondary muscle fibers form from 2 to 8 months of gestation in cattle (DU et al., 2010). Development of adipocytes starts early in gestation but subcutaneous and intramuscular adipocytes, which are more

important for meat quality, begin at the end of gestation and continue until about 250 days (DU et al., 2015). Although preadipocytes can proliferate and differentiate in adults, their capacity appears to be limited to the developmental stage early in life (MARTIN et al., 1998).

DU et al. (2010) also showed that nutrient restrictions during pregnancy can decrease the number of muscle fiber and muscle mass affecting performance and meat quality of progeny. And this is supported by BLAIR et al. (2013), where the cow's condition in the middle gestation affected offspring carcass characteristics. Fetal life is very important for muscle development because after birth progeny don't have an increase in the number of muscle fiber.

In a study carried out by DUARTE et al. (2014), comparing gene expression of skeletal muscle components of fetuses from overnourished or maintenance fed cows, they observed an increase in expression of gene favorable to fibrogenesis and adipogenesis in the fetus of the undernourished cows, without affecting myogenesis of these animals. This lack of effect on myogenesis may be related to the fact that control cows were not sufficiently restricted, once their maintenance requirements were reached.

UNDERWOOD et al. (2010) comparing the progeny performance of cows fed on native pasture or improved pasture found that steers whose dams were raised in better pasture condition had greater slaughter weight, higher fat thickness, and no change in marbling score. In this case there was an interference of maternal nutrition in the intramuscular and subcutaneous fat deposits.

Maternal diet effects on offspring may be variable according to restriction length and severity (COPPING et al., 2014). Under adverse conditions, such as nutrient restriction, progeny may change the metabolism to adapt and make a better use of available nutrients. Muscle and intestine work together on this metabolism adaptation.

This was demonstrated in the study of MEYER et al. (2010), where maternal nutrition during fetal development affected intestinal development in mass, length and intestinal morphology as a tool to increase nutrient absorption after birth. On the other hand in the study carried out by GIONBELLI et al. (2016), despite the difference in intestinal mass and length, calves whose dams received over nutrition had higher intestinal villi length of jejunum and ileum.

Intestinal proliferation is dependent on growth factors and hormones and not just on nutrients (PLUSKE et al., 1996). According to HAMMER et al. (2011) undernourished goats during pregnancy produced less colostrum after getting birth, however, progeny were more efficient at absorbing IgG. In this study, the animals were slaughtered with 24 hours of life and although there was no control of the amount ingested, all calves had access to colostrum in this period.

Myogenesis and adipogenesis are originated from the same group of mesoderm cells and for this reason, they are considered competitive with each other during fetus development (DU et al., 2015). These complexes processes have a large number of genes and transcriptions factors regulating muscle and adipose tissues formation which can lead to differences in meat characteristics and quality (ANDERSEN et al., 2005; LEE et al., 2012). Commitment phase of adipose tissue concurs with secondary myogenic period and the formation of adipocytes occurs mostly at the end of gestation and shortly after birth (DU et al., 2015). Moreover, maternal nutrition during gestation may affect adipocyte hyperplasia, which alters the development of adipose tissue, as well as the density of intra-muscular adipocytes and consequently marbling (DU et al., 2015).

GREENWOOD et al. (2005) comparing postnatal performance of progeny of well-fed or severely restricted nutrient cows found that maternal malnutrition may

represent a reduction of up to 35% in progeny birth weight. In addition, animals with lower birth weight have limited capacity for compensatory growth.

Maternal diet also affect meat quality. Calves whose dams had a positive energy balance during gestation presented greater thickness of subcutaneous fat and lower classification in *Yield Grade* (BLAIR et al., 2013). The same were observed by SUMMERS et al. (2015), when bulls born from cows without supplementation had higher empty body fat, greater subcutaneous fat thickness, greater tenderness, higher marbling score and, consequently, poorer classification in *Yield Grade*. In addition, progeny of cows that received 70% of nutritional requirements during gestation had a greater diameter of subcutaneous adipocytes and lower *Yield Grade* in the study carried out by LONG et al. (2012). Maternal over nutrition also improves meat quality, as was described by WILSON et al. (2016), when cows receiving 129% of protein nutritional requirements produced calves with higher subcutaneous fat and lower *Yield Grade* at slaughtering. These animals had an even lesser quantity of insulin after feeding in relation to the progeny of the cows that received 100% of protein requirements.

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SECOND CHAPTER - ARTICLE

PERFORMANCE, MYOGENESIS AND LIPOGENESIS OF PROGENY OF NELLORE COWS SUPPLEMENTED DURING MID AND LATE GESTATION

Article formatted according to the Journal animal Science guidelines

PERFORMANCE, MYOGENESIS AND LIPOGENESIS OF PROGENY OF NELLORE COWS SUPPLEMENTED DURING MID AND LATE GESTATION

ABSTRACT

Little is known about developmental programming in *Bos indicus* particularly Nellore animals, which is the predominant beef breed in Brazil. The aim of the present study was to determine the effect of maternal protein suplementation on cow performance, growth, myogenesis and lipogenesis on progeny from birth until weaning. Cows grazed pastures (8.2 % CP and 68.5 % NDF) and forty-six pregnant cows were fed only mineral (NS) and the other forty-six were fed a protein supplement (36 % CP, 0.2 % BW) from middle of gestation until calving. At calving, six calves from each treatment were slaughtered, body components were measured and samples of muscle and jejunum were collected for histological and gene expression analyses. In addition, 10 calves from each treatment had muscle biopsy sampled at 11 ± 4 days and at weaning. Least square means of all data were analyzed using the GLM procedure of SAS. Cow BW and BCS did not differ during the study, however, cows that were supplemented with protein gained more BW (P = 0.03) and body condition (P = 0.05) during gestation. As a result, pregnancy rate in the subsequent breeding season had a trend (P = 0.10) to be greater for supplemented cows. On the other hand, BW gain during lactation was greater for cows from the NS group (P = 0.05). Calves from cows supplemented with protein had greater birth BW compared to calves from cows not supplemented (P = 0.05) without differences in BW at weaning or ADG during this period. Body components from slaughtered calves did not differ between treatments except for small intestine length that were greater for NS calves (P = 0.05) with small crypt size (P < 0.01). NS calves had greater *SLC27A4* (P = 0.04) expression and a trend for greater expression of *SLC5A1* (P = 0.08) in the jejunum than

calves whose dams were supplemented. Calves from supplemented cows had a trend for more cells per field ($P = 0.07$) despite the smaller ribeye area ($P = 0.07$) and the smaller area ($P = 0.06$) in this longissimus muscle at birth. Muscle gene expression data suggested that undernourished offspring had early adipogenesis and supplemented offspring had greater expression of myogenic, adipogenic and fibrogenic markers at birth and at weaning. In conclusion, supplementation of pregnant Nellore cows leads to a positive effects for subsequent pregnancy rate and for potential for muscle growth of their calves. Nutrient restricted calves presented changes in intestine development as a way to improve nutrients absorption.

