



SARA ADRIANA SALINAS LAURA

**EFFECT OF INJECTED VITAMIN A DURING PREGNANCY
OR IN NEWBORN CALVES ON PERFORMANCE, BEEF
QUALITY, AND MUSCLE LIPOGENESIS**

LAVRAS-MG

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

Prof. Dr. Marcio Machado Ladeira
Orientador

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SARA ADRIANA SALINAS LAURA

**EFEITOS DA INJEÇÃO DA VITAMINA A DURANTE A GESTAÇÃO OU RECEM
NASCIDOS NO DESEMPENHO, QUALIDADE DA CARNE, E LIPOGENESIS
MUSCULAR**

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APROVADA em 15 de abril de 2026.

Dr. Daniel Rume Casagrande - DZO/UFLA

Dra. Erick Darlisson Batista- DZO/UFLA

Dr. Marcio Duarte-DZO/UFV

Dr. Karol Nascimento- DZO/UFSJ

Documento assinado digitalmente
 **MARCIO MACHADO LADEIRA**
Data: 25/07/2026 18:28:03-0300
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Muita obrigada!

(1% melhor cada dia)

RESUMO

Este estudo avaliou os efeitos da injeção de vitamina A (VA) em vacas gestantes ou em bezerros neonatos sobre o desempenho da progênie, a qualidade da carne bovina e a expressão de genes no tecido muscular de animais Angus × Nelore terminados em confinamento. Trinta e seis bezerros foram distribuídos aleatoriamente em um delineamento fatorial 3×2 (3 protocolos de vitamina A e sexo). Os protocolos de vitamina A foram realizados da seguinte forma: sem injeção de VA (Controle); injeção de VA em vacas aos 250 dias de gestação (VAcow; dose única de 2.000.000 UI); e injeção de VA nos bezerros logo após o nascimento e aos 60 dias de idade (VAcalf; 200.000 UI em cada dose). Os animais foram levados ao confinamento com peso corporal médio de $213 \pm 25,7$ kg, distribuídos em 12 baias, com três animais por baia. O período de confinamento teve duração de 207 dias. Os machos apresentaram maior ganho médio diário (GMD) ($P = 0,03$). A espessura de gordura subcutânea foi maior nas fêmeas ($P < 0,001$), com tendência a valores mais elevados nos animais VAcow ($P = 0,08$). A área de olho de lombo e o teor de gordura intramuscular foram maiores ($P = 0,04$) nos machos do grupo VAcow, sendo o teor de gordura intramuscular semelhante ao das fêmeas, independentemente do protocolo de vitamina A. Além disso, a expressão dos genes CPT2, DKL1 e ZNF423 foi reduzida ($P < 0,05$) nos animais VAcow. Enquanto isso, o gene RXRA apresentou menor expressão ($P = 0,05$) nos animais VAcow e VAcalf. Em relação ao sexo da progênie, a expressão dos genes PPARG, SREBF1, CPT2, ACOX, GHR, MYH1 e MYH2 foi maior ($P < 0,05$) nas fêmeas. Entretanto, a expressão de PAX7 foi maior ($P = 0,05$) nos machos. Em conclusão, a injeção de vitamina A durante a gestação pode melhorar a deposição de gordura intramuscular em machos e alterar a expressão de diversos genes, demonstrando que essa vitamina exerce efeitos de longo prazo sobre a progênie. Além disso, a injeção de vitamina A não aumentou a gordura intramuscular nas fêmeas.

Palavras-chave: Adipogenesis, miogenesis, programação fetal

ABSTRACT

This study evaluated the effects of VA injection in pregnant cows or neonatal calves on offspring performance, beef quality and expression of genes in the muscle tissue of Angus × Nellore animals finished in the feedlot. Thirty-six calves were randomly assigned in a 3 × 2 factorial design (3 vitamin A protocols and sex). Vitamin A protocols were done as follows: no VA injection (Control); VA injection in cows at 250 d of gestation (VAcow; 2.000.000 IU single dose); VA injection in calves just after birth and 60 d of age (VAcalf; 200.000 IU each dose). Animals were taken to the feedlot with an average body weight of 213±25.7 kg, distributed into 12 pens, with three animals per pen. The feedlot lasted 207 d of feeding. Steers had greater ($P = 0.03$) average daily gain (ADG). Back fat thickness was greater in heifers ($P < 0.001$) with a tendency for higher values in VAcow animals ($P = 0.08$). The ribeye area and intramuscular fat content was greater ($P = 0.04$) in VAcow steers. Being intramuscular fat content similar to the heifers, regardless vitamin A protocol. Besides, the expression of *CPT2*, *DKL1* and *ZNF423* genes were downregulated ($P < 0.05$) in VAcow animals. Meanwhile, the *RXRA* gene was less expressed ($P = 0.05$) in VAcow and VAcalf animals. Regarding progeny sex, the expression of the *PPARG*, *SREBF1*, *CPT2*, *ACOX*, *GHR*, *MYH1*, *MYH2* genes were higher ($P < 0.05$) in females. However, *PAX7* expression was higher ($P = 0.05$) in males. In conclusion, VA injection during gestation can improve intramuscular fat in steers and alter the expression of several genes, demonstrating that this vitamin has a long-term effect on the progeny. In addition, vitamin A injection did not increase intramuscular fat in heifers.

Keywords: adipogenesis, myogenesis, fetal programming

INDICADORES DE IMPACTO

Este trabalho investigou os efeitos da injeção da vitamina A em vacas no terço final da gestação e ao nascimento de animais Angus x Nelore F1, terminados em sistema intensivo. Procurando entender os efeitos sobre o desempenho, qualidade da carne e a lipogênese muscular. Resultados mostraram que a injeção fornecida nas vacas gestantes de progênie machos acrescentou a deposição de gordura intramuscular sendo similar aos resultados das fêmeas. O qual pode estar relacionado à maior expressão do gene lipogênicos ACACA ou a existência dum dimorfismo sexual no desenvolvimento fetal. Por outro lado, não teve diferenças significativas nos parâmetros de qualidade da carne de pH, força de cisalhamento, e coloração da carne entre os tratamentos, a exceção dos níveis do valor b. Além, fêmeas tiveram expressão maior dos genes SREBF, CPT2, ACOX, GHR, MYH1, MYH2, e somente PAX7 foi maior nos machos. Esses achados sugerem que a Vitamina A em vacas pode melhorar a qualidade da carne bovina de machos, gerando uma maior quantidade de carne com gordura intramuscular. A pesquisa permitiu contribuir na melhor dosagem para ter resultados benéficos na progênie durante a programação fetal.

IMPACTS INDICATORS

This study investigated the effects of vitamin A injections administered to cows during the last third of gestation and at birth in Angus × Nellore F1 cattle finished under an intensive production system. The objective was to understand the effects on performance, meat quality, and muscle lipogenesis. The results showed that vitamin A injections administered to pregnant cows carrying male progeny increased intramuscular fat deposition, resulting in values similar to those observed in females. This effect may be associated with higher expression of the lipogenic gene ACACA or with the existence of sexual dimorphism during fetal development. On the other hand, no significant differences were observed among treatments for meat quality parameters, including pH, shear force, and meat color, except for the b* value. Furthermore, females exhibited greater expression of the genes SREBF, CPT2, ACOX, GHR, MYH1, and MYH2, whereas only PAX7 expression was higher in males. These findings suggest that vitamin A administration to cows may improve the meat quality of male offspring by increasing intramuscular fat deposition and, consequently, producing a greater amount of marbled beef. This research also contributes to defining more appropriate vitamin A dosages to achieve beneficial effects in the offspring through fetal programming.

SUMMARY

Chapter 1	11
Literature review	11
1. INTRODUCTION	11
2. LITERATURE REVIEW	12
2.1. Fetal and Neonatal Programming.....	12
2.2 Adipogenesis.....	14
2.3 Angiogenesis.....	16
2.4 Vitamin A in developmental programming	17
BIBLIOGRAPHY.....	22
CHAPTER 2.....	31
ARTICLE	31
Abstract:.....	31
1. INTRODUCTION	32
2. MATERIAL AND METHODS	33
2.1 Animal welfare.....	33
2.2 Experimental management, treatments and animals.....	33
2.3 Feedlot growing and finishing	34
2.4 Harvesting, carcass quality measurements, and sample collection	35
2.5 Meat quality and histological analysis	35
2.6 Gene expression analysis.....	36
2.7 Statistical analysis.....	37
3. Results.....	38
4. Discussion	39
5. Conclusion	43
6. Bibliography.....	43

Chapter 1

Literature review

1. INTRODUCTION

Improving beef quality characteristics by increasing intramuscular fat (IMF), commonly known as marbling, can be achieved through various factors, including genetics, nutrition, sex and management practices (NGUYEN *et al.*, 2021). IMF is important because it enhances tenderness, juiciness and flavor of meat to overall acceptance of consumers (MILLER, 2020; THOMPSON, 2004), and also add value to meat cuts (HUDSON *et al.*, 2020).

Adipogenesis begins during the prenatal phase, and it will have a long influence on fat deposition and meat quality (LADEIRA *et al.*, 2018). Marbling starts with the maternal influences, around midgestation and late gestation in ruminant animals (AHN *et al.*, 2023; DU, TONG, *et al.*, 2010). In this case, it overlaps with the secondary myogenesis and goes until 250 days of age, which stops during the weaning stage (DU *et al.*, 2013). This period is known as marbling window in cattle stage (DU *et al.*, 2013; KEOGH *et al.*, 2021). Nutritional strategies on this stage could have positive response, increasing the number of intramuscular adipocytes without affecting other fat depots (HUANG *et al.*, 2024). Therefore, nutrigenomic can be used to manipulate molecular mechanism and IMF deposition in beef cattle impacting transcription factors, expression of genes involved in adipogenesis and lipogenesis and cell differentiation (BAIK *et al.*, 2023; WANG *et al.*, 2016).

The adipose tissue is highly vascularized, so adipogenesis is also linked to the development of its vasculature (CAO, 2013; YU *et al.*, 2022). Leading to suspicions that adipocytes derive from cells associated with blood vessels (ROSEN & SPIEGELMAN, 2014) and adipose tissue endothelial cells promote preadipocyte differentiation (Christiaens & Lijnen, 2010).

The studies about the use of vitamin A (VA) as a new technology for beef industry are promising outlook. Numerous studies have already demonstrated that VA, when applied during marbling window, positively influences intramuscular fat deposition and myogenesis (HARRIS *et al.*, 2018; PENG; SMITH; LEE, 2021; JIN *et al.*, 2022; MACIEL *et al.*, 2022). This effect is due to the recruitment of a greater number of intramuscular adipose progenitor cells through adipogenesis and angiogenesis (WANG *et al.*, 2016; YU *et al.*, 2022). By further increasing the

production of quality beef through VA use, this would boost Brazil's competitive ability, providing more opportunities to expand exports and create new markets.

In this context the hypothesis of this work is that VA injection during the final third of gestation in cows will be as effective as VA injection in newborn calves to produce more intramuscular fat in beef from steers and heifers finished in feedlot. Therefore, the aim of this study was to evaluate the effects of VA injection, during gestation or at birth, on performance of Angus × Nellore steers and heifers, and the development of muscle and adipose tissue, chemical composition and beef quality.

2. LITERATURE REVIEW

2.1. Fetal and Neonatal Programming

First studies with humans showed that embryo and fetus' development are influenced by mother's health and nutrition during pregnancy and also external factors, which can program permanent structural and physiological modification in the fetus, so affect neonate develops into adulthood (BARKER, 2004; MEYER, 2025; WATHES, 2022). Additionally, neonatal period is critical for programming related to metabolic functions (DEBARBA *et al.*, 2020). This is known as developmental programming, and in the last years, this study area has been increasing in livestock. Its crucial to consider programming of the neonatal period in ruminant livestock as well.

“Fetal programming” refers to any stimulus during pregnancy that alters uterine environment for the growing fetus, modifying the developmental trajectory in postnatal life (CHRISTOFARO FERNANDES *et al.*, 2023; NATHANIELSZ; POSTON; TAYLOR, 2007a). Eutherians (placental mammals) are particularly sensitive to the direct and indirect effects of maternal nutrition at all stages between oocyte maturation and birth (FERGUSON, 2005). These changes can affect the expression of fetal genome and carry lifelong consequences.

