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Associative effects of nutrition and production in grazing beef cows and calves

Pedro Henrique Borba Pereira
Doctor Scientiae

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Thesis submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Claudia Batista Sampaio

Co-adviser: Edenio Detmann

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Assent:

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Undoubtedly, the thesis is dedicated to Mr. José da Silva Pereira and Mrs. Maria Inês Borba Pereira (my parents), who have been my pillars throughout life.
I dedicate.

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Thank you, so much!

"Do you really wanna go back in time?
I still have some pictures on my wall
I still know the places where they're from
I still find all answers in my dreams
I still feel the gum under my shoe
How about
Holding a palm full of miracles
How about
Building a house on the moon
Do you really wanna go back in time?
(…)"
Miracles (Back in Time)
The DØ

ABSTRACT

PEREIRA, Pedro Henrique Borba, D.Sc., Universidade Federal de Viçosa, July, 2025. **Associative effects of nutrition and production in grazing beef cows and calves**. Adviser: Claudia Batista Sampaio. Co-adviser: Edenio Detmann.

For this thesis, three (03) chapters were developed, integrating studies with beef cows and calves raised under grazing systems. The first study was conducted under grazing conditions, evaluating the effect of calf sex and creep-feeding supplementation on the productive performance and blood metabolism of Nellore cows. The second study applied a meta-analysis approach to assess protein supplementation of grazing Nellore cows during the peripartum period and its effects on energy metabolism. The third chapter was a literature review addressing the effects of vitamin A supplementation on maternal performance and the nutri-epigenetic effects on calves. In the first chapter, the objective was to evaluate the interaction between calf sex and creep-feeding supplementation on body weight (BW), body condition score (BCS), milk yield, and blood metabolic profile of grazing Nellore cows. A total of 55 multiparous Nellore cows were used, with an average BW of 520 ± 59.2 kg, average BCS of 4.5 ± 0.46, and approximately 230 ± 6 days of gestation. Fetal sex was determined, resulting in 29 males and 28 females. Cow-calf pairs (considering fetal sex) were randomly assigned to eight paddocks in a completely randomized design with a double-error structure, with both male and female calves coexisting within each paddock. Four paddocks were allocated to the Control calves' group (CON; mineral mix *ad libitum*), and four paddocks to the Supplemented calves' group (SUP; protein-energy supplementation containing 260 g/kg crude protein on a dry matter basis, offered at 6 g/kg BW per animal). Cows received a protein-energy supplement containing 350 g/kg crude protein (CP) on a dry matter (DM) basis infrequently during the last 30 days of gestation, and after calving, they received only a mineral mix *ad libitum* until weaning. Maternal BW and BCS were recorded at -30, 1, 30, 100, and 240 days relative to calving (day 0). Milk yield (MY) was estimated by double milking at approximately 45 and 240 days relative to calving, with adjustment to daily yield. Calf BW was measured approximately 12 h after birth and at 30, 100, and 240 days of age. Blood samples from cows were collected at -30, 1, 30, 100, and 240 days relative to calving to determine plasma concentrations of glucose, ß-hydroxybutyrate (ß-OHB), non-esterified fatty acids (NEFA), total protein, albumin, blood urea nitrogen, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (GGT). No interaction (P > 0.05) was detected between calf sex and creep-feeding supplementation for BW, BCS, MY, or blood metabolites of cows. However, supplementation interacted with calf BW (P < 0.001), and calf sex interacted with calf BW (P < 0.001). Only the effect of time influenced cow performance and blood metabolites (P < 0.05). Thus, calf sex and creep-feeding supplementation did not impair productive performance or metabolic profile of grazing Nellore cows. In the second chapter, the objective was to evaluate the effects of maternal supplementation during pre- and/or postpartum on performance and blood concentrations of glucose, NEFA, and ß-OHB in Nellore cows grazing tropical pastures, using a meta-analytical approach. The database was compiled from 17 experiments conducted in Brazil between 2016 and 2025, with 654 observations, totaling 80 treatments across 150 experimental units. All studies adopted maternal supplementation in pre- and postpartum as the fixed-effect model (control: mineral mix *ad libitum*; supplemented: average of 309 g/kg CP in DM during prepartum and average of 319 g/kg CP in DM during postpartum), adjusted for animal category (multiparous cows and primiparous heifers). Prepartum supplementation increased BW at calving (P < 0.001), improved average daily gain (P = 0.01), and reduced plasma NEFA concentrations (P < 0.001), compared with non-supplemented cows. Based on the results, the estimated composition for prepartum supplementation is 309 g/kg CP in DM, with an allowance of 3.00 g/kg BW per day, to ensure positive performance responses in Nellore cows. No interaction was detected between prepartum and postpartum supplementation (P > 0.05). Postpartum supplementation had no significant effect on BW or blood concentrations of glucose, NEFA, and ß-OHB (P > 0.05). Therefore, prepartum protein supplementation of Nellore cows grazing tropical pastures modulates circulating NEFA concentrations and improves maternal performance, without affecting energy metabolism or postpartum performance. In the third chapter, the objective was to discuss the dynamics of vitamin A supplementation and its effects on gestational/maternal and neonatal metabolism in beef cattle, through a literature review. Vitamin A, a fat-soluble vitamin, regulates reproductive functions, growth, and tissue maintenance through gene expression. In ruminants, this micronutrient may be supplemented orally as ß-carotenoids or intramuscularly as exogenous retinol. In pregnant beef cows, oral supplementation during mid- or late-gestation increases plasma retinol concentrations, enhancing fetal development, colostrum production, and postpartum fertility. In newborn beef calves, intramuscular administration of vitamin A stimulates lipogenesis, muscle growth, and meat quality. Given the increasing evidence of the importance of vitamin A for maternal and neonatal health, there is strong potential to optimize supplementation strategies. Future studies should investigate the long-term effects of different vitamin A supplementation protocols on hepatic retinol retention in dams and their impacts on progeny, particularly regarding fetal development and postnatal growth under grazing conditions. The improvement of such nutritional interventions contributes to more efficient and sustainable beef production systems, with greater economic benefits for producers.