Keywords: adipogenesis, fetal programming, fibrogenesis, histology, myogenesis

INTRODUCTION

Beef production is determined by adequate muscle deposition and is affected by calves development and growth. Environmental conditions that change feed availability, such as winter, dry season and periods of food shortage, may impair progeny development and harm the production system. In addition, pregnant nutrition is important for fetal growth and cow fertility in the subsequent breeding season. Failure of the cow to become pregnant and the postpartum anestrus interval are the most impairing factors in cow-calf system, and they are strongly related with undernutrition (NASEM, 2016).

The beef cattle herd in Brazil and other tropical countries is primarily raised in an extensive system, using low fertilized grass pasture as the main feed source. Usually, pregnant cows are kept in pasture and mid to late gestation happens during the dry season, with a high chance of cows being nutrient restricted. Protein supplementation can be a tool to increase intake and digestibility of low-quality forage and supply amino acids for

pregnancy. Although there is no concrete information on amino acids requirements for pregnant cows, it is known that methionine is one of the first limiting and essential amino acid for DNA methylation, indispensable for proper embryonic development through transcription regulation and remodeling of chromatin (Chmurzynska, 2010).

The hypothesis of this study was that feeding a protein supplement to pregnant cows during the dry season would improve cow nutrition resulting in better reproduction performance in the next breeding season, as well as greater weaning weight and myogenesis and adipogenesis of the progeny. Therefore, this study had the objectives to evaluate the effects of dietary crude protein (CP) supplementation during mid to late gestation on cow pregnancy rate, calves growth and myogenesis, lipogenesis and fibrogenesis in longissimus muscle of the progeny.

MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use of State University of São Paulo, Jaboticabal Campus. All procedures were conducted in accordance with the ethical guidelines adopted by the Brazilian Guidelines for the Care and Use of Animals for Scientific and Educational Purposes (CONCEA, 2013). The study was conducted at the Experimental Farm of Agência Paulista de Tecnologia dos Agronegócios, Regional Center of Colina (APTA-Colina) and at Federal University of Lavras, in Brazil.

Animals and diet

Ninety-two multiparous cows with initial body weight of 391±45kg were used in this study. All of them were inseminated with the male sexed semen from the same sire in two fixed timed artificial insemination. Cows were allocated in two groups

(supplemented and non-supplemented) and were allotted in two side-by-side paddocks with *Brachiaria brizanta* cv Marandu. Each paddock had 25 hectares with similar conditions and the cows were switched fortnightly to eliminate the paddock effect. Stocking rate for both paddock were 1.9 AU/ha. Cows started to receive supplementation in the beginning of dry season, at 124 ±21 days of pregnancy. Forty six cows received only mineral salt *ad libitum* (NS); and forty six were fed with 0.2% BW of a protein supplement (SUPP) (Table 1).

Table 1 - Ingredients and composition of the supplement

Ingredient, % as fed		Chemical composition, %DM	
Soybean meal	50.9	DM, % as fed	90.6
Corn	29.6	Ash	30.5
Urea	4.9	CP	36.9
Limestone	7.5	EE	4.27
Mineral salt	1.9	NDF	42.8
Monocalcium phosphate	3.1	NDA	11.8
Vitamin-mineral premix	2.1	Lignin	5.2
		IVDMD	86.1

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; IVDMD: *In vitro* dry matter digestibility.

Throughout gestation, body weight and body condition score (BCS) of cows were assessed monthly. BCS was undertaken using a scale of 0 to 9, with 0 being very thin and 9 being grossly fat. Cows were allocated to one of two nutritional groups using a stratified randomization by body weight and body condition score before supplementation start.

Calving and Weaning Management

After birth all calves were weighed and identified with the same number of the dam, and were treated with Doramectin (Dectomax® injectable; Zoetis Inc, Kalamazoo, MI) for internal and external parasites. The first six calves born from each treatment were separated from their dams 24 hours after birth and slaughtered by the concussion technique and exsanguination of the jugular vein. All the slaughter procedures were in accordance with good animal welfare practices and followed the Sanitary and Industrial Inspection Regulation for Animal Origin Products (Brasil, 1997). The remaining calves were weighed again at 130 ± 20 days after birth and adjusted for 120 days and at weaning at 245 ± 4 days.

Sample collection

After calves slaughter, components of digestive tract (small intestine, large intestine, rumen and reticulum, omasum and abomasum), organs (lung, heart, liver, kidney, and pancreas), and carcass and non-carcass components of each calf were dissected and measured. The components weighed were correlated with the birth weight and expressed in percentage. After exsanguination, samples from *longissimus thoracis* and small intestine of the calves were quickly collected and stored in an ultra-freezer at -80° C for gene expression analyses. Another sample of *longissimus* muscle was collected for chemical composition analyses using the FoodScan Meat Analyser TM® (FOSS, Hillerød, Denmark) with near-infrared spectrophotometer technology (AOAC, 2007) method 2007.04.

Two samples of intestine were collected from the jejunum. One of those was fixed in 10% formalin solution (pH=7.4) for histologic analysis, and another one was quickly

collected and stored in an ultra-freezer at -80°C for gene expression analysis. A sample of longissimus muscle also was collected for histological analyses but was treated with a cryoprotectant (talcum and isopentane), and cryopreserved in nitrogen and stored at -80°C until cryotomy.

From birth to weaning, remaining calves were kept with the cows in the same pasture used during gestation and, the supplemented group stopped recieving the protein supplementation and received only mineral salt at weaning. Therefore, both groups were held together until weaning at 245 ± 4 days of age.

Reproductive performance in the subsequent breeding season

Around sixty days after calving cows were used in another FTAI protocol. Cows had at least two FTAI and after the second AI, all cows were exposed to clean-up bulls for 40 d period that began 3 d after AI. Overall pregnancy rates were analyzed via trans-rectal ultrasonography at 40 d after bull removal.

Muscle Biopsy

Ten calves from each treatment were biopsed in the *Longissimus thoracis* muscle between the 12th and 13th rib in the right side, 11±4 days after birth. After hair removal, the skin was cleaned and disinfected, calves received a local anesthetic (lidocaine HCl). A sterile cloth drape were placed over the biopsy site and a 1 cm incision was made with a scalpel. A sterile Bergstrom biopsy needle (Eskilds Tuna, Sweden) was used to obtain 2 to 3g of muscle tissue. A topical antibiotic spray was applied to the incision site and then covered with a spray-on aluminum bandage. All calves were monitored for swelling during a 24 h post-biopsy period. The same procedure were repeated in the same calves before weaning, around 200 ± 4 days, on the left side.

Histological analyses

Longissimus dorsi muscle sections of 12 μm thickness were obtained in a cryostat at -20°C, which were placed on slides. Three cuts were made in the horizontal line and three in the transverse line of each sample to guarantee optimal muscle cuts for each sample. Intestinal samples were dehydrated with increasing concentrations of alcohol and xylol, embedded in paraffin, and cut into a 4 μm with a microtome. Two slides were made for each tissue sample (four cuts). For morphological analysis of fiber number and diameter in the muscle, and for villus height and crypt depth in the jejunum, the staining technique used was Hematoxylin and Eosin (Pluske et al., 1996).