Manipulation of maternal nutrition during gestation can induce fetal programming, exerting profound effects on the offspring. Beginning with the lifetime impacts on development of fetal organs and tissues, reproduction, reduce passive immunity, which may be correlated to feedlot performance, carcass value, and profitability (BROADHEAD *et al.*, 2019). During this stage, differentiation of skeletal muscle, adipose and connective tissue cells develop from a common progenitor cell with effects on offspring growth, performance, production efficiency,

and determine the meat quality (DUARTE, 2021; DU *et al.*, 2010). Alterations in maternal nutrient supply can modify the intrauterine environment and impact the processes of myogenesis, adipogenesis and fibrogenesis, and these tissues are critical for determining meat yield and quality in beef cattle (COSTA *et al.*, 2021).

The majority of muscle fibers form between 2 and 6 months of gestation, when primary and secondary myogenesis occurs (Du, Yin, *et al.*, 2010; SANTOS *et al.*, 2022) being this last one which generates most muscle fibers (Bailey *et al.*, 2001), whereas adipose cells are detectable about day 80 of gestation and development of subcutaneous adipocytes occurs between mid to late fetal stage to month 8, with intramuscular adipocytes beginning to appear in the mid to late fetal stage to 250 days of life (DU *et al.*, 2013). Programming postnatal body composition and growth involve alteration in adipocyte morphology cellularity and metabolism. Transcription factors can regulate gene expression through activation or inhibition (CASTELLANOS *et al.*, 2020), changing tissues composition and improving meat quality. This molecular process has not been thoroughly studied and neither how epigenetic changes can be regulated (ABEBE *et al.*, 2023a). Epigenetic mechanisms are defined as heritable changes affecting gene and genome function without involving changes in the DNA sequence. They are essential for embryonic development and cellular differentiation, sustaining gene expression and making this decision irreversible (CHAVATTE-PALMER *et al.*, 2018). Development in epigenetics have permit understand molecular basis of process, as IMF regulation, making a key area for research (ABEBE *et al.*, 2024). Study epigenetic changes is crucial for understanding the regulation of genes at the molecular, cellular, tissue, and organ levels. Epigenetic changes lead to heritable modifications in gene expression by DNA methylation, histone modification or the influence of non-coding RNA causing gene activation or silencing.

In fact, in livestock, compromised fetal or neonatal growth has shown to lead to poor body composition (WU *et al.*, 2006). As COSTA; GIONBELLI; DUARTE, (2021) explained that pregnant ruminants raised in pastures, under feed restriction due to forage seasonality, may impair the offspring development, because fetal vital tissues are prioritized to use maternal nutrients, impairing skeletal muscle and other tissues' development. This concept of fetal and neonatal programming has wide-ranging implication for the animal sciences. Thus, appropriate fetal programming is essential for maximizing the growth and production potential of animals.

2.2 Adipogenesis

Adipocytes are cells derived from the differentiation of mesenchymal stem cells (MSCs), by a process known as adipogenesis (HAUSMAN *et al.*, 2009). Additionally, adipocytes and fibroblasts share the same pool of stem cells known as fibro-adipogenic progenitors (FAPs), which, through genetically controlled competitive processes, will give rise to the two-cell lineage (DU *et al.*, 2015). This occurs during the stages of MSC commitment into preadipocytes and their differentiation into mature adipocytes (Figure 1) (DU, YIN, *et al.*, 2010; LIU *et al.*, 2020). Adipogenesis is regulated by factors such as genetics, nutrition, and the environment, which generate key signals regulating it (HARPER & PETHICK, 2004; TAN & JIANG, 2024). To understand these cellular differentiation processes, increased attention has been given to the study of transcription factors and gene expression patterns that regulate these processes (LEE *et al.*, 2011; Liu *et al.*, 2020; Zhang, 2015), such as *peroxisome proliferator-activated receptors* isomers (*PPAR*), *zinc finger proteins 423* (*ZFP423*), *sterol regulatory element-binding proteins* (*SREBP*), and *CCAAT/enhancer-binding proteins* (*C/EBPs*) (LADEIRA *et al.*, 2016; LIU *et al.*, 2020a).

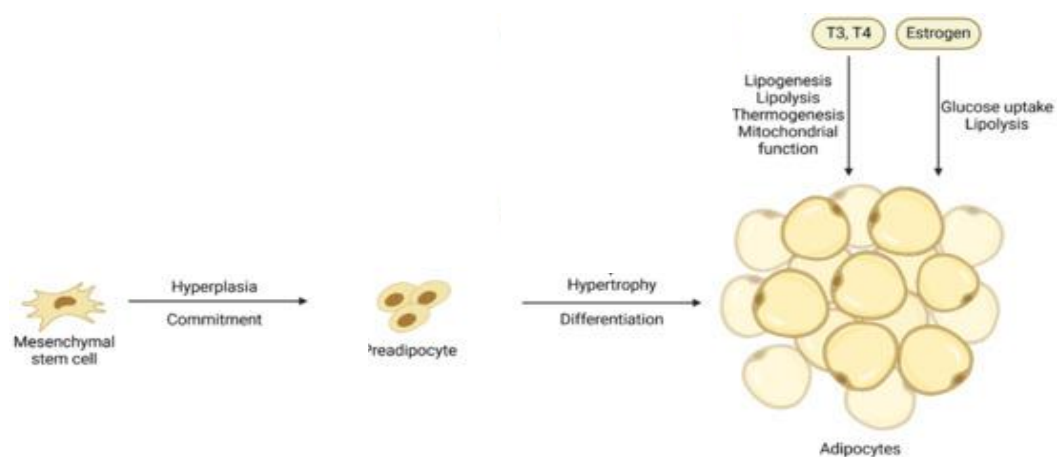


Figure 1. Key transcriptional and hormonal regulators of the commitment of mesenchymal stem cells or adipose progenitor cells to preadipocytes and the differentiation of preadipocytes into adipocytes. Arrows denote stimulation and T-shaped lines denote inhibition (adapted from TAN; JIANG, 2024)

At the stage of commitment or determination of preadipocytes, the expression of Zinc Finger Protein 423 is a key transcription factor in the commitment of MSCs to preadipocytes (HUANG *et al.*, 2012; WANG *et al.*, 2016); ZFP423 induces the expression of *PPARG* e *CEBPA*, resulting in the differentiation stage (GUPTA *et al.*, 2010; HUANG *et al.*, 2012). Subsequently, *PPAR-γ* and *C/EBP* proteins are crucial transcription factors to activate the expression of genes for the later stage of adipogenesis, and the formation of mature adipocytes (ROSEN & MACDOUGALD, 2006). Meanwhile, the primary role of *PPAR-γ* is to act on lipid

metabolism and regulate genes associated with fatty acid (FA) storage, such as *Fatty Acid Binding Protein 4 (FABP4)*, *Acyl-CoA Synthetase (ACSL)*, and *Lipoprotein Lipase (LPL)* (BIONAZ *et al.*, 2012). Following this, sterol regulatory element-binding protein 1c (SREBP-1c), is a key factor in *de novo* fatty acid synthesis, absorption, and storage, positively regulating the expression of genes involved in fatty acid synthesis, such as *Acetyl-CoA Carboxylase (ACACA)*, *Fatty Acid Synthase (FASN)*, and *Stearoyl-CoA Desaturase (SCD1)* (SHIMANO, 2001). The molecular processes and interactions between these transcription factors and target genes are still being studied, along with the exploration of how epigenetic changes can be regulated (ABEBE *et al.*, 2023).

Lipogenesis is the physiological process of endogenous FA synthesis and occurs in the cytosol. Both lipogenesis, which synthesizes lipids, and adipogenesis, responsible for adipocyte development, work together to deposit fat in beef cattle (WANG *et al.*, 2023). For this deposition to occur, triglycerides must be incorporated into the animal's adipose tissue after fatty acid absorption or *de novo* synthesis of other fatty acids (RICHARD *et al.*, 2020; ROLLIN *et al.*, 2003).

The deposition of adipogenic cells begins before the middle third of gestation in beef cattle, with the first adipocytes being detected at 80 days of pregnancy (TAGA *et al.*, 2012). However, there is an order of adipose tissue deposition as follows: first visceral adipose tissue, followed by intermuscular, subcutaneous, and finally intramuscular tissue (BONNET *et al.*, 2010). Adipogenesis in bovine intramuscular tissue expand from the late gestation to around approximately 250 days of the animal's life, overlapping with the period of secondary myogenesis, where maternal nutrition enhances the formation of intramuscular adipocytes, resulting in adipocyte hyperplasia, decreased connective tissue, and improved production efficiency postnatally and meat quality (DU, 2023; DU & YIN, *et al.*, 2010). This provides an opportunity to specifically enhance intramuscular adipocyte formation during the late gestation, neonatal and early weaning stages without affecting other fat depots (DU *et al.*, 2015; HUANG *et al.*, 2024). The development of adipose tissue cells depends on the processes of mesenchymal steams cells commitment, hyperplasia and hypertrophy (CIANZIO *et al.*, 1985). In hyperplasia, tissue expansion occurs through the increase in the number of adipocytes, where preadipocyte precursors differentiate into mature adipocytes. In hypertrophy, lipid droplets and adipocytes expand through lipid synthesis or uptake (NGUYEN *et al.*, 2021; ROBELIN, 1986; WANG *et al.*, 2023).

Because of the difference metabolism between adipose tissues, IMF has slowest rates of lipogenesis and it uses glucose as an important substrate or acetyl donor (GARDAN *et al.*, 2006). Even though, Smith *et al.*, (2018) found that glucose is an important acetyl units donor to fatty acid biosynthesis in intramuscular adipose tissue (PARK *et al.*, 2018) In addition, adipocyte hyperplasia is especially important to provide new sites for lipid deposition (HARRIS *et al.*, 2018).

2.3 Angiogenesis

Adipose tissue is constantly evolving during lifetime and its composition changes along the life. Containing several cell types such as stromal vascular cells, which includes adipose stem cells and endothelial progenitor cells (DI STEFANO *et al.*, 2019), it is characterized to be a tissue highly vascularized, and the density of blood vessels plays a crucial role in regulating adipocyte function and adipogenesis (HEROLD & KALUCKA, 2021). So, it is regulated via vascular endothelium (RUPNICK *et al.*, 2002).

Angiogenesis, is a multistep process which involves endothelial cell proliferation, migration, invasion and new blood vessels develop from pre-existing vessels (KORYBALSKA, 2018; MADER *et al.*, 2006). Besides nutrients and oxygen, the vessels also transport growth factors required for adipocyte survival and functions, (CAO, 2013), showing that exists close communication between the blood vessels and adipocytes and the expansion of adipose tissue is angiogenesis-dependent (CAO, 2013; RUPNICK *et al.*, 2002). Before birth, rat and mouse fat showed a poorly differentiated mesenchymal tissue, consisting fibroblast-like cells in a loose connective matrix with small capillaries. By the other hand, at postnatal day (until 8th day), showed a circumscribed area with adipocytes, the area is delimited by fibroblast like cells and contain numerous large capillaries (TRAN *et al.*, 2012). After that, capillars began to develop within mature lobular architecture and mesenchymal cells compact, to begin adipose differentiation. Blood vessel growth precedes adipocyte differentiation and maturation during epididymal adipose tissue formation (HAN *et al.*, 2011).

The vascular system controls the adipose tissue through influences on the adipocyte function and preadipocyte differentiation (CAO, 2013). Blood vessel growth precedes adipocyte differentiation and maturation during epididymal adipose tissue formation (HAN *et al.*, 2011). The vascular system controls the adipose tissue through influences on the adipocyte function and preadipocyte differentiation (CAO, 2013). Fat depot-specific difference in

angiogenic gene expression results from the difference in adipocyte size (YAMADA *et al.*, 2010). Moreover, there are limited studies that evaluated angiogenesis in adipose tissue from livestock.

Adipose tissue expresses various angiogenic growth factor as Adipocyte-derived vascular endothelial growth factor-A (VEGF-A), fibroblast growth factor (FGF) and leptin (CAO, 2007). VEGF/VEGF-receptor system is the main regulator of the angiogenic activity in adipose tissue and is expressed in the stroma vascular fat (LEMOINE *et al.*, 2013), and VEGF-A stimulates proliferation and migration of endothelial cells. Furthermore, platelet-derived growth factor- β (PDGF- β) recruits pericyte muscle cells to stabilize the vessel, and with transforming growth factor beta (TGF- β) stimulates the differentiation of mesenchymal stem cells to promote extracellular matrix production and vessel maturation (JOHNSTON E ABBOTT, 2022) These stimulates adipose tissue vascularization and improves metabolic health (SUN *et al.*, 2012). Endothelial cells communicate with adipocytes via releasing or exchanging extracellular vehicles, which contain proteins and lipids that capable of modulating cellular signaling pathways (CREWE *et al.*, 2018). Peroxisome proliferator-activated receptor gamma (PPAR- γ) can influence angiogenesis, no affecting proliferation of endothelial cells and increasing capillary density and sprouting(GEALEKMAN *et al.*, 2012).