Keywords: creep-feeding; metabolism; Nellore; supplementation; vitamin A

RESUMO

PEREIRA, Pedro Henrique Borba, D.Sc., Universidade Federal de Viçosa, julho de 2025. **Efeitos associativos da nutrição e produção de vacas e bezerros de corte**. Orientadora: Claudia Batista Sampaio. Coorientador: Edenio Detmann.

Para esta tese, foram elaborados três (03) capítulos que associam estudos com vacas e bezerros de corte criados em sistema de pastejo. O primeiro estudo foi desenvolvido em condições de pastejo analisando o efeito do sexo e a suplementação via creep-feeding do bezerro sobre o desempenho produtivo e metabolismo sanguíneo de vacas Nelore; o segundo estudo foi desenvolvido por meio da metodologia de meta-análise com a suplementação proteica de vacas Nelore sob pastejo de pastagens tropicais no período de periparto e seus efeitos no metabolismo energético; e o terceiro capítulo foi desenvolvido com base em uma revisão de literatura, abordando os efeitos da suplementação de vitamina A no desempenho materno e os efeitos nutri-epigenéticos nos bezerros. No primeiro capítulo, objetivo foi avaliar a interação entre o sexo do bezerro e a suplementação via creep-feeding sobre o peso corporal, escore de condição corporal, produção de leite e perfil do metabolismo sanguíneo de vacas Nelore em pastejo. Foram utilizadas 55 vacas Nelore pluríparas, com peso corporal (PC) médio de 520±59,2 kg, escore de condição corporal (ECC) médio de 4,5±0,46, com aproximadamente 230± 6dias de gestação. O sexo dos fetos foi determinado, resultando em 29 machos e 28 fêmeas. Os pares vaca-bezerro (considerando o sexo do feto) foram distribuídos aleatoriamente em oito piquetes por meio de um delineamento inteiramente casualizado com dupla estrutura de erro, com bezerros machos e fêmeas coexistindo em cada piquete. Quatro piquetes foram destinados para o grupo de bezerros Controle (CON; mistura mineral *ad libitum*) e quatro piquetes foram destinados ao grupo de bezerros Suplementados (SUP; suplementação proteica-energética contendo 260 g/kg de proteína bruta com base na matéria seca com oferta de 6g/kg de peso corporal por animal). As vacas receberam um suplemento proteico-energético contendo 350 g/kg de proteína bruta com base na matéria seca de forma infrequente, nos últimos 30 dias médios da gestação e após o parto receberam apenas mistura mineral *ad libitum* até o desmame. O PC materno e o ECC foram mensurados aos -30, 1, 30, 100 e 240 dias em relação ao parto (dia 0). A estimativa da produção de leite (PL) foi realizada por duas ordenhas no período médio de 45 e 240 dias em relação ao parto, com correção para a produção diária. O peso corporal dos bezerros foi mensurado aproximadamente 12 horas após o parto e aos