The samples were analyzed under a light microscope, OLYMPUS CX31 (Olympus Corp., Tokyo, Japan), coupled to an OLYMPUS SC30 (Olympus Corp., Tokyo, Japan) camera for image capture. Five images per sample were obtained with 4x and 20X objective for jejunum and muscle respectively, and they were analyzed in the ImageJ® analyzer software (National Institutes of Health, Bethesda, Maryland, USA). To measure histological characteristics of the muscle, the number of cells per field was quantified with the *Cell counter* tool, cell density was measured using the *Grig* tool of the same software that allowed to quantify the number of fibers in 15,000 μm^2. For the muscle cell diameter (μm), the *Straight* tool was used to measure cell diameter, and then cell area (μm^2) were calculated.

For jejunum histological measurements, fifteen villi and fifteen crypts per animal were measured, to evaluate villus height, crypt depth and crypt depth/villus height ratio.

Gene expression

Total RNA was extracted from the samples using QIAzol (QIAGEN, Valencia, CA, USA), treated with DNA-free DNAse (Ambion, Austin, TX, USA) and reverse

transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Target genes were analyzed and their respective primers sets are shown in Table 2 and 3. RTqPCR was performed on an Eppendorf Realplex system (Eppendorf, Hamburg, Germany) using SYBR Green detection system (Applied Biosystems, Foster City, CA, USA). PCR reactions were incubated in a 96-well plate at 95°C for 10 min, followed by 40 cycles of 95°C for 15s and 60°C for 1 min. After amplification, a melting curve (0.01 C/s) was used to confirm product purity. The amplification efficiency of each reaction was measured using the PCR program LinRegPCR version 2017.1 (Ruijter et al., 2009; Tuomi et al., 2010; Ruijter et al., 2014). The relative expression levels were calculated according to the method described by Pfaffl (2001) which is based on Ct values that are corrected for the amplification efficiency for each primer pair.

Table 2. Primers sets used to quantify mRNA expression of genes in *longissimus* muscle tissue using quantitative real-time PCR

Gene	Abbreviation	Primer sequences	Accession number	Amplicon, bp	R^2	Efficiency
Enhacer Biding Protein alpha	*C/EBPA*	**F** GGCAACGACTTTGACTACCC **R** TCGTTGCTGTTCTTGTCCAC	NM_176784.2	83	0.995	92.8
Constitutive Coactivator of PPAR-gamma-like protein 1	*PPARG*	**F** CGACCAACTGAACCCAGAGT **R** TCAGCGGGAAGGACTTTATG	NM_181024.2	83	0.997	97.5
Zinc finger protein 423	*ZFP423*	**F** AGACAGGAACAGCGTGACAA **R** CTGACAGTGATCGCAGGTGT	NM_001101893.1	91	0.996	97.8
Cluster of differentiation 36	*CD36*	**F** GTGATGAGAAGGCGGAAATG **R** ACCACACCAACACTGAGCAA	NM_001278621.1	94	0.996	94.5
Adipocyte-type fatty acid-binding protein	*FABP4*	**F** GGATGATAAGATGGTGCTGGA **R** ATCCCTTGGCTTATGCTCTCT	NM_174314.2	73	0.998	92.9
Wingless-type MMTV integration site family member 10B	*WNT10B*	**F** AGCCTTTCAACCCCGTCTT **R** GGGTCTCGCTCACAGAAGTC	XM_010827688.2	87	0.998	96.1
Cadherin-associated	*β-catenin*	**F** GCACAATCTTTCCCACCATC	NM_001076141.1	90	0.997	98

protein, beta 1		**R** ACTGGTGAACCGAGCATCTT				
Transforming growth factor beta 1	*TGFβ1*	**F** CTGGGCTGGAAGTGGATTC **R** TCCAGGCTCCAGATGTAAGG	NM_001166068.1	83	0.990	95.9
Collagen type III, alpha 1	*COL3A1*	**F** AACCAGAACCGTGCCAAATA **R** TGGGGCAGTCTAATTCTTGG	NM_001076831.1	90	0.997	94.5
Fibronectin 1	*FN1*	**F** GGGGGCAGTCCTACAAGATT **R** TTTGCCATTACCCAGACACA	NM_001163778.1	92	0.990	98.5
Myogenic differentiation 1	*MyOD*	**F** CGACGGCATGATGGACTAC **R** CGCCTCGCTGTAGTAAGTGC	NM_001040478.2	82	0.997	94.0
Myogenin (Myogenic factor 4)	*MyOG*	**F** CCTACAGACGCCCACAATCT **R** TATGGTTTCATCTGGGAAGG	NM_001111325.1	93	0.990	98.6
Insulin-like growth factor 1 receptor	*IGFR1*	**F** TGCGGTTCTGTTGATAGTGG **R** TGGAGTGCTGTATGCCTCTG	HQ703508.1	101	0.993	97.5
Mammalian Target of Rapamycin	*MTOR*	**F** CATGGAAATGGCATCCAAG **R** GAGTTTGAGGTGAAGCGAGC	XM_015475105.1	90	0.993	94.3
Myosin heavy chain type I	*MyHC-I*	**F** AGGAGAAACACGCCACAGAG **R** CTTTTCCTTGGTCAGCTTGG	NM_174117.1	92	0.998	95.7
Myosin heavy chain type IIa	*MyHC-IIa*	**F** GCCCAAGGAATCTTTTGTCA **R** CTGTCAGAGTCGCTCCTCCT	NM_001166227.1	89	0.998	92.8
Myosin heavy chain	*MyHC-IIx*	**F** AAGCTGTCAAGGGTCTACGC	AB059399.2	94	0.999	95.6

type IIx		**R** TCCTGGAGCCTGAGAATGTT				
β-actina	*BACT*	**F** GTCCACCTTCCAGCAGATGT **R** CAGTCCGCCTAGAAGCATTT	BC142413.1	90	0.999	94.9
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	**F** CGACTTCAACAGCGACACTC **R** TTGTCGTACCAGGAAATGAGC	NM_001034034.1	96	0.990	95.3
Ribossomal Protein 18S	*RP18S*	**F** CCAGTAAGTGCGGGTCATAA **R** CCATCCAATCGGTAGTAGCG	NM_001033614	84	0.985	96.5

Table 3. Primers sets used to quantify mRNA expression of genes in jejunum tissue using quantitative real-time PCR

Gene	**Abbreviation**	**Primer sequences**	**Accession number**	**Amplicon, bp**	**R^2**	**Efficiency**
Cluster of differentiation 36	*CD36*	**F** GTGATGAGAAGGCGGAAATG **R** ACCACACCAACACTGAGCAA	NM_001278621.1	94	0.996	94.5
Sodium-glucose linked transporter	*SLC5A1*	**F** ACCGCCCTTTACACAATCAC **R** AGGATGAAAGACCCCAGGAG	AF508807.1	92	0.989	94
Glucose transporter 2	*SLC2A2*	**F** GTTCTTTGGAGGGTTGCTTG	NM_001103222.	100	0.997	94.5

		R CGAAAACCCCATCAAGAGAG	1			
Fatty acid transport proteins 4	*SLC27A4*	**F** GGATGATAAGATGGTGCTGGA **R** ATCCCTTGGCTTATGCTCTCT	NM_174314.2	90	0.998	90.5
Splicing factor 3a subunit 3	*SF3A3*	**F** CGTGTTGGTGGTCTGTCTTG **R** CGGAGCGTGGACTTCTTAGT	XM_019957796.1	99	0.995	94.5
β-actina	*BACT*	**F** GTCCACCTTCCAGCAGATGT **R** CAGTCCGCCTAGAAGCATTT	BC142413.1	90	0.999	94.9
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	**F** CGACTTCAACAGCGACACTC **R** TTGTCGTACCAGGAAATGAGC	NM_001034034.1	96	0.990	95.3

Six reference genes were tested and the best individual or combination of endogenous control was chosen using the web-based tool RefFinder in which integrates the currently available major computational programs including the four commonly used algorithms geNorm (Vandesompele et al., 2002), NormFinder (Andersen et al., 2004), BestKeeper (Pfaffl et al., 2004), and comparative ΔCt method (Silver et al., 2006), to evaluate the most stable reference across study groups. RefFinder selected the *β-actin*, *GAPDH* and *RP18S* genes as more stable to be used in muscle gene expression and *SF3A3, β-actin, GAPDH* as more stable for jejunum as reference in the calculation of the comparative expression.