Besides, blood flow is important for overall skeletal muscle growth and function. The increment of capillary density can enhance skeletal muscle, and satellite cells are increased along with the capillary density, indicating blood vessels are important for muscle growth (MÄNNISTÖ *et al.*, 2025).

2.4 Vitamin A in developmental programming

Vitamin A (VA) is a fat-soluble vitamin and is considered an essential micronutrient for cattle, as it is involved in several physiological processes, such as organ and tissue morphogenesis, especially in neonatal and growing animals (NAPOLI, 1996). Besides, it regulates biological functions related to adipose tissue development and fat deposition (CONDRON *et al.*, 2017). In this latter role, VA can impact each stage of adipogenesis. This micronutrient exists in three different forms in the body depending on its oxidation state: Retinol (hydroxyl form), Retinal (aldehyde form), and Retinoic Acid (carboxylic form). Dietary, it is obtained from plant sources, such as carotenoids (mainly β -carotene in pasture-based diets), or as retinol esters from animal sources and supplements (CAÑETE *et al.*, 2017). Then, they are secreted into the lymph and bloodstream (SPIEGLER *et al.*, 2012; Vogel *et al.*,

1999). For the activation of retinol, it undergoes two oxidative processes: first, a reversible oxidation by alcohol dehydrogenases (ADHs) and microsomal retinol dehydrogenases (RDH), and then retinaldehyde is irreversibly oxidized to retinoic acid by retinal dehydrogenases (RALDHs) (PARÉS *et al.*, 2008). Upon entering the bloodstream, VA undergoes structural rearrangements, leading to the release of retinyl ester as retinol bound to retinol binding protein (RBP) (ARNOLD E KWITEROVICH, 2003). Carotenoids are converted to retinol before storing it as retinyl esters in different organs and tissues. After consumption, these components are digested in the gastrointestinal tract, brush-border hydrolyze retinyl esters into retinol and free FAs. Then, taken up into enterocytes are transported via facilitated diffusion. The majority of retinol in the enterocyte is re-esterified into retinyl ester by the action of lecithin:retinol acyltransferase (LRAT) and then incorporated into chylomicrons for delivery. Excessive retinol is stored in the liver, where endogenous retinoids are stored as all-trans retinoic acid esters. Adipose tissue is the second storage site, containing up to 20% of the body's total retinol (TSUTSUMI *et al.*, 1992).

Fetal programming may be triggered by various perturbation in the maternal environment, such as maternal nutrition and effects on uteroplacental blood flow. This perturbation must affect the fetus through the placenta (JANSSON & POWELL, 2007). The placenta is a fundamental organ in mammalian development during gestation and plays a crucial role in mammalian embryogenesis, due to the exchange of gases, nutrients, and embryonic waste between the mother and the fetus (JANSSON & POWELL, 2007; WATSON & CROSS, 2005). The different factors involved in placental transfer and fetal storage of VA in beef cattle have not been fully explored. It is known that fetal growth accelerates after 150 days of gestation, requiring more nutrients, including VA, for growth. In ruminants, the physiological exchanges between the mother and the fetus occur through the caruncular-cotyledonary unit of the placenta, called the placentome (VONNAHME *et al.*, 2015). The embryo depends on maternal transfer of nutrients, such as VA, through placental transfer (SPIEGLER *et al.*, 2012). VA and its derivatives (retinoids) (Figure 2) are transferred via the bloodstream through the placenta by various proteins, some of which overlap with those involved in lipoprotein uptake. According to QUADRO *et al.*, (2020) the transfer of β -carotene circulating in the maternal bloodstream from the placenta to the fetus is mediated by lipoproteins and regulated by the availability of the VA precursors with a feed-forward mechanism. Even though, this mechanism is still largely unknown.

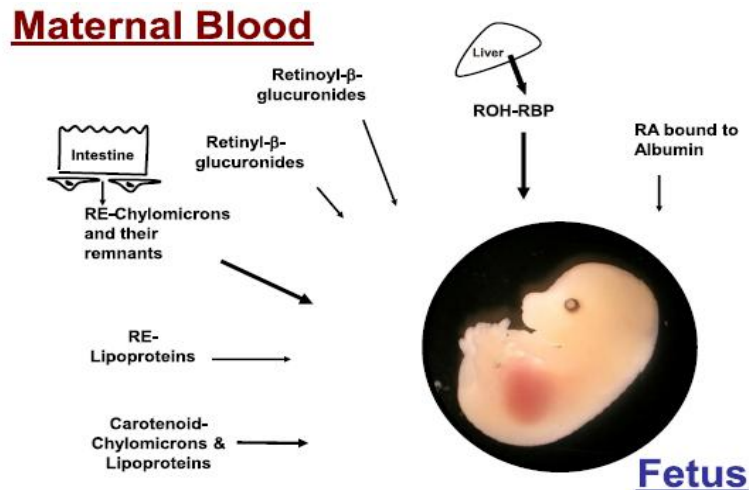


Figure 2. Circulating forms of retinoids and carotenoids available for embryonic development (SPIEGLER *et al.*, 2012).

The study by Lorente e Miller, (1977) has shown that retinol (ROH) passes through the placenta, but the cellular mechanism may involve three possibilities: 1. Direct transfer of retinol-retinol binding protein (ROH-RBP) via cellular uptake and protein-ligand complex (as in liver and skin tissues); 2. Transfer of retinyl esters bound to lipoproteins, modified by lipoprotein lipase; 3. Cellular uptake of ROH through the signaling receptor and retinol transporter (STRA6).

In the maternal bloodstream, retinoid forms as ROH are bound to RBP whose function is to mobilize and transport retinol from hepatic retinoid stores, distributing it to peripheral tissues and supporting retinoic acid-dependent functions, such as in embryos (BLANER *et al.*, 2016; QUADRO *et al.*, 2005). ROH passes from the mother's bloodstream into the trophoblasts and reaches the yolk sac cavity, where VA uptake occurs (WARD *et al.*, 1997). It is required and mediated for circulation by the STRA6, a large membrane protein (KAWAGUCHI *et al.*, 2007), which is a specific receptor for the ROH-RBP complex. This receptor is regulated in response to maternal VA diet in embryos and helps maintain retinoid homeostasis for tissue development (KAWAGUCHI *et al.*, 2007). In rodents, RBP expression is detected early at the junction between the uterine wall and placenta (SAPIN *et al.*, 1997). However, in cows, it has been observed that the placenta can secrete RBP into the allantoic fluid (LIU *et al.*, 1990). RBP is synthesized in epithelial cells of the liver, from where it is secreted into the bloodstream, with the purpose of transporting retinol to specific tissues (WARD *et al.*, 1997). RBP binds one molecule of retinol with a serum protein, transthyretin, and circulates as a 1:1 molar complex

(MONACO *et al.*, 1995), which increases RBP's affinity for retinol (BELLOVINO *et al.*, 2003). Maternal retinol binds to RBP and is released at the maternal-fetal interface. This binding to yolk sac RBP can be secreted into the fetal circulation, where most ROH is stored in the liver as retinol esters (RE). To reach target tissues, it is hydrolyzed and bound to RBP as part of a blood complex (SAPIN *et al.*, 1998). Therefore, maternal status or dietary VA intake affect its availability to cross the placenta and reach the fetus. Placenta can buffer retinoid delivery, its store VA until embryonic liver be functional and also protect embryo from a potential toxic excess of maternal retinoid (SPIEGLER *et al.*, 2012). Therefore, based on the literature cited above, it is possible to hypothesize that retinoic acid can reach the fetus through the placental flow.

Studies have shown that supplementation with Vitamin A, specifically its active metabolite retinoic acid (RA), during marbling window in cattle may be a promising nutritional strategy, since RA shows a beneficial effect on lipogenesis and adipogenesis metabolism (HARRIS *et al.*, 2018; PENG; SMITH; LEE, 2021; TAN; JIANG, 2024). Retinoic acid is a potent agent capable of induce alteration in epigenetic modification that produce effects on the phenotype (BAR-EL & REIFEN, 2017), without changing the DNA sequence (ZHANG *et al.*, 2023). Phenotype of animals may be modified by nutritional modulations through epigenetic mechanisms, which is termed nutritional epigenetic (BAR-EL & REIFEN, 2017). VA epigenetic mechanisms include: DNA methylation, which its mediated by DNA methyltransferases (DNAMTS) and leads to recruitments of members of methyl 5-carbon of cytosine ring located next guanidine nucleotide (CpG dinucleotides) binding domain family of protein (DNAMTS 1, 3A and 3b) (Fazi, *et al.*, 2005), also include histone modifications form nucleosome structure allowing gene transcription and expression (Hake, 2011), and the last epigenetic metabolism is miRNAs which can regulate both mechanisms. miRNAs are endogenous, small, non-coding RNAs that regulate gene expression by binding to their target mRNAs, leading to degradation or translation repression (CHEN *et al.*, 2014). Sometimes, RA regulates the expression of miRNA through its receptors such as retinoic acid receptos (RAR α and RAR β) which bind to the regulatory region of certain miRNAs to alter their expression levels or miRNA may target receptors of RA and affect the effect of RA (WANG *et al.*, 2020). These mechanisms can impact transcription factor and gene expression, affecting cell function (ABEBE *et al.*, 2023; WANG *et al.*, 2019).

Retinoic Acid serve as a ligand for RAR α , RAR β and RAR δ). They partner with retinoic X receptors (RXR α , RXR β and RXR δ) to bind to retinoic acid response elements on target gene loci, acting as transcription factors for target genes in adipogenesis and lipogenesis (Blomhoff & Blomhoff, 2006). In addition, when retinoic acid bind to PPAR β / δ stimulate cell proliferation (WANG *et al.*, 2016). Besides, exists some intracellular binding proteins for VA states (ROH, RAL and RA) as cellular retinol-binding protein (CRBP I, II and III), having specific role in VA transport and metabolism (NOY, 2000). So, it's known that transcription factor affects adipogenic differentiation, RARs are transcription factors that potentially IMF deposition in beef cattle (LIU *et al.*, 2020). The preferential binding of retinoic acid between RAR and PPAR β/δ determines RA's effect, regulated by Cellular Retinoic Acid-Binding Protein 2 (CRABP2), directing RA to RAR and fatty acid binding protein type5 (FABP5) to PPAR β/δ resulting in positive effects on intramuscular preadipocyte commitment (WANG *et al.*, 2016).

Researchers have showed VA regulates adipogenesis, angiogenesis and apoptosis. In this sense, some studies have already demonstrated that VA, when applied during marbling window positively influences intramuscular fat deposition and myogenesis. Harris *et al.*, (2018) demonstrated that injecting VA twice (at birth and one moth of age) with 150,000 or 300,000`IU increased IMF at weaning and slaughter. Maciel *et al.*, (2022), evaluated a single VA injection at birth (300,000 IU), observed that only one VA injection increased IMF deposition. Jin *et al.*, (2022) administrated VA orally 40,000 IU/kg as-fed during 3 months and found increase satellite cells. Song *et al.*, (2023) gave weekly intramuscular injection of 7,500 IU VA from birth to 3 weeks of age in newborn lambs and found an increase in lamb muscle growth by promoting the myogenic potential of satellite cells. VA can also promote vascular system development, increases the progenitor pool in offspring, enhances adipocyte hyperplasia, and supports better metabolic health in progeny (Wang *et al.*, 2017). These studies evaluated the application of VA in different forms of use, injected or oral, and both forms have promising use.