30, 100 e 240 dias de vida. As coletas de sangue das vacas foram realizadas aos -30, 1, 30, 100 e 240 dias relativo ao parto para análise das concentrações sanguíneas de glicose, ß-hidroxibutirato (ß-OHB), ácidos graxos não esterificados (AGNE), proteínas totais, albumina, nitrogênio ureico no sangue, aspartato aminotransferase (AST), alanina aminotransferase (ALT) e gama-glutamiltransferase (GGT). O sexo do bezerro e a suplementação via creep-feeding não apresentaram interação ($P > 0,05$) para o PC, ECC, PL e metabólitos sanguíneos das vacas. Houve interação da suplementação e do PC do bezerro ($P < 0,001$) e para o sexo do bezerro com o PC ($P < 0,001$). Apenas o efeito do tempo possibilitou modificações no desempenho das vacas e nos metabolitos sanguíneos ($P < 0,05$). Dessa forma, o sexo do bezerro e a sua suplementação via creep-feeding não compromete o desempenho produtivo e/ou o perfil metabólico de vacas Nelore em pastejo. No segundo capítulo, o estudo teve como objetivo avaliar os efeitos da suplementação materna durante o pré e/ou pós-parto sobre o desempenho e as concentrações de glicose, ácido graxo não esterificado AGNE e ß-OHB no sangue de vacas Nelore em pastagens tropicais, utilizando uma abordagem de meta-análise. O banco de dados foi compilado a partir de 17 experimentos realizados no Brasil entre 2016 e 2025, com 654 observações, totalizando 80 tratamentos em 150 unidades experimentais. Todos os estudos possuíam como modelo básico de efeito fixo a suplementação materna no pré e pós-parto (controle: mistura mineral *ad libitum*; e suplementado: média de 309 g/kg de PB na MS no período de pré-parto e média de 319 g/kg de PB na MS no período pós-parto), ajustado para a categoria animal (vacas multíparas e novilhas primíparas). A suplementação pré-parto aumentou o PC ao parto ($P < 0,001$), melhorou o ganho médio diário ($P = 0,01$) e reduziu as concentrações sanguíneas de AGNE ($P < 0,001$), em comparação a vacas não suplementadas. Com base nos resultados, a composição estimada para a suplementação no pré-parto é de 309 g/kg de PB na MS com oferta de 3,00 g/kg de PC por dia para vacas Nelore apresentarem desempenho positivo. Não foi observado interação entre a suplementação pré e pós-parto ($P > 0,05$). O efeito da suplementação pós-parto não apresentou efeito significativo para o PC e concentrações de glicose, AGNE e ß-OHB no sangue ($P > 0,05$). Assim, a suplementação proteica no pré-parto em vacas Nelore mantidas em pastagens tropicais modula os níveis de AGNE circulantes no sangue e melhora o desempenho materno, sem afetar o metabolismo energético ou o desempenho no período de pós-parto. No terceiro capítulo, o estudo teve como objetivo abordar a dinâmica da suplementação com vitamina A e seus efeitos sobre o metabolismo gestacional/materno e neonatal em bovinos de corte, por meio de uma revisão de literatura. A vitamina A, uma vitamina lipossolúvel, modula funções reprodutivas, crescimento e manutenção tecidual por meio da regulação gênica. Em ruminantes, esse micronutriente pode ser suplementado via oral por ß-carotenoides ou via intramuscular de forma exógena. Em vacas de corte gestantes, a suplementação oral no terço médio ou final da gestação eleva os níveis plasmáticos de retinol, beneficiando o desenvolvimento fetal, a produção de colostro e a fertilidade no período de pós-parto. Em bezerros de corte recém-nascidos a administração intramuscular da vitamina A favorece a lipogênese, crescimento muscular e a qualidade da carne. Diante das crescentes evidências da importância da vitamina A para a saúde materna e neonatal, existe grande potencial para otimizar as estratégias de suplementação. Estudos futuros devem investigar os efeitos de longo prazo de diferentes protocolos de suplementação da vitamina A sobre a retenção de retinol no tecido hepático na matriz e os impactos na progênie quanto ao desenvolvimento fetal e crescimento pós-natal, especialmente em condições de pastejo. O aperfeiçoamento dessas intervenções nutricionais torna o sistema de produção de carne bovina mais eficiente e sustentável, com melhores resultados econômicos para os pecuaristas.