Statistical Analysis

Data were analyzed as a completely randomized design, and animal was considered the experimental unit. Cow's performance were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with maternal nutrition (MN), fixed timed artificial insemination (FTAI), and MN × FTAI interaction as fixed effects. The covariance structure was chosen according to the Bayesian information criterion, by comparing 4 covariance structures for each variable (compound symmetry, autoregressive order one, heterogeneous autoregressive order one, and unstructured), and the structure that yielded the smallest Bayesian information criterion was used. The least squares means (LSMEANS) statement was used to calculate the adjusted means for treatments.

Binomial data, such as subsequent reproduction performance, were analyzed using the GLIMMIX procedure of SAS. The least squares means function of SAS was used to separate treatment means.

Gene expression of muscle biopsy samples were analyzed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). The normality of the data was verified

through the Shapiro-Wilk test. Data that had no normal distribution were transformed using the RANK procedure of SAS. The analysis of variance was performed by the GLM procedure of SAS, and the means obtained were compared by the Tukey test at 5 %. Statistical differences were considered with $P \leq 0.05$ and tendencies with $0.05 < P < 0.10$.

RESULTS

In Figure 1 is presented the average temperature and precipitation in the Research Unit Farm during the experimental period. In addition, in Table 4 is presented the data of the pasture quantity and quality. Pasture deferment was effective to maintain the availability of forage during experimental period. July, the core of dry season, was the period of worst forage quality and availability when the forage mass reached 3678 kg DM/ha with 5.9 % of CP and 55.4 % IVDMD.

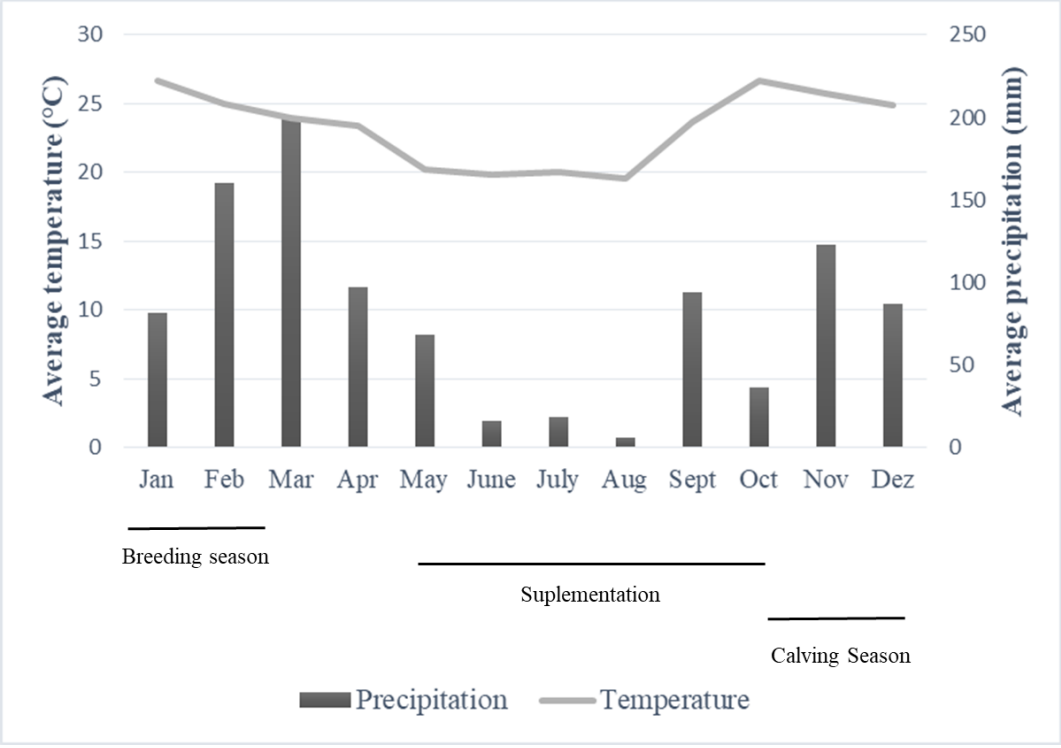

Figure 1. Climate data collected in the research unit during experimental period (Colina City, São Paulo State, January to December of 2015)

Table 4. Quantitative and qualitative characteristics of Marandu pasture

	Supplementation period						
	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.
Forage mass, kg DM/ha	5028	7418	3923	3678	5815	6046	6272
Green forage mass, kg DM/ha	4621	6350	2785	2624	1083	525	788
Green leaf, %	45.8	28.6	16.3	16.3	6.2	4.5	10.2
Forage allowance, kg DM/kg BW	5.6	9.2	4.3	4.0	6.4	6.6	6.9
Chemical composition of pasture[1]							
DM, %	32.5	36.4	34.2	59.8	46.8	27.3	24.2
CP, %	7.9	7.7	7.5	5.9	7.4	10.1	10.4
NDF, %	70.4	64.6	68.0	71.3	71.9	65.7	61.7
ADF, %	35.9	33.2	33.1	35.6	43.2	39.6	34.2
Lignin, %	5.0	4.8	7.2	5.8	7.1	7.6	5.9
IVDMD, %	68.4	70.5	68.7	55.4	56.7	59.2	72.5
Ash, %	6.7	7.6	8.4	8.4	6.2	7.2	8.8
Fat, %	1.6	1.9	1.7	1.7	1.4	1.7	1.6

[1] simulated pasture; DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; IVDMD = in vitro dry matter digestibility.

There was no difference in cow weight on day 0, pre-calving, post-calving or at weaning. However, when we consider the weight gain during pregnancy and during the lactation period, cows from supplemented group had greater weight gain during gestation and lesser in lactation. The same was observed for BCS, no differences in BCS at day 0 or in pre-calving. But supplemented cows had an increase in BCS during gestation.

Pregnancy rate in the subsequent breeding season had a tendency to be greater for cow in the supplemented group.

Table 5. Performance of cow from conception to weaning and pregnancy rate in the next breeding season

Item	NS	SUPP	SEM	*P*-value
Cow weight, kg				
Day 0[1]	390.2	384.6	9.03	0.69
Pre-calving[2]	468.7	479.9	8.29	0.34
Post-calving[3]	424.1	436.6	8.30	0.28
Weaning	479.0	465.6	15.53	0.54
Weight gain - gestation	79.2	95.3	5.27	0.03
Weight gain - lactation	59.1	26.4	11.78	0.05
Day 0 BCS	5.23	5.02	0.12	0.25
Pre-calving BCS	5.24	5.39	0.12	0.36
Change in BCS	0.01	0.36	0.13	0.04
Pregnancy rate %	62.1	78.6	-	0.09

[1] During FTAI; [2] 270 days after FTAI; [3] around 45 days after calving; NS = non-supplemented; SUPP = supplemented; SEM = standard error mean.