Tan *et al.*, (2016) evaluating VA supplementation orally in mice has showed that maternal supplementation has increased VA content in the breastmilk and improved the status in the offspring comparing with VA administered to neonates, showing as probable strategy in managing fat deposition related to obesity. However, in beef cattle, there are only two studies evaluating VA supplementation during late-stage of gestation and both had VA application orally. Jo *et al.* (2020) supplemented VA (78,000 IU/d) after 225 days of gestation until

delivery, and Dean *et al.* (2024) supplemented (12.200 IU/kg) from 180 d of gestation until parturition. In both studies VA showed a positive effect, on birth weight and commitment of fibro-adipogenic progenitors FAP cells to adipogenic lineage. Dean *et al.*, (2024) also found, in repeated carcass ultrasound, greater intramuscular fat content throughout all the evaluated stages of their post-natal life and no effect on subcutaneous fat, hot carcass weight and carcass yield and dressing. However, despite the cited studies, according to our literature research, no research has also evaluated the use of injected VA on pregnant cows on performance and beef quality.

Vitamin A can be considered as a potential feed nutrient used to improve intramuscular adipogenesis at initial stages of life. Even though, there is a lack of information concerning VA administration in pregnant cows or in newborn calves and the best dosage as well as the timing to be applicated or the breed effect to maximize IMF and muscle deposition.

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CHAPTER 2

ARTICLE

Effect of injected vitamin A during pregnancy or in newborn calves on performance, beef quality, and muscle lipogenesis

¹Sara Adriana Salinas Laura, ¹Marcio Machado Ladeira

*mladeira@dzo.ufla.br

¹Departamento de Zootecnia, Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais 37200-000, Brasil

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Abstract: This study evaluated the effects of VA injection in pregnant cows or neonatal calves on offspring performance, beef quality and expression of genes in the muscle tissue of Angus × Nelore animals finished in the feedlot. Thirty-six calves were randomly assigned in a 3 × 2 factorial design (3 vitamin A protocols and sex). Vitamin A protocols were done as follows: no VA injection (Control); VA injection in cows at 250 d of gestation (VAcow; 2.000.000 IU single dose); VA injection in calves just after birth and 60 d of age (VAcalf; 200.000 IU each dose). Animals were taken to the feedlot with an average body weight of 213±25.7 kg, distributed into 12 pens, with three animals per pen. The feedlot lasted 207 d of feeding. Steers had greater ($P = 0.03$) average daily gain (ADG). Back fat thickness was greater in heifers ($P < 0.001$) with a tendency for higher values in VAcow animals ($P = 0.08$). The ribeye area and intramuscular fat content was greater ($P = 0.04$) in VAcow steers. Being intramuscular fat content similar to the heifers, regardless vitamin A protocol. Besides, the expression of *CPT2*, *DKL1* and *ZNF423* genes were downregulated ($P < 0.05$) in VAcow animals. Meanwhile, the *RXRA* gene was less expressed ($P = 0.05$) in VAcow and VAcalf animals. Regarding progeny sex, the expression of the *PPARG*, *SREBF1*, *CPT2*, *ACOX*, *GHR*, *MYH1*, *MYH2* genes were higher ($P < 0.05$) in females. However, *PAX7* expression was higher ($P = 0.05$) in males. In conclusion, VA injection during gestation can improve intramuscular fat in steers and alter the expression of several genes, demonstrating that this vitamin has a long-term effect on the progeny. In addition, vitamin A injection did not increase intramuscular fat in heifers.

Keywords: adipogenesis, myogenesis, fetal programming

1. Introduction

Fetal programming occurs when an organism responds to an environmental challenge during intrauterine growth (Nathanielsz et al., 2007), influencing aspects of offspring development (Zago et al., 2020). Maternal nutrition can alter different processes of fetal metabolism, which epigenetic mechanisms may regulate (Santos et al., 2022). In this case, the gestational phase is crucial for muscle and fat tissues development in the cattle offspring, since skeletal muscle growth begins during mid gestation, while intramuscular fat formation starts in the last trimester (Du et al., 2010). Therefore, nutritional and management manipulation during fetal stages can generate long-term alteration on growth performance and beef quality of offspring (Ahn et al., 2023).

Different strategies have been studied aiming regulate or manipulate tissues development in beef cattle. Nutrigenomics, which studies the influence of nutrients on the genome, showed that it is possible to regulate gene expression and modify several metabolic pathways (Baik et al., 2023). Vitamin A (VA) is known to alter the recruitment of undifferentiated cells, resulting in the increment of adipogenic lineage, mainly intramuscular adipocytes (Harris et al., 2018). This effect is due to the recruitment of a greater number of intramuscular adipose progenitor cells (Wang et al., 2016; Dean et al., 2024; Yu et al., 2022). The VA may induce the expression of preadipocytes markers and regulate gene transcription (Z. Tan & Jiang, 2024a). Retinoic Acid (RA), the active metabolite of VA, regulates gene transcription by acting as a ligand of nuclear proteins called retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which then bind to retinoic acid response elements (RAREs) on target genes (The et al., 1990; Mark et al., 2006; Wang et al., 2016). In addition, RAR can act as heterodimers with the peroxisome proliferator-activated receptor alpha (PPAR α), increasing adipogenesis. These transcription factors govern adipocyte differentiation essential for intramuscular fat (IMF) accumulation (Du et al., 2010). Therefore, VA works as a signal transmitter, controlling the differentiation process, stimulating adipose progenitor cells development, which is essential for IMF accumulation effects on postnatal (Abebe et al., 2023). Furthermore, according to Fazi, Travaglini, Carotti, Palitti, Diverio, Alcalay, McNamara, Miller, Coco, et al. (2005), retinoic acid reduces the expression and activity of DNA methyltransferase (DNMT), correlating with demethylation at specific sites in certain genes, which may explain the long-term effect of VA on the offspring metabolism.

In this sense, some studies have already demonstrated that VA, when applied during marbling window, positively influences intramuscular fat deposition and myogenesis (Harris et al., 2018; Jin et al., 2022; Maciel et al., 2022; Peng et al., 2021b; Song et al., 2023). These studies evaluated the application of VA in different forms of use, injected or oral, and both forms have promising use. However, there are only two studies evaluating VA supplementation during late-stage of gestation and both had VA application orally. Dean et al. (2024), found greater intramuscular fat content in the offspring from VA supplemented pregnant cows throughout all the evaluated stages of their post-natal life and no effect on subcutaneous fat, hot carcass weight and carcass yield and dressing. However, despite the cited studies, according to our literature research, no research has evaluated the use of injected VA on pregnant cows on performance and beef quality.

In this sense, this study hypothesizes that VA injection in pregnant cows will be as effective as VA injection in newborn calves to produce more intramuscular fat in the beef from steers and heifers finished in feedlot. Therefore, the study aimed to evaluate the effects of VA injection in pregnant cows or newborn calves on the performance, beef quality, and expression of genes involved in adipogenesis, myogenesis, lipogenesis and retinoic receptors of Angus × Nellore animals finished in the feedlot.

2. Material and Methods

2.1 Animal welfare

This study was carried out in the feedlot of the Beef Cattle Sector, and the Animal Research Laboratory of the Federal University of Lavras (UFLA). All the procedures and handling of animals were conducted in accordance with the guidelines of the Ethics Committee for Animal Experimentation (Protocol No. 014/21) and according to the guidance on the operation of the Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63.

2.2 Experimental management, treatments and animals

Thirty-six F1 Angus x Nellore male and female calves from multiparous and primiparous cows that underwent from two fixed-time artificial inseminations (AI) groups and fed the same basal diet were randomly assigned in a completely randomized 3×2 factorial design (vitamin treatments and sex), to one of three treatments at 250 d of gestation: 15 did not receive VA injection (Control; 7 male and 8 female); 7 calves from VA injection in pregnant

cows at 250 d of gestation (VAcow; 2.000.000 IU single dose, 3 males and 4 females); 14 calves that received VA injection just after birth and 60 d of age (VAcalf; 200.000 IU each dose, 9 males and 5 females). During the experimental procedures in the cow/calf operation, a small number of experimental units in the VAcow treatment group were lost due to health-related issues and calf mortalities. These events were promptly identified, and affected units were excluded to maintain the integrity of the dataset. After births, cows and calves from the three treatments were kept in the same paddock, and from approximately 90 d of age started the creep feeding supplementation until weaning at 210 d.

2.3 *Feedlot growing and finishing*

After weaning, animals were transported 300 km to the Federal University of Lavras for growing and finishing. They were allocated into 12 pens (3 animals per pen) with an average body weight of 213 ± 25.7 kg, and were stratified by treatment, body weight (BW), and sex. Before the trial began, animals were treated for endo- and ecto-parasites (Dectomax. Zoetis. Brazil). The first 15 days were used for diet adaptation, using the step-up protocol, starting with a forage:concentrate ration of 50:50 for 5 d, then 40:60 for 5d and 30:70 for 5d. The experimental period lasted 207 d of feeding (77d of growing diet + 130 d of finishing diet). Each animal was considered an experimental unit, with exception for the variables dry matter intake and feed efficiency, where pens were the experimental units. Animals from all treatments were fed the same experimental diet for growing and finishing phase. Diets were formulated according to NASEM, (2016) (Table 1), provided *ad libitum* twice a day, at 7:30 am and 4:00 pm. For the finishing phase, steers were immunocastrated, receiving three doses of 1 mL each of anti-GnRF immunocastration vaccine (Bopriva. Zoetis), at 84, 112, and 196 d of feeding. To determine the average daily gain (ADG) and feed efficiency, the animals were weighed at the beginning and end of the experimental period after a 16-h fast.

Samples of the dietary ingredients were collected every 15 days. From these samples, a composite sample was made, which was pre-dried in a forced-air oven at 55°C for 72 h and then ground in mills with a 1-mm-mesh sieve. The chemical analysis of the feed samples was performed according to the Association of Official Analytical Chemists (AOAC, 1990) for crude protein (method 920.87), ash (method 924.05), ether extract (method 920.85), and moisture (method 934.01). The starch content was analyzed according to HALL, (2009) and the neutral detergent fiber was analyzed according to VAN SOEST; ROBERTSON; LEWIS, (1991) using amylase (ISO 16472, 2006). The nonfibrous carbohydrates content was calculated according to SNIFFEN et al., (1992).

2.4 *Harvesting. carcass quality measurements. and sample collection*

After feedlot period, when heifers and steers reached the averaged weights of 449 ± 10.85 and 498 ± 10.85 kg of live body weight, respectively, they were transported to a packing plant distant 240 km of UFLA (Frivasa. Itajubá, Minas Gerais, Brazil), and were harvested using halala method, followed by skinning and evisceration. In the slaughterhouse, carcasses were identified, then divided longitudinally into two halves to obtain the hot carcass weight and hot carcass yield. Just after skinning, approximately 10 g of the *longissimus thoracis* (LT) muscle containing muscle fibers and intramuscular fat samples, and 5 g of muscle and adipose tissue were collected of the left-carcass in the 13th rib region. The biggest sample was placed in liquid nitrogen for transport and then stored at -80° for gene expression analysis. The other samples were placed in formol for histology analysis.

After 24h of refrigeration at 2°C , the subcutaneous fat thickness and ribeye area were measure between the 12th and 13th ribs of the left half carcass. The first one, was measured using a graduated caliper at three-fourths of the length of the ribeye from the cranial portion. The other one, was outlined in transparent inkjet film and later measured using the area meter LI-3100 (LI-COR. Lincoln. NE. USA). After cooling, two steaks (2.54cm width) of LT were removed from the 13th rib from the left half-carcass to analyze chemical composition, color and tenderness. The samples were transported, vacuum packaged and stored at -20°C until analysis in the Meat Quality Laboratory of UFLA.

2.5 *Meat quality and histological analysis*

Before processing sample, from the first steak, backfat was removed and 100 g of steak was grounded to analyze chemical composition (protein, ether extract, ash and moisture). Analysis was performed by near infrared according to the 2007-04 AOAC method using FoodScanTM equipment (FOSS, Hillerod, Dinamarca). The other steak was age for 14 d at 4°C , and used to analyze color, cook loss and Warner-Bratzler square Shear Force (WBsSF).

To determine CIE color indices, the steaks were removed from vacuum package and exposed to atmospheric air for 30 min, allowing for myoglobin oxygenation (blooming). Meat surface reflectance data were recorded from the average of five consecutive measurements performed on the surface of steaks using a CM-700 spectrophotometric colorimeter (Konica Minolta Sensing Inc., Osaka, Japan) and using the CIE $L^*a^*b^*$ system, with 8 mm aperture size, illuminant A, 10° observer angle and in specular component excluded mode (SCE). From

the readings obtained in SCE mode, lightness (L^*), redness (a^*), and yellowness (b^*) values were obtained.