Palavras-chave: creep-feeding; metabolismo; Nelore; suplementação; vitamina A.

Summary

1 Introduction

The beef cow-calf production under tropical pasture conditions faces nutritional limitations primarily imposed by climatic seasonality, which directly affects forage quality and availability throughout the year. These nutritional challenges negatively impact maternal performance, reproductive efficiency of the dams, and offspring growth.

During the final third of gestation, prepartum, and the early lactation, in beef cows, known as the peripartum period, maternal metabolism undergoes a marked increase in energy demand. This requires physiological, endocrine, and metabolic adaptations to meet the maintenance requirements of the dam, support fetal development, and enable colostrum/milk production (Bauman and Currie, 1998; Mulliniks et al., 2013; Lopes et al., 2020).

These adaptations in beef cows are referred to as homeorhesis (Gionbelli et al., 2024), involving the mobilization of body reserves through the oxidation of adipose and muscle tissue (Soleto et al., 2018; Ferreira et al., 2022; Saraiva et al., 2024). These metabolic changes also affect hepatic metabolism and may impair the reproductive efficiency of the cows (D’Occhio et al., 2019; Barucelli et al., 2025).

Another limitation concerns the availability of rumen-degradable protein during the dry season, which may restrict microbial protein synthesis and fiber digestibility (Detmann et al. 2009, 2014). This constraint may compromise the supply of net energy to the cows (Ayres et al., 2014; Hill et al., 2018; Gionbelle et al., 2024).

In this context, nutritional strategies based on protein and protein-energy supplementation have been widely studied to reduce the negative effects of the peripartum period on maternal performance. Studies have shown that providing supplements during the final third of gestation improves calving body weight (Barcelos et al., 2022), enhances the body condition of the dams (David et al., 2024), and reduces body reserves, aiding in the recovery of energy metabolism at early lactation (Ferreira et al., 2022; Lopes et al., 2022; Melo et al., 2024).

In addition to maternal nutrition management, interventions applied to the offspring, such as creep feeding, have proven effective in enhancing calf body weight gain due to the increased nutritional requirements of the category (Owens et al., 1993). This strategy increases body weight at weaning (Carvalho et al., 2019), hastens the age at puberty (Paixão et al., 2024; Catussi et al., 2024), and positively influences the metabolism of adipose and muscle tissues (Santos et al., 2023), thereby promoting precocity.

Evidence demonstrates that male and female beef calves exhibit distinct response dynamics to creep feeding. Where, male beef calves tend to have greater weight gain and body development when supplemented, due to their higher nutritional requirements and faster postnatal growth rate (Carvalho et al., 2019). However, Lopes et al. (2016) observed that the interaction between creep feeding use and progeny sex does not affect the metabolic performance of cows, despite the differing nutritional demands of the calves.

Moreover, an innovative aspect within cow-calf system interactions concerns supplementation with bioactive nutritional components, such as vitamin A. The compounds exhibit metabolic and epigenetic actions, being capable of modulating physiological processes in both the dam and the offspring.

Maternal or neonatal administration of this vitamin A is associated with greater hepatic retention of retinol, prevention of the negative effects of oxidative metabolism, and stimulation of cellular differentiation during the fetal and postnatal phases (Harris et al., 2018; Peng et al., 2020; Campos et al., 2020).