Calves born from supplemented cows had greater body weight at birth (Table 6). However, no differences were found in the other time-point analyses, 119 days and weaning. Additionally, there was no difference in the average daily gain of calves from birth to 119 days, 120 days to weaning or overall ADG from birth to weaning.

Table 6. Body weight and average daily gain of the calves from the birth to the weaning

Item	NS	SUPP	SEM	*P*-value
Body weight, kg				
Birth	33.7	35.8	0.78	0.05
119 days	115.0	114.6	3.71	0.92
Weaning	205.5	207.3	4.42	0.77
ADG, kg				
Birth to 119d	0.693	0.668	0.02	0.53
120d to weaning[1]	0.527	0.536	0.01	0.47
Birth to Weaning	0.746	0.747	0.02	0.79

[1] ajusted, NS = non-supplemented; SUPP = supplemented; SEM = standard error mean.

Data from slaughtered calves 24 hours after birth are presented in Table 7. There was no difference between organs percentage, and carcass percentage or non-carcass percentage components of the slaughtered calves. *Longissimus* muscle composition did not differ between treatments either, but there was a tendency of greater ribeye area for NS calves. Despite the lack of difference in total digestive tract, the length of small intestine in calves whose dams were not supplemented was longer than SUPP group Calves from NS group had a trend to increase the weight of kidney *(P = 0.08)* and fat in the kidney *(P = 0.10)* than calves from SUPP group.

Table 7. Body components, jejunum histology and *Longissimus* muscle composition from calves slaughtered at 24h after birth, relative value from body weight

	NS	SUPP	SEM	*P value*
Total digestive tract, %	3.22	3.28	0.11	0.74
Small intestine, %	1.76	1.89	0.09	0.30
Small intestine, m	17.2	15.9	0.04	0.05
Large intestine, %	0.54	0.52	0.03	0.74
Large intestine, cm	247	218	12.2	0.12
Jejunum histology				
Villus height, μm	127.2	135.5	0.00	0.50
Crypt depth, μm	13.1	19.5	0.61	<0.01
HV/DC rate	1.01	0.73	0.48	0.01
Organs, %	4.31	4.24	0.09	0.61
Non carcass component, %	32.6	33.4	0.77	0.47
Carcass, %	53.3	49.9	3.64	0.64
Ribeye area, cm^2	14.9	11.8	1.33	0.07
Composition,				
Colagen, g/kg	0.66	0.65	0.04	0.86
Protein, g/kg	19.9	20.4	0.24	0.21
Fat, g/kg	1.04	0.90	0.11	0.39
Moisture, g/kg	77.2	76.7	0.28	0.27
Mineral, g/kg	1.87	1.88	0.17	0.97

NS = non-supplemented; SUPP = supplemented; SEM = standard error mean, HV / DC = villus height/depth of crypt.

Calves from supplemented cows had lower height of villus/depth of crypt ratio (HV/DC), due to the greater depth of crypt (Table 7). However, there was no difference in villus height. Supplemented offspring had lower expression of lipid transporter *SLC27A4* ($P = 0.04$) and there was a tendency of lower gene expression in small intestine *SLC5A1* ($P = 0.08$), with no difference in *CD36* ($P = 0.94$) and *SCL2A2* ($P = 0.49$) (Figure 2).

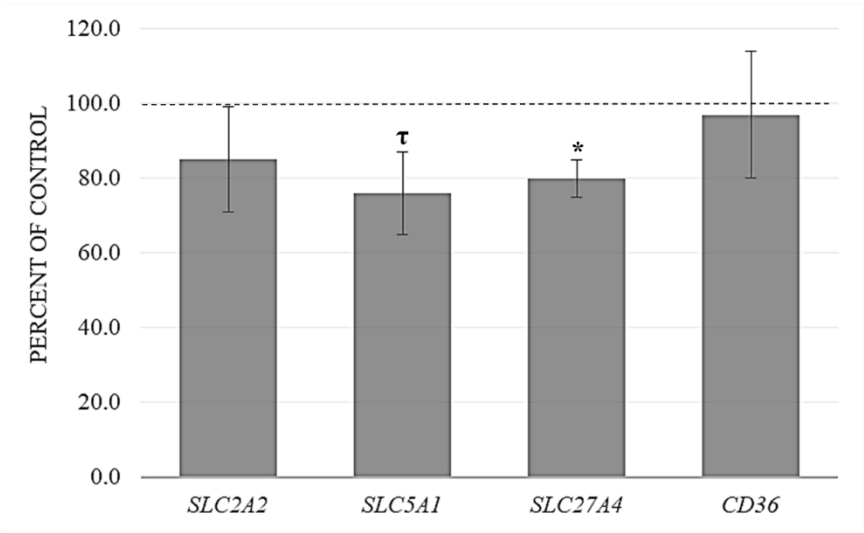

Figure 2. Influence of maternal diet on gene expression of transporters in the small intestine of the offspring. Data are expressed as a percentage of NS group control. Bars represent Standard Error Mean. *$P = 0.04$, $^{\tau}P = 0.10$.

Calves from supplemented cows had a tendency to have greater number of cells per field in *Longissimus dorsi*, but with smaller area than the offspring of non-supplemented cows (Table 8 and Figure 3). Nevertheless, the density and diameter of the muscle fibers of those calves did not differ.

Table 8. Histological characteristics of *Longissimus dorsi* muscle in calves according to the maternal diet

	NS	SUPP	SEM	*P value*
Cells per field	440	602	0.39	0.07
Density[1]	15.2	16.5	0.43	0.51
Diameter (μm)	23.4	21.4	0.39	0.18
Area (μm ^{2})	504	407	0.36	0.06

[1] Density at 15,000 μm^2; NS = non-supplemented; SUPP = supplemented; SEM = standard error mean.

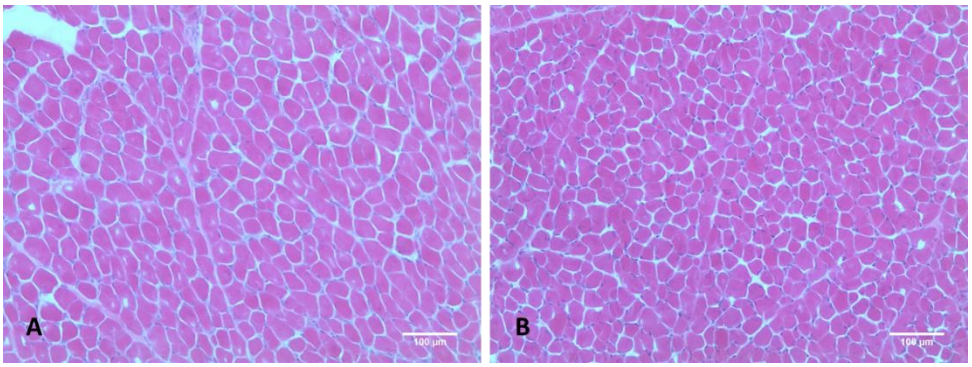

Figure 3. Images of *Longissimus dorsi* muscle stained with hematoxylin and eosin from calves born from supplemented dams (A), and non-supplemented dams (B) 24h after birth.