Cooking loss (CL) was obtained as the percentage difference between thawed and grilled weight of the samples. Steaks were weighed (initial weight; IW) and grilled until they reached an internal temperature of 71°C, which was monitored using a portable digital thermometer (TD-880 with K-type thermocouple; ICEL, Manaus, Brazil) inserted in the center of each steak. After grilling, each steak was weighted once the temperature stabilized at room temperature (final weight; FW)(AMSA, 1995). Warner-Bratzler square shear force (WBsSF) was performed on the same steaks used to calculate cooking losses according to the methodology described by SILVA et al., (2015). For each steak, six rectangular cores ($1.0 \times 1.0 \times 2.5$ cm) were obtained in the muscle fiber direction. Each rectangular core was sheared transversely at 200 mm/min by a Warner – Bratzler blade coupled at a TA.XTplus texture analyzer (Stable Micro Systems Ltd. Godalming, Surrey, United Kingdom). The cut resistance results were measured in Newtons from the average value of the six rectangular cores.

Histological analyses of muscle and adipose tissue were performed at the Laboratory of Cytology and Histology of the UFLA Veterinary Department. Fractions of these tissues were fixed in 4% formaldehyde solution for 72 h and later stored in 70% ethanol until the preparation of histological sections. The samples were dehydrated in increasing concentrations of ethanol, diaphanized in xylol and included in paraffin blocks. To prepare the histological slides, the paraffin blocks were sectioned in a four-micrometer microtome, dewaxed in a 65°C oven and subsequently stained with hematoxylin and eosin. Digital images were obtained by means of an image capture and analysis system consisting of a CX31 trinocular microscope (Olympus, Optical do Brasil Ltda, Sao Paulo, Brazil) and camera (SC30 Color CMOS Camera for Light Microscopy, Olympus, Optical do Brasil Ltda, São Paulo, Brazil). About 100 adipocytes and muscles per region evaluated for each animal were randomly analyzed by ImageJ software. The area, calculated from its delimitation was evaluated. About diameter it was calculated in base the cells in the area of 20000 μm^2 .

2.6 Gene expression analysis

The 26 genes were selected as markers to evaluate the following metabolic processes: angiogenesis, adipogenesis, myogenesis and lipogenesis. The primers (Table 2) were designed according to the sequences published in the GenBank public database, NCBI platform (National Center for Biotechnology Information), using Oligo Perfect™ Design software (Primer Quest

Tool). They were analyzed using Oligo Analyzer 3.1 and Premier Biosoftware. Then, they were synthesized by Invitrogen (Carlsband, CA, USA) and diluted in a sample pool to determine their efficiencies and regression coefficient (R^2).

Total RNA was extracted from frozen muscle tissue samples with the SV Total RNA Isolation System (Promega, Madison, WI, USA) following the manufacturer's recommendations. To evaluate the integrity and quality of the extracted samples, the RNA was subjected to electrophoresis in 1% agarose gel (w/v) and stained with GelRed Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA), which was visualized in an E-Gel® Imager Camera Hood (Life Technologies, Neve Yamin, Israel). The quantity (ng/ μ L) and quality (260/280-nm and 260/230-nm optical density ratios) of the RNA were evaluated using a nanospectrophotometer (DeNovix DS-11, Wilmington, DE, USA). Complementary deoxyribonucleic acid (cDNA) was synthesized with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) following the manufacturer's recommendations. After the process, the samples were stored in a freezer at - 20°C.

Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using the SYBR Green detection system (Applied Biosystems, USA) in an Eppendorf Realplex thermocycler (Eppendorf, Hamburg, Germany) according to the recommendations described by Oliveira et al. (2014). For each gene under study, the entire RT-qPCR experiment used different cDNAs (10 ng/ μ L), obtained from the 36 biological replicates, with two technical replicates each. The results were normalized using the threshold cycle (Ct) obtained for the expression of the reference gene *Actin beta* (*ACTB*). The *ACTB* gene was chosen based on the study of Coelho et al. (2022). The levels of relative expression were calculated using the 2 Delta Delta Ct method (Livak & Schmittgen, 2001).

2.7 Statistical analysis

Performance measurement, carcass characteristics, chemical composition, color, tenderness and skeletal muscle gene expression data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, USA) in a completely randomized design, with vitamin A administration (cow or calf), sex and the interaction between vitamin A and sex considered fixed effects. The cow parity, cow weight and artificial insemination lot were used as covariates. The animal was considered the experimental unit with exception for DMI and feed efficiency. The least-squares means were used to calculate the adjusted means for treatment. For gene expression, the Shapiro-Wilk test was performed to assess the normality of all collected data.

When data were not normally distributed, they were transformed using PROC RANK procedure (SAS Inst. Inc., Cary, USA). Tukey's test was used to analyze the differences between the means of treatments when sex $\times$ vitamin A administration interaction was significant. Means were considered significant when $P \leq 0.05$, and trends were discussed when $0.05 < P \leq 0.10$.

3. Results

Due to the large feedlot period, lasting 207 days, performance data was splitted in two phases: growing with 77 days and finishing with 130 days. Vitamin A increased weaning BW (initial) in male calves and decreased it in female offspring from cows that received it during gestation (Table 3; Figure 1; $P < 0.01$). In addition, final BW in growing and finishing phases were greater ($P < 0.04$) in VAcow in growing and no differences in finishing steers, and higher compared with heifers from all treatments, demonstrating that the effects at weaning were kept during the feedlot. VAcow heifers had the lowest weight during the three stages. There was a tendency on finishing on vitamin A and sex interaction on average daily gain (ADG), being VA calf male and VAcow female the lowest ($P 0.03$) in the finishing phase of the feedlot period. Males were greater ADG ($P < 0.01$) in growing phase compared to females. VAcow males had the greater DMI during growing phase than females and control males, VAcow and CON male had tendency to be similar than CON am VAcalf females at finishing DMI in the feedlot. On the other hand, feedlot feed efficiency was higher in male during the growing phase.

Carcass weight was heavier ($P < 0.01$) in steers compared to the heifers, and there was a tendency ($P = 0.07$) of greater dressing percentage in the carcass of the males (Table 4). However, back fat thickness was greater in heifers ($P < 0.001$). The ribeye area was greater ($P = 0.04$) in VAcow than in Control steers and heifers from all treatments, and VAcow animals, regardless of sex, also tended ($P = 0.08$) to increase backfat thickness (Figure 2).

Regarding beef chemical composition, there was a vitamin A and sex interaction for intramuscular fat ($P = 0.05$), where IMF content was greater in VAcow steers than in Control and VAcalf steers. However, there was no effect of VA on heifers and difference compared to VAcow steers. There was no effect of vitamin A or sex on cooking losses, shear force and color indices L^* and a^* ($P > 0.10$). Even though, b^* was lower ($P = 0.04$) in the muscle of VAcow than other VA protocols, even though being greater in heifers ($P = 0.04$). By the other hand, Shear force was higher in VA treatments in female than male treatments.

There was VA $\times$ sex interaction effect only in the expression of two genes (Table 5), where the greater expressions of *RXRG* was found in the males Control and females VAcow (Figure 3). For the *ACACA* gene, VAcow males had similar expression than female treatments. The expression of *CPT2*, *DLK1*, and *ZNF423* genes were downregulated ($P < 0.05$) in VAcow animals, regardless the sex, compared to CON and VAcalf animals for *DLK1* and only control for *ZNF423* (Figure 4). Meanwhile, the *RXRA* gene was less expressed ($P = 0.05$) in both VAcow and VAcalf animals. Regarding progeny sex, the expression of the *PPARG*, *SREBF1*, *CPT2*, *ACOX*, *GHR*, *MYH1*, *MYH2* genes were greater ($P < 0.05$) and *MYH7* was greater ($P = 0.08$) in females. On the other hand, only the *PAX7* expression was greater ($P = 0.05$) in males.

About histology analysis, there was interaction between VA and sex in adipose cell number, diameter and area; and muscle cell number (Figure 5 and 6). VA cow steers had the highest muscle cell number per area (Figure 5). VAcow steers had a greater adipose area, whereas control and VAcalf calves showed greater adipocyte diameter (Figure 6). By the other hand, in adipose cell number, all steers treatments and VAcow heifers had higher values.

4. Discussion

Researchers found that vitamin A application on neonatal phase increases marbling (Harris et al., 2018; Maciel et al., 2022; Scapol et al., 2023). This is an important parameter in meat quality, which it is highly related to sensory quality of the beef, known as intramuscular fat (IMF) (Baik et al., 2023; Polkinghorne & Thompson, 2010). We hypothesized that injection on dam or in the post-natal phase of the offspring will have similar IMF content and higher than Control. However, our results show that this effect only occurred in the offspring steers from dams receiving VA at 250 days of pregnancy, with no effect of Vitamin A protocols on heifers. Actually, the IMF of the VAcow steers was similar to the heifers, regardless the VA protocol, demonstrating the injection during gestation was able to produce a steer beef with the same quality of heifers. VAcow steers had similar *ACACA* gene expression as heifers, which could explain the higher IMF deposition in this group, this is a *de novo* fatty acid synthesis gene that produce lipogenesis (Ladeira et al., 2016). This gene stimulates adipocyte differentiation, resulting in a positive correlation with intramuscular fat (Jeong et al., 2012).

In addition to the higher IMF content, we observed that VAcow steers where the heaviest animals and steers had the highest DMI and ribeye area, but among the treatments, control steers tended to be more efficient. Regarding sex, differences in average daily gain between males and females is aligned due to variations in growth curves, tissue deposition, and

fattening (McIntyre et al., 2009). According to QUIGLEY; DREWRY, (1998) supplementation of cow diet with VA is important to provide colostrum that contains adequate amounts of VA and this consumption could be affected by sex factor. Limited studies investigated the influence of maternal diet and fetal sex on offspring performance, being the majority focused on females, even though there is evidence of sexual dimorphism in fetal developments (McCoski et al., 2021), in the transport, abundance and utilization in the retinoid liver content in fetal mice during late gestation (Carvalho & Gonçalves, 2014). Preliminary experiment evaluating gestational supplementation of Vitamin A has also found heavier birth weight on newborns calves from supplemented dams. However, different of our results, it was not sustained long enough (Harris et al., 2018; Jo et al., 2020). Even though Dean et al., (2024) showed no differences between bull calves offsprings final BW, ADG and DMI coming from multiparous cows.

The active metabolite of vitamin A (Retinoic acid, RA) is a key transcriptional regulator and catalyst of differentiation events (Christine Schleif et al., 2022). RA has been shown to generate effects on cell proliferation and differentiation, fetal morphogenesis, organ development and muscle fiber type composition (Duester, 2008). Therefore, weight increase could be related to VA during pregnancy and its positive effect on the growth performance of bull calves. Researchers have found that VA stimulate the myogenesis process (Peng et al., 2021; Song et al., 2023; Wang et al., 2018a). This occurs because VA promote the increment of the number of myogenic progenitors and the myogenic potential in the early stage of muscle development but not by affecting muscle protein synthesis in the late stages (Song et al., 2023). Oliveira (2025) also found in, the first part of this study, that VACow steers had greater expression of *MYF5*, *PAX7* and *DLK1* at weaning. Supporting the changes in BW phenotypes and also our histological results showing higher muscle cell numbers on VACow steers (figure 6), contrary to what Wang et al. (2018) found. Even though other works cited before did not show difference on sex, which could be related to the interval on dose time of the application done, which was the longest. Studies until now just evaluate application at birth and 30 days of calves. Besides, according to Knutson et al., (2020) genotype plays an important role in the response to VA supplementation.

Besides, males had higher expression of myogenic marker gene *PAX7*, which is associated with higher satellite cells (Song et al., 2023; Wang et al., 2018b). Satellite cells, myogenic stem cells are responsible for muscle regeneration, self-renewal and hypertrophy (Kim et al., 2023). These cells can fusion with existing muscle fibers to increase growth of meat

animals. Instead of, females presented higher expression of *GHR*, *MYH1*, *MYH2*. Muscle with higher proportion of Type I and IIa muscle fibers display smaller fiber size (Wang et al., 2018), capillary network is more developed and anticipate the overall blood stream and transport fat and oxygen for energy metabolism in the muscle, showing a positive correlation with IMF (Joo et al., 2017). Furthermore, lower shear force and enhance tenderness and flavor (Huo et al., 2021), indicate superior meat quality as it also is associated with IMF deposition (Mo et al., 2023) and being an indicator of alteration of color, enhancing the color of fresh meat (Wang & Du, 2024).