Specifically, the provision of β-carotene or injectable vitamin A during gestation has shown a potential to improve maternal fertility (Jo et al., 2020), enhance colostrum synthesis (Nishijima et al., 2017), and positively influence adipogenesis in the offspring (Pereira et al., 2024), resulting in greater intramuscular fat deposition and increased weaning body weight. These effects appear to be related to the modulation of gene expression in muscle (Harris et al., 2018) and adipose tissues (Li et al., 2023), reflecting improvements in carcass quality (Maciel et al., 2022) and subsequent animal performance.

Thus, it becomes necessary to coordinate nutritional strategies that target both dams and calves, promoting greater efficiency and sustainability in beef production. This combined approach minimizes the effects of tropical environmental constraints on maternal reproduction while simultaneously enhancing the growth and development of the offspring.

My objective was to evaluate the associative effects of different nutritional strategies applied to beef cows and their calves under grazing systems, with a focus on improving maternal productivity and metabolic performance, reproductive efficiency, and offspring growth.

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CHAPTER 1

Offspring Sex and Supplementation Influence Maternal Performance and Metabolism in Nellore Cows

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Abstract

This study aimed to evaluate the interaction between creep feeding supplementation and progeny sex on the productive performance and metabolic profile of grazing Nellore cows. Fifty-five multiparous Nellore cows with a body weight (BW) of 519±74, body condition score (BCS) of 4.50±0.5, and 230±6 days of gestation were used. Fetal sex was determined, resulting in 29 male and 28 female fetuses. Cow-calf pairs (considering fetal sex) were randomly assigned to eight paddocks in a completely randomized design with a double-error structure, with both male and female calves coexisting within each paddock. Four paddocks were allocated to the Control calves' group (CON; mineral mix) and four paddocks to the Supplemented calves' group (SUP; protein-energy supplementation via a creep-feeding system). Maternal BW and BCS were assessed at −30, 1, 30, 100, and 240 days relative to calving. Calf BW was measured approximately 12 hours after birth and at 30, 100, and 240 days of age. Maternal blood samples were collected to analyze glucose, NEFA, β-OHB, total protein, albumin, urea nitrogen, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). Milk yield was estimated at 45 and 240 days postpartum. The experiment followed a completely randomized design. No interaction ($P > 0.05$) between supplementation and calf sex was found for BW, BCS, milk production, or blood metabolites of the cows. These variables were only influenced by the time of assessment ($P < 0.05$). An interaction between supplementation and time was observed for calf BW ($P < 0.001$), as well as an interaction between sex and BW ($P < 0.001$). Thus, calf sex and creep-feeding supplementation did not impair the productive performance and/or metabolic status of grazing Nellore cows.

Keywords: β-hydroxybutyrate, Beef cattle, Creep-feeding, Energy metabolism, Non-esterified fatty acids

Implications

This study demonstrated that neither calf sex nor creep-feeding supplementation impaired the performance or metabolic profile of grazing Nellore cows from late gestation to weaning. Maternal body weight, body condition, and milk yield were mainly driven by physiological stage rather than by calf factors or supplementation. These findings suggest that creep-feeding benefits calf growth, particularly in males, without compromising cow health or productivity. This knowledge is relevant for beef producers in tropical systems, supporting management strategies that enhance calf performance while maintaining sustainable cow-calf productivity.

1. Introduction

The use of creep-feeding supplementation in beef calves enhances weaning body weight (BW) (Carvalho et al., 2019; Cruz et al., 2022; Paixão et al., 2023). Moreover, this nutritional strategy, combined with subsequent dietary plans, can promote earlier finishing of steers in feedlot systems (Corah and Bishop, 1975; Pordomingo and Pordomingo, 2021; Abitante et al., 2024) and reduce the age at puberty in heifers (Nepomuceno et al., 2017; Paixão et al., 2024; Catussi et al., 2024). These effects are driven by physiological mechanisms that enhance calf development during the suckling and post-weaning phases.

The benefits of calf supplementation in tropical environments are primarily attributed to increased nutritional demands during periods of accelerated growth (Carvalho et al., 2019; Lopes et al., 2023). Thus, supplementation during the suckling phase is aimed at complementing nutrient intake from maternal milk and forage (Lardy and Maddock, 2007).