In Figures 4, 5 and 6 are presented the relative gene expression in the calves of the slaughter study (24 h after birth) and muscle biopsy after birth (11 ± 4 days), and at weaning, respectively. 24 h after birth, calves from supplemented group had higher expression of *PPARG* (P = 0.03), *WNT10B* (P = 0.01), *CD36* (P = 0.04) and *TGFβ1* (P = 0.01) than calves from non-supplemented cows. However, there were no differences in the other genes.

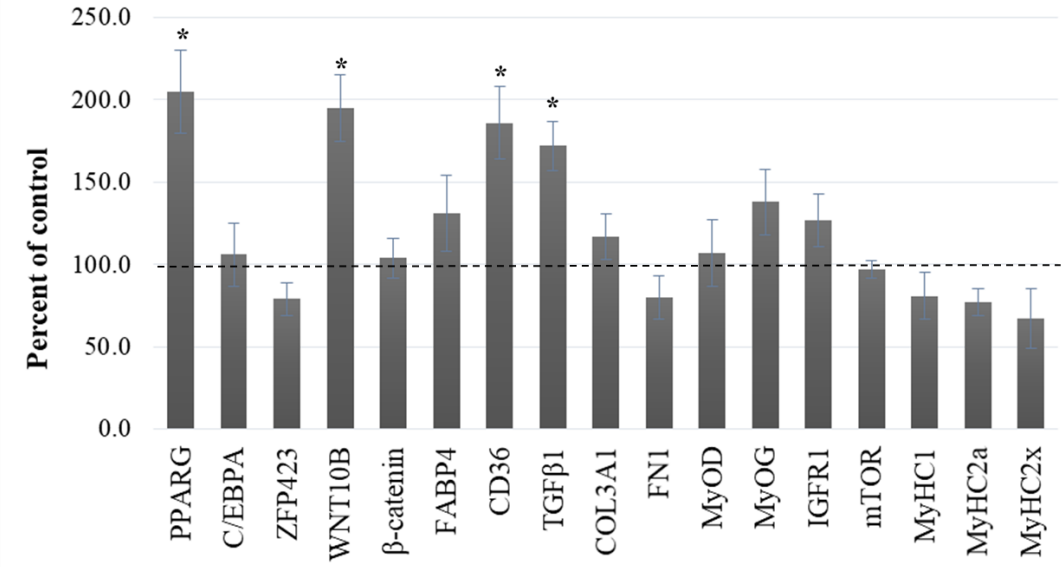

Figure 4. Effect of maternal diet on gene expression in the *longissimus dorsi* muscle of the offspring 24h after birth. Data are expressed as a percentage of NS control group. Bars represent Standard Error Mean. Values with asterisk are statistically different ($P \leq 0.05$).

Calves whose dams were supplemented had lesser expression of *C/EBPA* (P = 0.01) and *FABP4* (P = 0.07) than calves from non-supplemented cows 11 ± 4 days after birth. However, this difference did not remain through weaning, when calves from supplemented cows had a tendency of greater expression of *PPARG* (P = 0.08), *ZFP423* (P = 0.04) and *TGFβ1* (P = 0.02) than calves from NS cows. Expression of other genes were not affected by maternal diet in the offspring at birth or weaning.

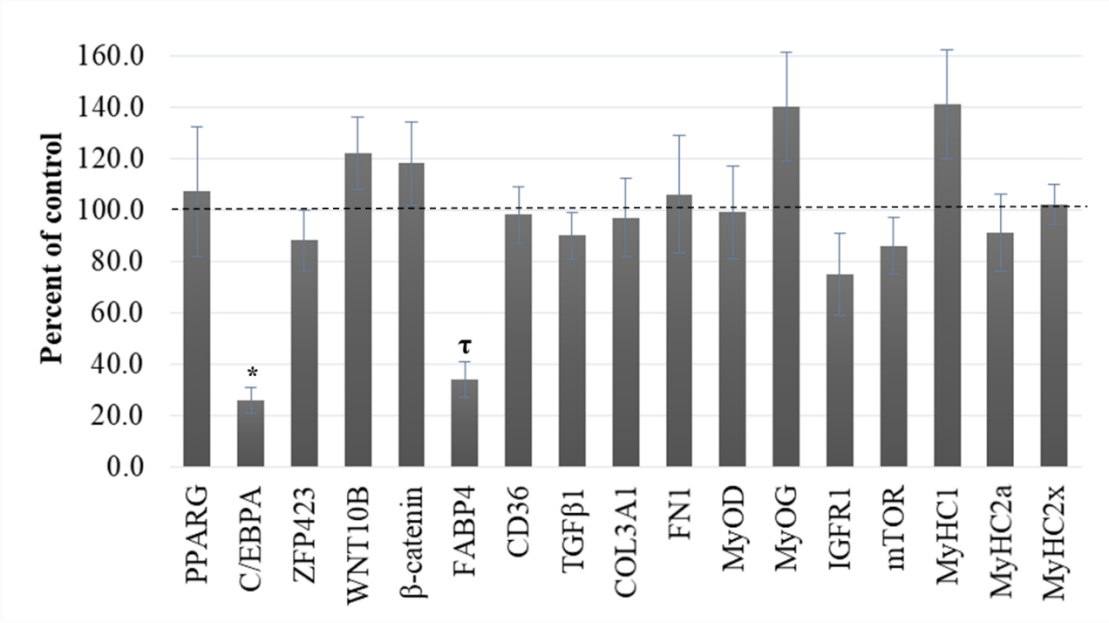

Figure 5. Influence of maternal diet on gene expression in the *Longissimus dorsi* muscle with biopsy 11 ± 4 days after birth. Data are expressed as percentage of NS control group. Bars represent Standard Error Mean. Values with asterisk are statistically different ($P \leq 0.05$), and with **τ** has a tendency to be different ($0.05 \leq P \leq 0.10$).