About carcass characteristics the tendency in VAcow male to increase heavy hot carcass weight and have higher rib eye area in males is related to the higher body weight. This treatment also tended to increase back fat thickness, being higher in females. Other studies at neonatal phase showed no effect on these characteristics and chemical composition (Harris et al., 2018; Santucci, 2023; Yu et al., 2022). Moreover, in this study VA affected hypertrophy, when VAcow steers had the higher adipose area (figure 6), which is related to our results on VA effect on back fat thickness on this treatment. Contrary to YU et al., (2022) that indicated VA didn't increase hypertrophy, his study had smaller adipose diameter and higher frequency to VA.

It was not observed difference in other beef quality characteristics on offspring such as cooking losses, and shear force, as other studies (Dean et al., 2024; Harris et al., 2018; Knutson et al., 2020; Scapol et al., 2023). As marbling was increased in VAcow steers and heifers, shear force could be affected, showing a more tender beef (Scapol et al., 2023; Wang et al., 2007), because of changes in the metabolism and in the contractile structure of muscle fiber, evaluating VA on postnatal phase. Moreover, steaks from animals whose dams received VA have lower *b* value, but in general it was higher in heifers (12.4 vs 11.6). This could be related due heavier and lower fat deposition animals could have slower glycogen decline and benefit lactic acid formation (Poveda-Arteaga et al., 2023). Even though, bulls' carcasses usually have lower color values, luminosity and *b* value, due to less fat deposition and aggressive behavior (Nassu et al., 2017).

Vitamin A has shown effect on genes identified to affect fat deposition via involvement in fatty acid synthesis, metabolism and oxidation (Du et al., 2023; Harris et al., 2018). The higher intramuscular fat in heifers is related to the higher expression of *PPARG*, *CPT2* and *ACOX*. Lipid deposition result from balance between lipids, absorbed from the diet, biosynthesis, and lipid catabolism. *CPT2* and *ACOX* can participate in FA oxidation playing an important role on lipid deposition by the balance of the expression of others genes (Teixeira et

al., 2017). As *CPT2* expression can interact with *PPARG*, when *PPARG* is silenced *CPT2* is reduced (Du et al., 2023). *PPARG* being the most important factor for regulating lipid deposition and manifest terminal differentiation stage of adipocyte (García-Rojas et al., 2010). Being highly expressed in adipocytes. It seems that preadipocytes from females have greater propensity to differentiate into mature adipocytes. Even though, Maciel et al., (2022) didn't found higher expression of *PPARG* because of the high expression of WNT in preadipocyte cells holding adipocyte precursor in the preadipocyte stage but without affecting synthesis of IMF. By the other hand, *ACOX* already correlated with the meat quality of livestock, as a regulator of intramuscular adipogenesis (F. Zhang et al., 2021).

CPT2 was reduced in VAcow as *DLK1* and *ZNF423*, being genes of preadipocytes. It was contrary to other results (Dean et al., 2024), who evaluated *DLK1* at 10d of age of male calves, which was associated to maternal supplementation of VA increase *DLK1* expression and development of preadipocyte and no effect on *ZFP423* in vivo to induce mature adipocytes. When *ZFP423* promotes adipogenic commitment into preadipocyte formation (Wang & Du, 2024), vitamin A increases the number of adipose progenitors or preadipocytes. But this statement cannot be generalized, Dean et al., (2024) and Oliveira 2025 suggested that probably there is a timing-dependent relationship between VA and its effects, but a clear reason still unclear and require investigation in specific epigenetics effects as DNA methylation.

RA-mediated pathway occurs when all-trans RA binds to RAR, allowing these receptors to form heterodimers with RXR. These complexes generate response elements on DNA (Johnson et al., 2025). *RXRA* gene in control animals were more expressed, even though this is more abundant in tissues as placenta and visceral tissues (Li et al., 2021). Meanwhile, *RXRG* was more expressed in muscle, and higher in females VAcow (0.77). It was proposed that *PPARG* binds to RXR, and regulate the transcription of genes crucial for adipocyte development, lipid uptake and storage, but Oliveira (2025) found greater *RARB* and the tendency of *RARA* and *MTOR* in VAcow female calves at weaning phase of this animals, showing that vitamin A supplementation can increase level of retinoic acid in the offspring. VA has different variable response depended on different factor as environment, breed, nutrition status, administration, as well as receptor sensitivity that varies with age as animal matures it focus on metabolic stability and immune function (Johnson et al., 2025). Moreover, further investigation related to VA effect on adipogenesis and myogenesis is warranted.

5. Conclusion

The application of vitamin A in cows during gestation increases intramuscular fat in steers, but not in females. The dose and frequency used in our study to inject vitamin A in neonatal calves did not increase intramuscular fat, demonstrating that both factors influence the vitamin A response. The application of vitamin A alters the expression of several genes, demonstrating that this vitamin has a long-term epigenetic effect on the progeny, whether its application occurs during the gestational or the neonatal phase.

6. Bibliography

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Table1. Ingredients and chemical composition of the experimental diets

Ingredient composition, %DM	Growth (%MS)	Finishing (%MS)
Corn silage	42.8	27.6
Corn grain high moisture silage	41.1	59.7
Soyben meal	10.8	8.15
Urea	0.99	0.85
Mineral-vitamin premix ¹	4.30	3.70
Chemical Composition, %DM		
Dry matter	77.1	59.9
Crude protein	14.6	13.4
NDFap	24.3	17.9
Starch	39.6	47.1
Ashes	7.04	5.97
Ether extract	3.62	3.96

¹ Nutrient assurance levels (per kilogram of the premixed product): 110 g Ca. 61.60 mg Co. 519 mg Cu. 25 g S. 160 mg Fl. 16 g P. 30 mg I. 17g Mg. 1.412 mg Mn. 7.60 mg Se. 65 g Na. 2.200 mg Zn. 1.100 UI Vitamin D1. 10.000 10E6 UFC *Saccharomyces cerevisiae*. Monensin 4.000mg

Table 2. Primers sequences (5' – 3') used in the RT-qPCR analysis

Gene symbol	NCBI Accession Number	Primer sequence
<i>ACTB</i>	NM_173979.3	F: GTCCACCTTCCAGCAGATGT R: CAGTCCGCCTAGAAGCATTT
<i>CEBPA</i>	NM_176784.2	F: GGCAACGACTTTGACTACCC R: GGTCATTGTCACTGGTCAGC
<i>DLK1</i>	NM_174037.2	F: CTCTGTGCGGTCTGTTCTTATC R: CATCTGGCAATTACGTTGGTTG
<i>FABP4</i>	NM_174314.2	F: GCTGCACTTCTTTCTCACCT R: TGGACAACGTATCCAGCAGA
<i>GAPDH</i>	NM_001034034.2	F: CACAGTCAAGGCAGAGAACG R: ATTCTCAGTGTGGCGGAGAT
<i>GHR</i>	NM_176608.1	F: CCAGCTTTCCTTGTCAGAGC R: GAAGTTAGCTTGGCAGGGTG
<i>IGF1R</i>	NM_001244612.1	F: GACTCCTGTTTTTCTCCGCC R: CAGTTCCGAAGCGATGTTGT
<i>MTOR</i>	XM_002694043.7	F: GCACATGCAGCACTTTGTTC R: GGATTTTGTGTTGGCTGCGTTG
<i>MYF5</i>	NM_174116.1	F: AGCGTCTACTGTCCTGATGTA R: GTTGGTGATCCGATCCACTATG
<i>MYF6</i>	NM_181811.2	F: TACCCTGCAGCCCTTAGAAG R: TACAAGCCCAAAGCCGAAAG
<i>MYH1</i>	NM_174117.1	F: CCACTTTGTACGCTGCATCA R: GTGGCGTGTTTCTCCTTCTC
<i>MYH2</i>	NM_001166227.1	F: TCGCAACGCAGAAGAGAAAG F: AGCATCAGGACACGATCACT
<i>MYH7</i>	NM_174727.1	F: CAGAAGAACGCTGTGACCAG R: TCTTGTTCTCGCGCTTGAAG
<i>MYOD1</i>	NM_001040478.2	F: CCTGAGCAAAGTCAACGAGG R: GTAAATCGGGTTGGGGTTCG
<i>MYOG</i>	NM_001111325.1	F: CACAGATGCCACCACTTCTG R: TTCAGCACAGAGACCTTGGT

<i>PAX7</i>	XM_027522151.1	F: GACCCTCCAGTTTCCTCATTT R: CCAGTTATGAAACCCTCCTCTG
<i>PDGFRA</i>	XM_024993021.1	F: CGAGATGGGAGTTTCCAAGAG R: GACAGGTTGAGACCGACTTAAT
<i>PPARD</i>	XM_024983411.1	F: CAGCTACACAGGGCTTCTT R: CACTTGTTGCGGTTCTTCTTC
<i>PPARG</i>	NM_181024.2	F: GACATCAAGCCCTTCACCAC G: GGGGACTGATGTGCTTGAAC
<i>PPARA</i>	NM_001034036.1	F CAATGGAGATGGTGGACACA R TTGTAGGAAGTCTGCCGAGAG
<i>SREBF1</i>	NM_001113302.1	F GAGCCACCCTTCAACGAA R TGTCTTCTATGTCGGTCAGCA
<i>CPT2</i>	NM_001045889.2	F CTATTCCCAAACCTTGAAGAC R TTTTCCTGAACTGGCTGTCA
<i>SCD1</i>	NM_173959.4	F ACCATCACAGCACCTCCTTC R ATTTCAGGGCGGATGTCTTC
<i>ACOX</i>	BC102761.2	F GCTGTCCTAAGGCGTTTGTG R ATGATGCTCCCCTGAAGAAA
<i>ACACA</i>	NM_174224.2	F TGAAGAAGCAATGGATGAACC R TTCAGACACGGAGCCAATAA
<i>RARA</i>	XM_027517082.1	F: CAGCCATTTCTCCTCAGTT R: AGTGGTTAGGCAGAGAGAGA
<i>RARB</i>	XM_059882389.1	F: TCTGTGAGAATCCTGGGAGC R: CCAGCAGTGGTTCTTGTAGC
<i>RARG</i>	NM_001130756.1	F: GCAAGTACACCACGAACTCC R: ATGAGCATCCTGGGGAACAT
<i>RXRA</i>	XM_027556851.1	F: CACAGAGATGACATACGGAGAAA R: CGCCACATCCTCCAAGTTAT
<i>RXRB</i>	NM_001083640.1	F: CTGTGACCAACATCTGCCAG R: CTCCATGAGGAAGGTGTCTGA
<i>RXRG</i>	NM_001075408.1	F: CAGGAAAGCACTACGGTGTG R: GAAACCGAGCGATGGGAAAA
<i>VEGFA</i>	NM_174216.2	F: ACTTGAGTTGGGAGGAGAATG

		R: GCTGCCGTAAGAGGGATAAA
		F: AGAGTCTGCAGCTGGTACTC
<i>WNT1</i>	NM_001114191.1	R: CTGTACGTGCAGAAAGTTGGG
		F: GCACCAACTCTCCCATCTT
<i>ZNF423</i>	NM_001101893.1	R: TTCCGCCTTGGACTTCTTAC

Actin beta (*ACTB*). CCAAT Enhancer binding protein alpha (*CEBPA*). Delta like non-canonical notch ligand 1 (*DLK1*). Fatty acid binding protein 4 (*FABP4*). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Growth hormone receptor (*GHR*). Insulin like growth factor 1 receptor (*IGF1R*). Mechanistic target of rapamycin kinase (*MTOR*). Myogenic factor 5 (*MYF5*). Myogenic factor 6 (*MYF6*). Myosin heavy chain 1 (*MYH1*). Myosin heavy chain 2 (*MYH2*). Myosin heavy chain 7 (*MYH7*). Myogenic differentiation 1 (*MYOD1*). Myogenin (*MYOG*). Paired box 7 (*PAX7*). Platelet derived growth factor receptor alpha (*PDGFRA*). Peroxisome proliferator activated receptor delta (*PPARD*). Peroxisome Proliferator Activated Receptor gamma (*PPARG*). Peroxisome Proliferator-Activated Receptor α (*PPARA*). Sterol Regulatory Element-Binding Protein-1c (*SREBF1*). Carnitine palmitoyl transferase 2 (*CPT2*). Stearoyl-CoA desaturase (*SCD1*). Acyl-coenzyme A oxidase 1 (*ACOX*). Acetyl CoA carboxylase alfa (*ACACA*). Retinoic acid receptor alpha (*RARA*). Retinoic Acid Receptor beta (*RARB*). Retinoic Acid Receptor gamma (*RARG*). Retinoid x receptor alpha (*RXRA*). Retinoid X Receptor beta (*RXRB*). Retinoid X Receptor gamma (*RXRG*). Vascular endothelial growth factor A (*VEGFA*). Wnt family member 1 (*WNT1*). Zinc finger protein 423 (*ZPF423*).