Previous studies have reported that calves managed under creep-feeding systems prioritize milk intake, followed by supplement consumption and then forage (Valente et al., 2012; Lopes et al., 2017). It is often hypothesized that this preference hierarchy could impact the performance of both primiparous (Nogueira et al., 2006) and multiparous (Fordyce et al., 1996) zebu cows. Nonetheless, several studies have shown that calf supplementation does not affect the productive performance of zebu (Aguiar et al., 2015; Martins et al., 2017; Lopes et al., 2016) or taurine beef cows (Aguiar et al., 2015; Cruz et al., 2022).

In the uterine environment, male fetuses exhibit more rapid development than female fetuses, which is associated with the expression of receptors involved in intermediate glycine metabolism, including citric acid, creatine, and sarcosine, which increase energy demands for fetal growth, resulting in higher maternal blood concentrations of sarcosine (Gimeno et al., 2024). Accordingly, male and female fetuses exhibit distinct growth dynamics and metabolic processes, differences that persist into the postnatal period, particularly in growth rate (Estrella et al., 2024; Nascimento et al., 2024). Although the combined effects of calf supplementation and sex on performance are somewhat understood (Carvalho et al., 2019), their interaction with maternal metabolism and performance in the pregnancy and lactation phase remains unclear.

In this context, we hypothesized that calf sex and creep-feeding supplementation modulate the productive performance and metabolic profile of grazing Nellore cows.

Therefore, the objective of this study was to body condition score, milk yield, and metabolic profile of grazing Nellore cows.

2. Material and methods

The experiment was conducted at the Beef Cattle Teaching, Research, and Extension Unit of the Department of Animal Science of the Federal University of Viçosa (UFV), in Viçosa, Minas Gerais, Brazil. All animal care and handling procedures were approved by the Ethics Committee on the Use of Production Animals of UFV (Protocol number 02/2023).

2.1 Animals, treatments, and experimental design

Fifty-five multiparous Nellore cows were used, with an average age of 6±2 years, 230±6 days of gestation, body weight (BW) of 519±74 kg, and an initial body condition score (BCS) of 4.50±0.5 (on a 1–9 scale).

Fetal sex was determined by transrectal ultrasonography (DP-2200Vet®, Mindray; 7.5-MHz linear transducer) at approximately 60 days of gestation, resulting in 29 male and 28 female fetuses. Sixty days before the expected calving date, cow-calf pairs (considering fetal sex) were randomly assigned to eight paddocks of *Urochloa decumbens* (6.11 ha each with 7-8 pairs/paddocks), which served as the experimental units. Moreover, the cows had *ad libitum* access to water throughout the entire experimental period, and all paddock was equipped with covered feeders.

A completely randomized design with a double-error structure was adopted. Four paddocks were allocated to the Control group (CON; mineral mix provided *ad libitum*) and four paddocks to the Supplemented group (SUP; protein-energy supplementation via creep-feeding). The effect of calf sex was evaluated within each supplementation treatment.

Calves in the SUP group received a protein-energy supplement daily at 1100 h from 100 to 240 days of age. The amount offered was 6 g of supplement/kg BW, as recommended by Carvalho et al. (2019) to bull calves and heifer calves. Calves were weighed every 28 days to adjust supplement allocation. The supplement was formulated to contain 260 g of crude protein (CP)/kg dry matter (DM), meeting the nutrient requirements of growing *Bos indicus* calves grazing tropical pastures (Valadares Filho et al., 2023). Calves had unrestricted access to the creep-feeding area through gated entrances that excluded cows. Calves from both treatments had *ad libitum* access to a mineral mix and water throughout the entire experimental period.

During the 60 days before calving, pregnant cows received 1.5 kg/day of protein-energy supplement containing 350 g CP/kg DM (Table 1), provided infrequently: 3 kg on Mondays, 3 kg on Wednesdays, and 4.5 kg on Fridays, always at 1100 h in a linear trough space of 0.70 m per animal to ensure homogeneous supplement intake among cows. The supplement was calculated to supply approximately 40% of the cow's protein requirements (Valadares Filho et al., 2023). From calving to weaning, all cows received mineral mix *ad libitum*.