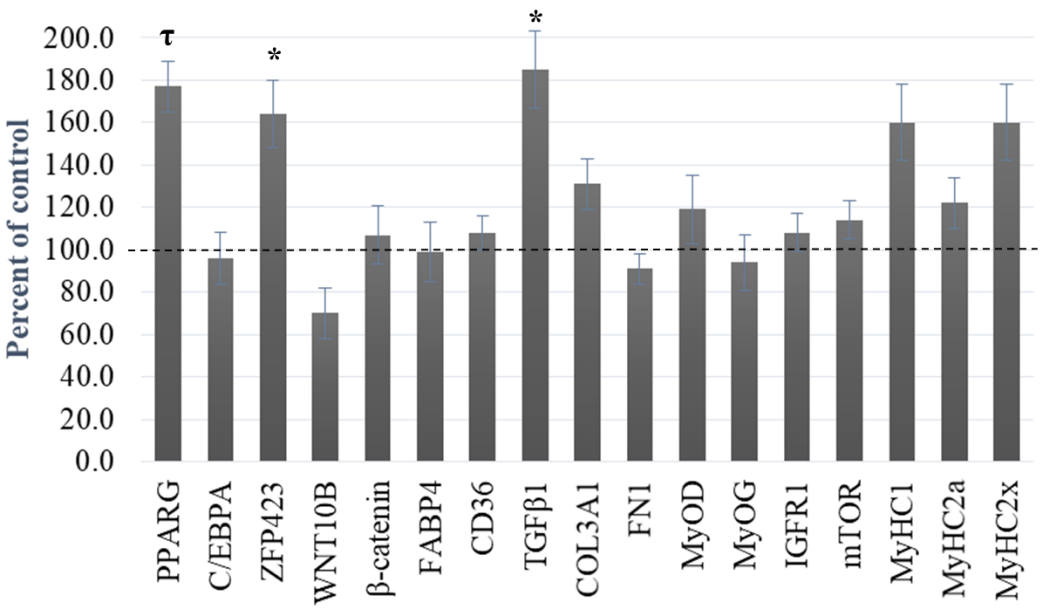

Figure 6. Influence of maternal diet on gene expression in the *Longissimus dorsi* muscle with biopsy at weaning. Data are expressed as percentage of NS control group. Bars

represent Standard Error Mean. Values with asterisk are statistically different ($P \leq 0.05$), and with **τ** has a tendency to be different ($0.05 \leq P \leq 0.10$).

DISCUSSION

Performance of grazing animals is related to the forage supply and grazing behavior, interfering in the quality and quantity of nutrient intake (Barros et al., 2014). According to Detmann et al. (2014) at least 12% CP is necessary to provide adequate equilibrium in the balance of ruminal nitrogen. In our study the deferment of the pasture was able to maintain forage mass above to 3600 kg DM/ha with a forage allowance greater than 4.0 kg DM/kg BW and crude protein levels around 5.9 % and 10.4 % during dry and dry-rainy transition season, respectively. The lowest protein content was observed in July. Those characteristics indicate that most of the time pasture had regular nutritional value, and it did not promote a severe nutritional restriction for non-supplemented pregnant cows throughout the dry period. There was an expectation that pasture conditions would provide more nutritional restriction in non-supplemented cows. However, there was no weight loss or body condition score change.

Considering changes in individual maternal and gestational tissues during supplementation period, calculated according to Gionbelli et al. (2015), it was observed that both groups mobilized body reserves to support pregnancy during the dry season. Associating pasture and supplement composition to estimate nutrient usage for pregnant cows (Gionbelli et al., 2016), supplemented cows had an estimated intake around 8.42 kg of DM, while non-supplemented cows had 9.55 kg of pasture DM intake. It means that non-supplemented cows increased forage intake to compensate the lack of supplementation. On the other hand, considering pasture and supplement composition, the amount of energy and protein supply for supplemented cows was 18.5 Mcal/d and

1064 g CP/d, and for non-supplemented cows was 18.06 Mcal/d and 773 g CP/d. These data suggests that the energy requirements were met in both groups, but the average protein requirements for pregnancy were met in 100% for supplemented cows, but just 75% for non-supplemented cows (Gionbelli et al., 2016), which was a moderate restriction.

Nutrition and reproduction are the major factors that affect financial and sustainability of cow-calf operations (Hess et al., 2005). Cows with adequate nutrition and sufficient body reserves are able to maintain a productive cycle producing healthy calves each year. Cow's reproductive performance is affected by nutritional plane and it is recommended that cows have a BCS of 5 to 6 prior to calving to maximize their performance (Bohnert et al., 2013). In this study, cow's body weight oscillated monthly because of pasture quality and quantity, with an increase throughout gestation without difference between treatments. However, considering the whole gestational period, supplemented cows had greater weight gain and BCS improvement than non-supplemented cows. The lack of differences in BW between treatments may be related to the absence of severe restriction, where non-supplemented cows increased pasture intake trying to compensate nutrient restriction. NS cows probably had compensatory weight gain during lactation period, but, according to Hess et al. (2005), pre-calving nutrition is more important for anestrus length than cow nutrition after calving and the interval between birth and first estrus is affected by a negative energy balance or nutritional deficiency at the end of gestation. For this reason non-supplemented cows had lower pregnancy rate in the subsequent breeding season. Moreover, cow nutrition in late gestation affects the development of follicles that mature in the subsequent breeding season (Hunter et al., 1991), oocyte quality (Krisher et al., 2004) and size and uterine

function (Lucy et al., 2003). This also explain the trend for supplemented cows to have greater pregnancy rate than non-supplemented.

In beef cattle, maternal nutrition studies shows variation in offspring birth weight (Greenwood et al., 2005; Larson et al., 2009; Wilson et al., 2016). In addition, it is known that lower birth weight is related to increase mortality, neonatal morbidity (Azzam et al., 1993), and lower productive efficiency during growth that may affect carcass traits in long-term (Greenwood & Cafe, 2007).

In the present study, calves from supplemented cows had greater BW at birth. This was in agreement with Vonnahme et al. (2007), where protein supplementation at 190 days of gestation increased fetus weight due to a greater transfer of nutrients through placenta. However, after birth there was no difference in the ADG. The difference in body weight at birth was about 6.3% live weight with greater weight for progeny of supplemented cows. In numerical terms this difference was maintained at weaning (±2kg), but the percentage represented only 1% of live weight and was therefore not significant.

Organs and tissue development during fetal phase may be affected by maternal nutrition (Funston and Summers, 2013). In this case, the lack of difference in calves' organs according to maternal nutrition plan can be explained by the moment of supplementation. Fetal organogenesis occurs in the first third of gestation (Du et al., 2010) and at this moment there was no nutritional restriction for cows in this trial. Therefore, these results show that, despite the fact that cows were not supplemented during first trimester, pasture was able to supply cow and fetus requirement.

However, there was an increase in the length of the small intestine in calves whose dams were nutrient restricted, without differences in small intestine mass as percentage of body weight. Intestinal length, as well as its morphological components, are important

for nutrient absorption. Likewise, intestinal development is quite malleable and therefore more susceptible to changes influenced by intrauterine conditions that remain until adulthood (Trahair et al., 1997). In addition, we observed differences in crypt depth and villus height/crypt depth ratio. Intestinal villus height is related to the number of enterocytes in the villus and the increase in crypt depth is indicative of the increased rate of enterocyte production and migration to the top of the villus (Pluske et al., 1996). It was hypothesized that the increased crypt depth in calves whose dams were supplemented is indicative of those calves still were performing villus growth, while restricted calves had finished this process. This earlier intestine development in calves from non-supplemented cows indicates an adaptation to better absorption of nutrients under a potentially impaired environmental condition after birth. On the other hand, supplemented calves, despite the smaller length of the intestine, still have cell proliferation in the gut wall that may be a potential for further development.

Moreover, calves born from non-supplemented cows had greater expression of *SLC5A1* and *SLC27A4* (Figure 2). *SLC5A1* encode the protein SGLT1 and is the major transporter of monosaccharides through the enterocyte membrane (Ladeira et al., 2016). *SLC27A4* is the gene that encodes FATP4, the only fat membrane transporter protein present in the intestine, with a significant role in maintenance and metabolism of fatty acids (Xu et al., 2012). Thus, nutritional restriction in cows during gestation allowed for greater expression of membrane transporters in offspring intestine, confirming that this organ was prepared to increase the efficiency of nutrients absorption after birth as an adaptative response to restriction.