Table 3. Performance during the growing and finishing phases of the progeny that received vitamin A in the neonatal phase (VAcalf), or of the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON).

Item	Male			Female			SEM	P-value		
	CON	VAcalf	VAcow	CON	VAcalf	VAcow		VA	Sex	VA*Sex
<i>Body weight. kg</i>										
Initial	198 ^{cd}	223 ^{ab}	242 ^a	210 ^{bc}	205 ^{bc}	178 ^d	9.24	0.48	<0.01	<0.01
Growing	331 ^b	362 ^a	391 ^a	319 ^{bc}	319 ^{bc}	294 ^c	14.6	0.39	<0.01	0.03
Finishing	482 ^{ab}	487 ^{ab}	526 ^a	466 ^b	463 ^{bc}	419 ^c	17.6	0.99	0.01	0.04
<i>Average daily gain. kg/d</i>										
Growing	1.71	1.80	1.93	1.41	1.47	1.48	0.140	0.42	<0.01	0.79
Finishing	1.20 ^w	0.96 ^z	1.02 ^{xyz}	1.15 ^{wx}	1.14 ^{wxy}	1.00 ^{yz}	0.081	0.03	0.48	0.09
<i>Dry matter intake. kg/d</i>										
Growing	6.60 ^c	7.21 ^b	7.98 ^a	6.62 ^c	6.79 ^{bc}	6.39 ^c	0.274	0.13	0.01	0.03
Finishing	7.18 ^x	7.03 ^x	8.00 ^w	7.29 ^{wx}	7.24 ^{wx}	6.95 ^x	0.282	0.54	0.29	0.09
<i>Feed efficiency. ADG/DMI</i>										
Growing	0.26	0.25	0.24	0.21	0.22	0.23	0.017	0.95	<0.01	0.33
Finishing	0.17	0.14	0.13	0.16	0.16	0.14	0.013	0.02	0.21	0.12

^{a, b, c, d} Means followed by the different lowercase letters on each row differ from each other for VA effect.

^{x, y} Means followed by the different lowercase letters on each row differ from each other for sex effect.

SEM = standard error of means

VA = vitamin A injection effect

VA×Sex = interaction treatment × sex

CON = Control treatment without VA injection in cows or calves.

VA calf = VA injection in newborn calves at birth and at 60 days of age.

VA cow = VA injection in pregnant cows at 250 days of gestation.

Table 4. Carcass traits and beef quality of the progeny that received vitamin A in the neonatal phase (VAcalf). of the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON).

Meat quality	Male			Female			SEM	P-value		
	CON	VA Calf	VA Cow	CON	VA Calf	VA Cow		VA	Sex	VA*Sex
Hot carcass weight. kg	254 ^{wxy}	262 ^{wx}	279 ^w	243 ^y	244 ^{xy}	219 ^z	9.97	0.86	<0.01	0.06
Dressing percentagem. %	52.7	53.9	53.1	52.2	52.8	52.4	0.682	0.15	0.07	0.79
Back fat thickness. mm	7.95	7.62	11.1	10.6	12.9	13.5	1.57	0.08	<0.01	0.33
Ribeye area. cm ²	82.7 ^c	93.6 ^{ab}	101 ^a	85.3 ^{bc}	77.5 ^c	85.6 ^c	4.17	0.08	0.01	0.04
<i>Beef chemical composition</i>										
Collagen. %	2.11	1.90	2.17	2.34	2.15	2.15	0.219	0.38	0.24	0.69
Fat. %	3.26 ^b	3.41 ^b	5.89 ^a	5.28 ^a	6.33 ^a	4.25 ^{ab}	0.846	0.61	0.10	0.05
Ash. %	2.13	2.06	2.00	2.10	2.00	2.50	0.211	0.44	0.28	0.19
Protein. %	22.7	22.9	22.3	22.70	23.0	22.7	0.397	0.39	0.32	0.57
Moisture. %	71.9	71.6	69.2	69.9	68.7	70.5	1.18	0.49	0.17	0.16
<i>Meat quality</i>										
L*	45.1	45.4	42.3	45.9	44.2	43.8	1.63	0.20	0.69	0.52
a*	14.2	13.2	14.1	15.6	12.7	14.3	1.56	0.19	0.76	0.61
b*	12.3	12.1	10.5	13.0	12.4	11.9	0.479	0.04	0.04	0.56
pH	5.59	5.56	5.6	5.60	5.59	5.63	0.058	0.63	0.44	0.26
Cooking loss. %	20.9	21.8	21.0	22.9	20.0	23.4	1.93	0.33	0.42	0.67
Shear force. N	4.00	4.21	4.45	4.04	4.66	5.07	0.699	0.86	<0.01	0.06

a, b, c, d Means followed by the different lowercase letters on each row differ from each other for VA effect.

e, f, g, h Means followed by the different lowercase letters on each row show tendency from each other.

x, y Means followed by the different lowercase letters on each row differ from each other for sex effect.

SEM= standard error of means

VA = vitamin A injection effect

VA×Sex = interaction treatment × sex

CON = Control treatment without VA injection in cows or calves.

VA calf = VA injection in newborn calves at birth and at 60 days of age.

VA cow = VA injection in pregnant cows at 250 days of gestation.

Table 5. Relative gene expression at slaughter of the progeny that received vitamin A in the neonatal phase (VAcalf) or the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON).

Gene	Male			Female			SEM [‡]	P-value		
	CON	VA calf	VA cow	CON	VA calf	VA cow		VA	Sex	VA×S
<i>Retinoic receptors</i>										
<i>RARG</i>	1.00	1.21	1.76	1.45	0.98	1.54	0.344	0.69	0.90	0.48
<i>RXRA</i>	1.00	0.83	0.72	0.92	0.74	0.64	0.122	0.05	0.38	0.98
<i>RXRB</i>	1.00	0.94	1.08	1.05	0.76	0.88	0.253	0.60	0.62	0.71
<i>RXRG</i>	1.00 ^{ab}	0.32 ^{bc}	0.11 ^c	0.42 ^{bc}	0.22 ^c	0.77 ^a	0.302	0.21	0.14	0.01
<i>Angiogenesis</i>										
<i>PDGFRA</i>	1.00	1.33	0.7	1.22	0.97	1.1	0.206	0.40	0.26	0.11
<i>VEGFA</i>	1.00	0.84	0.42	1.02	1.00	0.86	0.259	0.37	0.24	0.62
<i>Adipogenesis</i>										
<i>ZNF423</i>	1.00	0.7	0.5	0.89	0.65	0.44	0.152	0.05	0.47	0.91
<i>PPARG</i>	1.00	0.9	0.79	2.4	1.46	1.89	0.528	0.61	<0.01	0.89
<i>CEBPA</i>	1.00	1.22	1.17	1.01	1.36	3.17	0.79	0.95	0.21	0.86
<i>DLK1</i>	1.00	0.7	0.1	0.63	0.61	0.38	0.216	0.01	0.79	0.35
<i>Lipogenesis</i>										
<i>PPARA</i>	1.00	0.76	0.56	0.93	1.03	0.75	0.17	0.19	0.24	0.64
<i>SREBF1</i>	1.00	0.88	0.64	1.12	1.19	1.1	0.159	0.43	0.01	0.52
<i>CPT2</i>	1.00	0.96	0.65	1.18	1.63	1.1	0.159	0.03	<0.01	0.26
<i>SCD1</i>	1.00	0.67	0.65	0.77	1.11	1.2	0.252	0.92	0.14	0.17
<i>ACOX</i>	1.00	1.56	0.82	0.99	2.08	2.45	0.44	0.22	0.03	0.26
<i>ACACA</i>	1.00 ^{ab}	0.86 ^b	1.55 ^a	1.45 ^{ab}	1.52 ^a	1.04 ^{ab}	0.234	0.93	0.23	0.05
<i>Myogenesis</i>										
<i>MYF5</i>	1.00	0.85	1.06	1.2	0.85	0.93	0.257	0.78	0.79	0.85
<i>PAX7</i>	1.00	0.75	0.49	0.4	0.55	0.31	0.193	0.32	0.05	0.76
<i>IGF1R</i>	1.00	1.16	0.91	1.13	1.24	0.96	0.167	0.40	0.51	0.87

<i>MYH1</i>	1.00	1.02	1.2	2.6	3.08	3.3	1.006	0.93	0.01	0.73
<i>MYH7</i>	1.00	1.25	0.87	1.32	1.58	1.96	0.541	0.77	0.08	0.66
<i>MYH2</i>	1.00	1.11	0.57	1.56	1.33	1.19	0.274	0.21	<0.01	0.50
<i>MYOG</i>	1.00	1.54	1.88	1.45	1.26	1.21	0.521	0.92	0.96	0.84
<i>MYF6</i>	1.00 ^{wxy}	1.94 ^w	0.21 ^{wxy}	0.90 ^{wx}	0.08 ^y	0.72 ^{xy}	0.772	0.62	0.26	0.10
<i>GHR</i>	1.00	1.43	1.14	2.53	2.03	0.98	0.615	0.32	0.04	0.25
<i>WNT</i>	1.00	0.98	0.63	0.86	0.88	0.9	0.236	0.81	0.31	0.70

^{a, b, c, d} Means followed by the different lowercase letters on each row differ from each other for VA effect.

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SEM= standard error of means

VA = vitamin A injection effect

VA×Sex = interaction treatment × sex

CON = Control treatment without VA injection in cows or calves.

VA calf = VA injection in newborn calves at birth and at 60 days of age.

VA cow = VA injection in pregnant cows at 250 days of gestation.

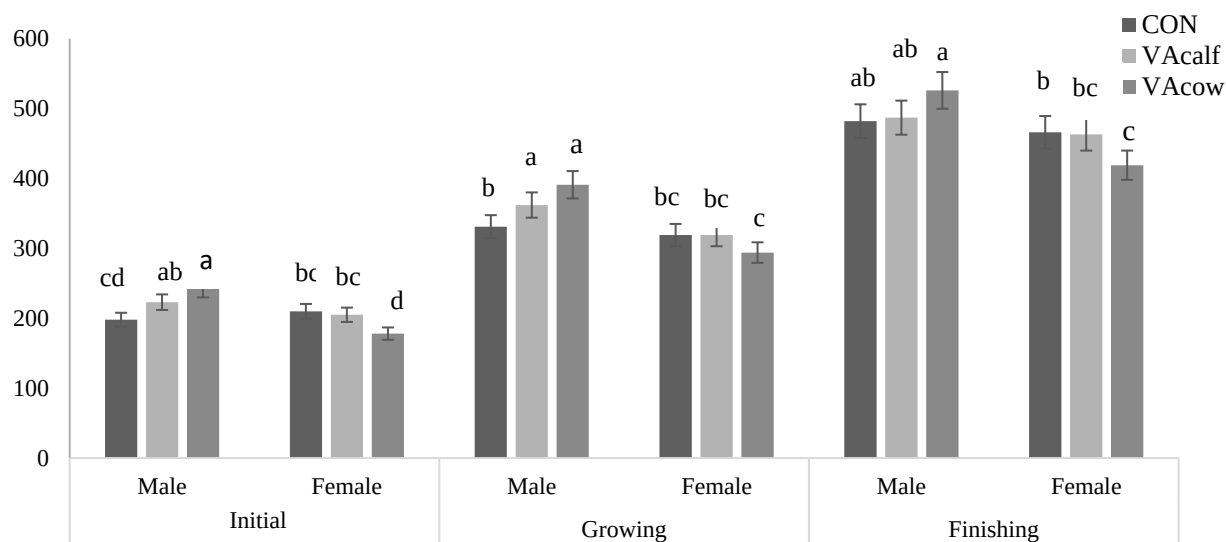


Figure 1. Weight of the progeny that received vitamin A in the neonatal phase (VAcalf), of the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON). Differences (^{a,b,c}) were considered when $P < 0.05$.