All cows underwent a fixed-time artificial insemination (FTAI) protocol at an average of 45 days postpartum. Each animal received a single-dose intravaginal implant (Primer®, Agener União, Embu-Guaçu, São Paulo, Brazil) containing 1.9 g of progesterone (CIDR-B®, Pfizer Animal Health, São Paulo, Brazil) combined with 2 mg of estradiol benzoate (Estrogin®, Farmavet, São Paulo, Brazil). Nine days after protocol initiation, the implant was removed, and 1.5 mL of equine chorionic gonadotropin (Ecogon®, Biogeneses Bago, Curitiba, Paraná, Brazil) and 2.0 mL of prostaglandin F2-α (Estron®, Agener União, Embu-Guaçu, São Paulo, Brazil) were administered. Twenty-four hours after implant removal and hormone application, 1 mL of estradiol benzoate (RIC-BE®, Agener União, Embu-Guaçu, São Paulo, Brazil) was administered. Then, 48 hours after implant removal, preovulatory follicle diameter was evaluated using ultrasound (DP-2200Vet®, 7.5 MHz linear-array transrectal probe; Mindray). Cows presenting preovulatory follicles larger than 11 mm were artificially inseminated. Doppler ultrasound (DP-2200Vet®, Mindray) was performed 22 days after insemination to assess corpus luteum functionality (Pugliesi et al., 2019). Pregnancy diagnosis was performed 30 days after insemination.

2.2 Experimental Procedures and Sampling

Cow-Calf Performance

Cows were weighed 30 days before the expected calving date, 12 hours after calving, and again at 30, 100, and 240 days postpartum (weaning). BCS was assessed at the same time points by three experienced evaluators using a 1–9 scale (NRC, 2000). Calves (both sexes) were weighed approximately 12 hours after birth and at 30, 100, and 240 days of age. All weight measurements were performed at 0800 h without feed or water restriction.

Cows' Milk Yield

Milk yield (MY, kg/d) was estimated at 45 and 240 days postpartum through a single mechanical milking. Cows were separated from their calves at 0500 h the day before milking. At 0600 h, calves were reunited with their dams to stimulate udder emptying through nursing. After one hour, the animals were separated again, and the cows were kept in a paddock without access to the calves for the next 12 hours. Milking occurred at 0600 h using a mechanical milking machine and 1 mL of exogenous oxytocin (10 IU/mL, Lactocina®, Patrocínio Paulista, São Paulo, Brazil) injected into the mammary vein to stimulate milk secretion. The milk weight and milking times were recorded for estimating 24-hour milk production, as described by Lopes et al. (2022).

Cows' Blood Samples

Blood samples were collected from the jugular vein using vacuum tubes with clot activator and gel separator (BD Vacutainer SST II Plus 9 mL, São Paulo, Brazil) and sodium fluoride (BD Vacutainer Fluoride 5 mL, São Paulo, Brazil) at 30 days before calving, 12 hours postpartum, and at 30, 100, and 240 days postpartum at 0800 h. The samples were analyzed for concentrations of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (β-OHB), total protein, albumin, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). After collection, samples were centrifuged at 3500xg for 15 minutes (Centribio®, Model 80-2B, 15 mL radius 10 cm, Mumbai, India) to separate serum and plasma, stored in 2 mL microtubes, and frozen at −20 °C for later analysis.

Forage Samples

To assess forage availability and quality, samples were collected every 28 days. Five random sampling points per paddock were clipped using a 0.5 × 0.5 m metallic frame, with plants cut approximately 1 cm above soil level to estimate forage mass. Simultaneously, forage samples were obtained via hand-plucking samples to analyze chemical composition (Table 2). Samples were weighed, dried in a forced-air oven (55 °C/72 h), ground in a knife mill (Willye® TE-680) with 1- and 2-mm sieves, and stored for further analyses.

2.3 Laboratory Analyses

Samples of supplement and forage, which were processed to pass through a 1-mm sieve, were analyzed for dry matter (DM, dried over-night at 105ºC, method G-003/2), organic matter (OM, combustion at 550 °C in a muffle furnace, method M-001/2), and crud protein (CP, Kjeldahl method N-001/2) contents according to the standard analytical procedures of the Brazilian National Institute of Science and Technology in Animal Science – INCT-CA (Detmann et al., 2021). The neutral detergent fiber (NDF) analysis was performed using a thermostable α-amylase without sodium sulphite (method F-012/1; Detmann et al., 2021). The NDF contents were expressed exclusive of ash and protein contaminants (apNDF). Supplement and forage samples, processed to pass through a 2-mm screen sieve, were evaluated for indigestible NDF (iNDF) content using non-woven textile bags (100 g/m²) and a 288-h *in situ* incubation procedure, as recommended by Valente et al. (2011).