On the other hand, the most vulnerable tissue to energy partition is skeletal muscle because it has low priority compared to vital organs (Zhu et al., 2006). Muscle fiber type and proportion of muscle, fat, connective tissue, number and size of muscle fibers may

also affect postmortem meat quality. Meat tenderness is influenced by the myofibrilar composition and by the cross-linking effect of the connective tissue (Miao et al., 2016). Large amount of connective tissue reduces meat tenderness, while intramuscular fat increase flavor and juiciness producing high quality beef (Ladeira et al., 2018). Likewise, animals with a larger number of moderate size fibers produce meat with better quality (Rehfeldt et al., 2000). According to Lee et al. (2010), the way to increase meat productivity without adding negative quality changes is by increasing the number of muscle fibers with moderate diameter. Despite the fact that, there was no differences in muscle composition, and expression of genes related to muscle fiber type, calves whose dams received supplementation had greater number of muscle cells per field after birth, while calves from restricted group compensated the smaller number of cells with the largest cells area and greater ribeye area (Figure 3 and Table 9). This is in agreement with other studies where restricted dams reduced fetus muscle hyperplasia, compromising the amount of muscle fibers, and increasing hypertrophy (Du et al., 2010; Duarte et al., 2014). This effect may be detrimental to animal development, since after birth the number of muscle cells does not change and thus impair the muscle growth potential (Du et al., 2010). According to Gondret et al. (2005), animals with larger number of myofibers results in a rapidly growing and better meat quality. In addition, the highest number of myofibers in supplemented calves group is in agreement with the greater expression of *Wnt10B* at birth in those calves. *WnT10B* is a transcription factor that regulates cell differentiation with positive effect on myogenesis (Ladeira et al., 2016).

It is also important to note that, DNA methylation is indispensable for proper embryonic development, and it depends on the availability of methyl donors group, such as methionine and folate (Chmurzynska, 2010). According to the NRC (2001), methionine is one of the limiting amino acids in ruminants' diet, mainly for animals in

pasture. Furthermore, fetal growth leads to an increase in the requirement of amino acids for pregnancy and the excess or deficiency of these nutrients can affect fetal metabolic pathways. Besides that, protein restriction during pregnancy can affect *PPARG* methylation in the liver and *PPARG* in adipose tissue (Chmurzynska, 2010).

In this study, supplemented cows may have affected offspring DNA methylation process through protein supplementation with an increase in methionine supply. *PPARG* expression increased in muscle of calves whose dams were supplemented, except at 11 days after birth, when there was no difference between treatments. This variations in *PPARG* expression may be related to different ages of calves from birth to weaning. At birth, all calves had access to colostrum before being slaughtered. At 11 days, calves were eating only milk. And at weaning, although calves still ingests milk, the main source of their nutrition was the pasture. PPARs isoforms use fatty acids as endogenous ligands, and they are nutritionally regulated (Bispham et al., 2005). Therefore, milk fatty acids could be affecting to *PPARG* expression in nursing calves.

CD36 is a downstream target of *PPARG* and works in the fatty acids transport and storage of triglycerides in adipose tissue (Lee et al., 2012). Therefore, the increase in *CD36* expression in muscle of calves from supplemented cows is in agreement with the greater expression of *PPARG* in those animals, 24 h after birth.

PPARG and *C/EBPA* are the main regulators of adipogenesis and act together on adipocyte differentiation as pleiotropic transcriptional activators of a large group of genes that produce the adipocyte phenotype (Del Pino et al., 2017). Despite this close relationship between *PPARG* and *C/EBPA*, *C/EBPA* expression was lower in the supplemented group at 11 days without changes in *PPARG* expression. According to Zamani and Brown (2011), increased expression of *C/EBPA* in the final phase of differentiation is followed by an increase in *FABP4* expression, leptin and other markers.

C/EBPA and *FABP4* were expressed to a lesser degree in supplemented offspring at 11 days, indicating that there was later adipogenesis in those calves compared to restricted calves. While the greater expression in restricted offspring would be indicative of the final phase of adipocytes differentiation. Also, higher *FABP4* expression may be an indicator of the number of adipocytes within the muscle tissue, which implies that there was a greater number of adipocytes (Del Pino et al., 2017). So, calves whose dams were nutrient restricted during gestation had lesser myogenesis during fetal phase and promoted more cell differentiation for adipogenesis earlier than supplemented offspring.

Moreover, the greater expression of *PPARG* and *ZFP423* at weaning in calves whose dams were supplemented indicates higher adipogenic differentiation in these animals at this time point. The greater expression of *ZFP423* is related to high adipogenic cells with low DNA methylation of *ZFP423* promoter, and consequently increasing overall fat, including intramuscular fat (Wang et al., 2016). The increase in intramuscular fat, in addition to the proper muscle development, indicates that beef quality may be positively impacted in the future, during the finishing phase.

TGFβ1 superfamily has several effects on adipogenesis such as preventing adipogenic differentiation through cooperation with the *WNT10B* pathway and suppressing *C/EBPA* and *PPARG* (Zamani and Brown, 2011). Regardless the suppressing effect of *TGFβ1* on *PPARG* expression described before, in the present study both markers were higher expressed in supplemented cows' offspring muscle at birth and weaning. These behavior can be explained by the autocrine property of *PPARG* (Choy et al., 2000), where *TGFβ1* inhibited adipogenic cell differentiation, without however totally blocking *PPARG* expression.

On the other hand, *TGFβ1* is the main regulator of fibrogenesis. At the beginning of development, mesenchymal cells are associated with the myogenic lineage. But part

of these cells remains as fibro-adipogenic progenitor cells located in the extracellular matrix of the muscle fiber, and intramuscular connective tissue is derived from those cells. The expression of *TGFβ1* is also affected by maternal nutrition, as was observed by Duarte et al. (2014), where fetuses of over-nourished cows had a greater expression of *TGFβ1*.

Summarizing, higher expression of *PPARG*, *CD36*, *WNT10B* and *TGFβ1* in progeny of supplemented cows indicates that 24 hours after birth these calves still had multipotent cells performing the differentiation for myogenesis, adipogenesis and fibrogenesis. Those mechanisms are competitive among themselves but this process is not entirely clear, mainly in newborn calves. In general, previous studies were carried out with fetuses before birth and little is known about the effects of colostrum on transcription factors and other gene expression. Eleven days after birth, nutritional restriction in non-supplemented cows shows greater *C/EBPA* and *FABP4* expression in muscle of the calves suggesting earlier adipogenesis, as a metabolic adaptation to increase body reserves. At weaning, calves from supplemented group increase expression of adipogenic genes supporting the hypothesis that these animals had a late adipogenesis.

CONCLUSIONS

Pregnant cow supplementation during dry season is a favorable strategy to maintain the productive cycle because it improves pregnancy rate in the subsequent breeding season, and offspring of supplemented cows had greater birth weight. Maternal nutrition during pregnancy also positively affects myogenesis, and adipogenesis of calves whose dams were supplemented. In addition, calves born from restricted cows presented some adaptations to a restricted condition as an increase in small intestine length and greater expression of membrane transporters in jejunum.

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