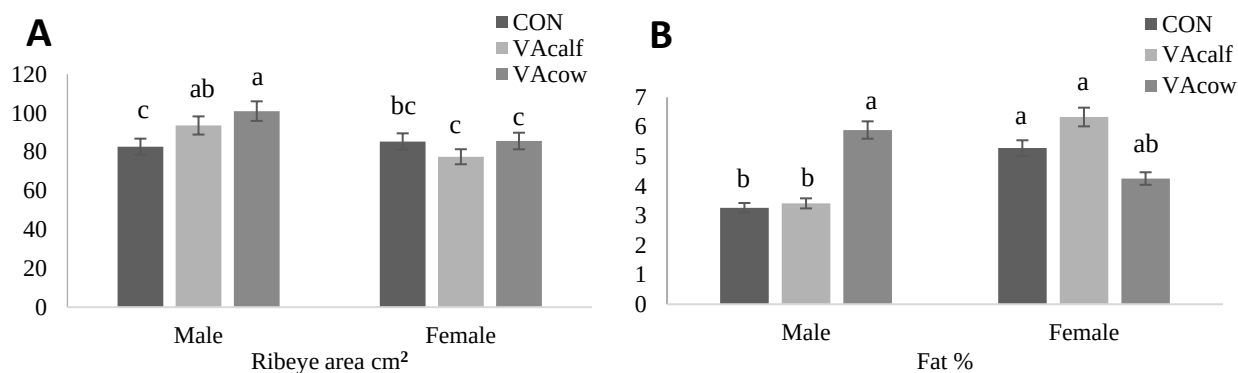


Figure 2. Meat characteristic at slaughter of the progeny that received vitamin A in the neonatal phase (VAcalf), of the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON). Differences (^{a,b}) were considered when $P < 0.05$. A. Ribeye area. B. Fat

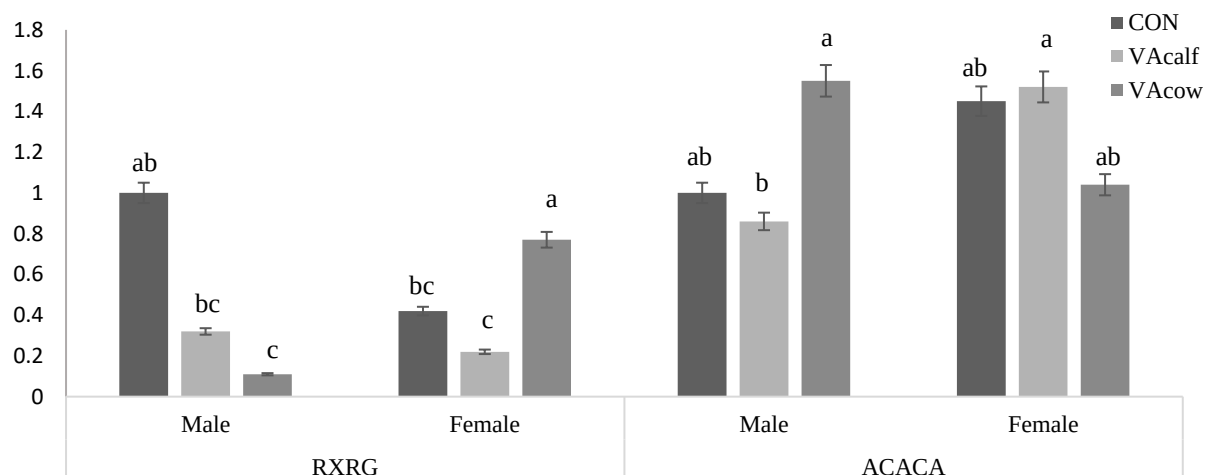


Figure 3. Relative gene expression at slaughter of the progeny that received vitamin A in the neonatal phase (VAcalf), of the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON). Differences (^{a,b,c}) were considered when $P < 0.05$.

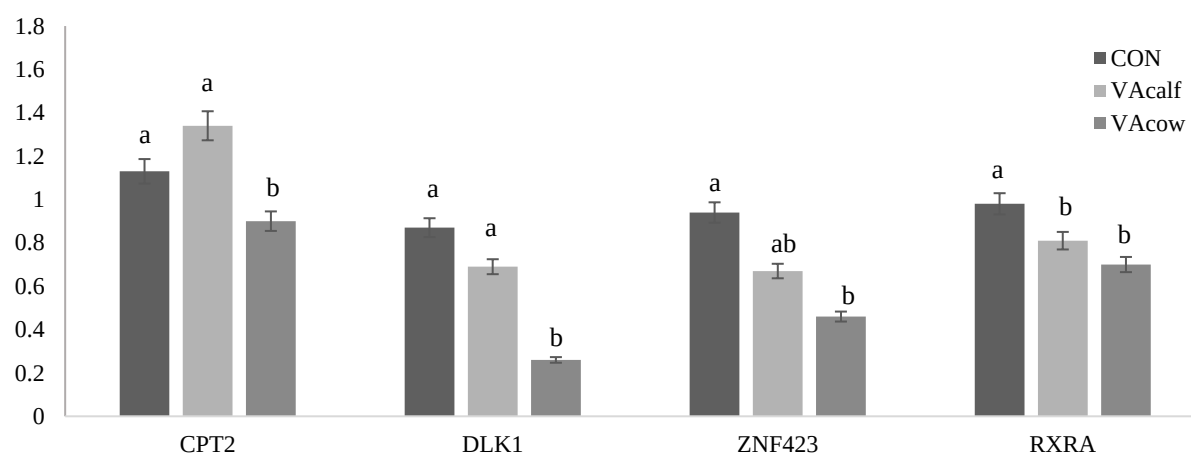


Figure 4. Relative gene expression at slaughter of the progeny that received vitamin A in the neonatal phase (VAcalf), of the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON). Differences (^{a,b}) were considered when $P < 0.05$.

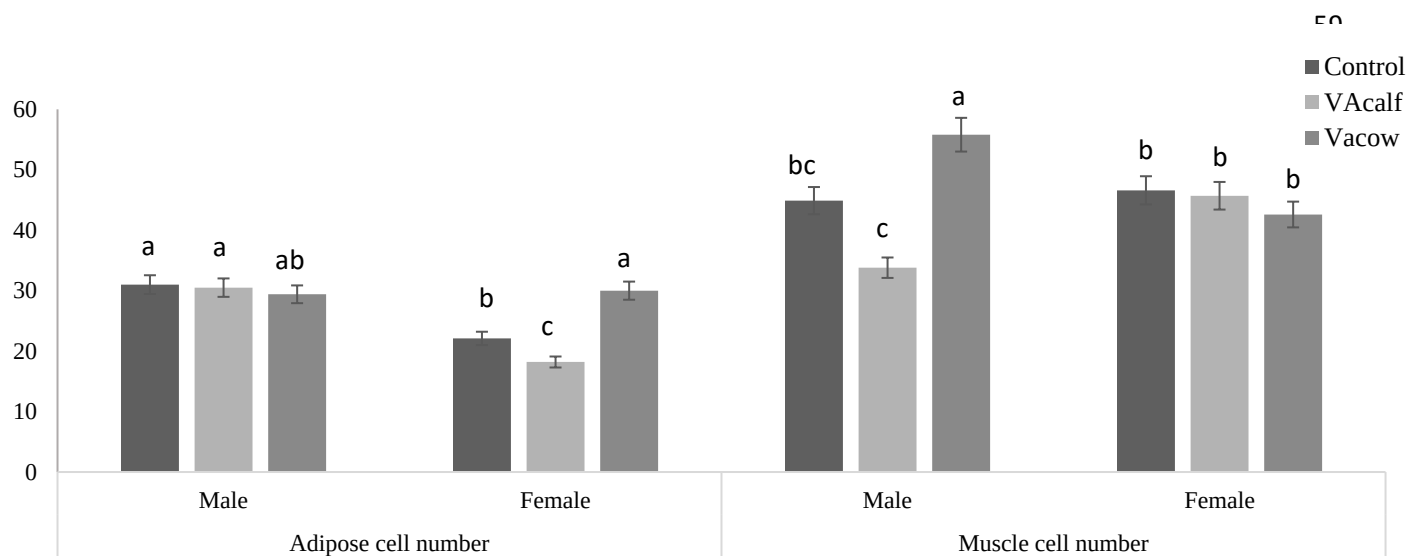


Figure 5. Adipose and muscle cell number of the progeny that received vitamin A in the neonatal phase (VAcalf), of the progeny from cows that received vitamin A at 250 days of gestation (VAcow) or without vitamin A injection (CON). Differences (^{a,b}) were considered when $P < 0.05$.

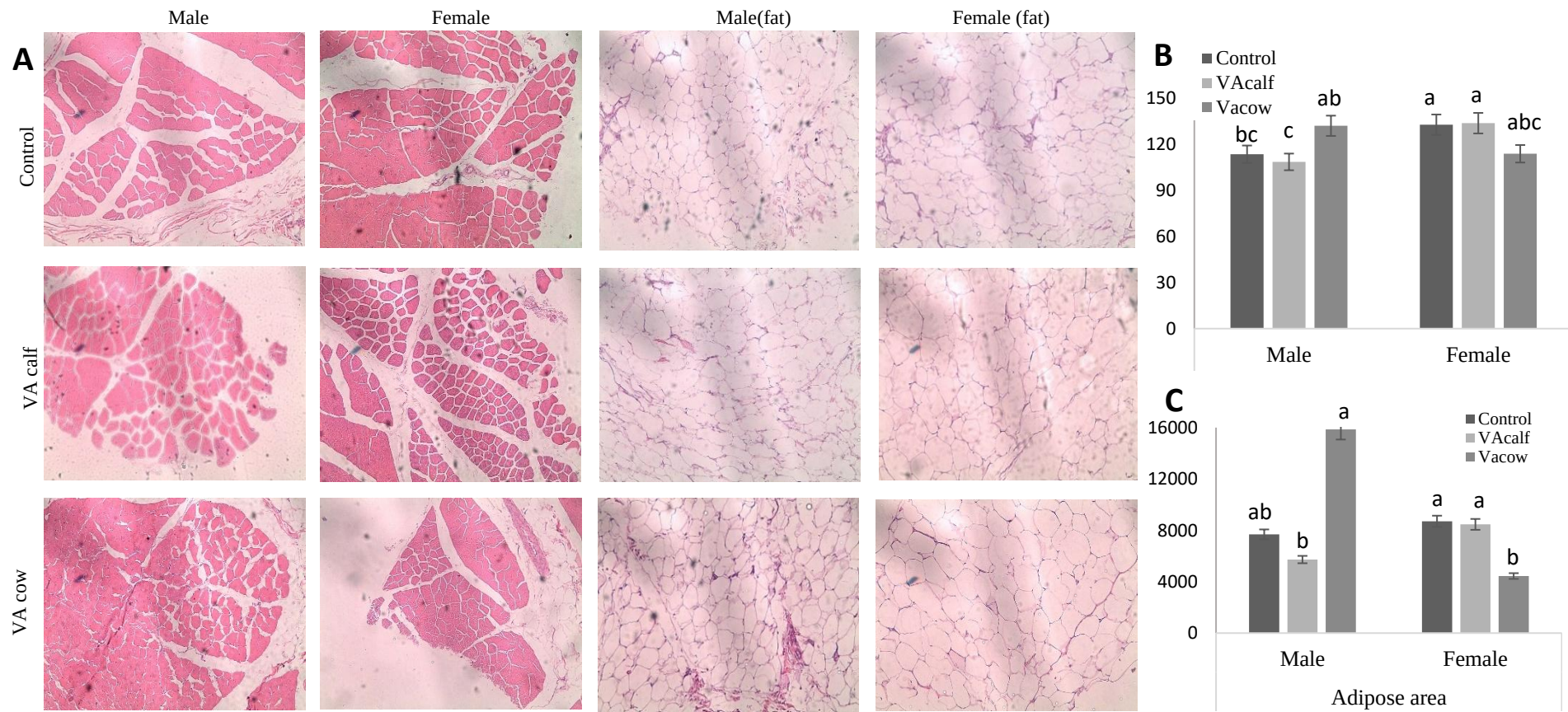


Figure 6. A. Representative images and adipocyte size distribution of subcutaneous fat and muscle at slaughter (scale bar = 10X). B. Statistical analysis of adipocyte diameter. C. Statistical analysis of adipose area. Differences (^{a,b,c}) were considered when $P < 0.05$.