Glucose concentrations were measured in plasma using the enzymatic glucose oxidase-peroxidase method (Bioclin®, K082). Serum samples were analyzed for NEFA (enzymatic method, Randox®, RB1008), β-OHB (colorimetric method, Randox®, FA115β-OHB), total protein (TP; biuret method, Bioclin®, K031), albumin (green bromocresol method, Bioclin®, K040), urea (BUN; enzymatic-colorimetric method, Bioclin®, K056), AST (UV kinetic method, Bioclin®, K048-7.3), ALT (kinetic method, Bioclin®, K049-6), and GGT (modified SZASZ-IFCC method, Bioclin®, K080-2), using an automated biochemical analyzer (BS200E, Mindray, China).

2.4 Statistical Analyses

Data were analyzed according to the model:

$$Y_{ijkl} = \mu + C_i + P_{(i)j} + S_k + (C \times S_{ik}) + \varepsilon_{(ijk)l}$$

where: Y_{ijkl} = observation taken in animal (*l*) with calf sex (*k*) within paddock (*j*) and submitted to creep-feeding supplementation (*i*); μ = general constant; $P_{(i)j}$ = effect of paddock or group of animal (*j*) nested within level (*i*) of creep-feeding supplementation (random); C_i = effect of creep-feeding supplementation (*i*) (fixed); S_k = effect of calf sex *k*; C x S_{ij} = effect of the interaction between creep-feeding supplementation (*i*) and calf's sex (*k*); and ε(ij) = random error assumed to be NIID (0, σ_{ε}^{2}).

Performance and blood characteristics measured over a long time were analyzed as repeated measures. The best (co)variance matrix was chosen based on the Schwarz criterion with correction. All analyses were performed using the MIXED procedures of SAS 9.4 (α = 0,05). When necessary, means were grouped based on Fisher's test significance difference.

3. Results

3.1 Cow-calf performance

Neither supplementation nor calf sex influenced ($P > 0.05$; Table 3) cows' body weight (BW), BCS, or milk yield (MY). Differences in maternal performance variables were attributed to the evaluation time ($P < 0.001$; Table 3, Figure 1). A similarity between BW and BCS trends over time was observed ($P < 0.05$; Figure 1A and 1B). Both variables decreased during the first 30 days postpartum, and subsequent periods exhibited a similar temporal pattern. A comparable decline was observed for MY throughout the lactation period ($P < 0.001$; Figure 1C).

An interaction was observed between creep-feeding supplementation and calf sex ($P < 0.001$; Table 3), as well as between calf sex and time of evaluation ($P < 0.001$; Table 3) on male calves. Accordingly, creep-feeding calves had higher BW at weaning compared to calves in the control group ($P < 0.05$, Figure 2A). Similarly, male calves presented greater BW at weaning ($P < 0.05$, Figure 2B).

3.2 Cows' metabolic profile

No interaction effect between creep-feeding supplementation and calf sex was observed on the maternal metabolic profile ($P > 0.05$; Table 3). However, the time of collection had a significant effect ($P < 0.001$; Table 3) on blood concentrations of glucose, NEFA, β-OHB, total protein (TP), albumin, BUN, AST, ALT, and GGT in the cows.

Glucose and NEFA concentrations in the blood followed similar temporal patterns, both peaking significantly ($P < 0.05$) on the day of calving before gradually declining throughout the subsequent periods (Figures 3A and 3B). Conversely, blood β-OHB concentrations were higher ($P < 0.05$) during the prepartum period (30 days before calving) and steadily decreased thereafter (Figure 3C), reflecting the cow's intrinsic metabolic shift. Therefore, no differences were observed among treatments ($P > 0.05$).

Total protein concentrations increased ($P < 0.05$; Figure 3A) throughout the evaluation period. Albumin levels rose ($P < 0.05$; Figure 3B) from the prepartum period until 30 days postpartum, then decreased at 100 days and increased again at the end of the evaluation. BUN concentrations decreased ($P < 0.05$; Figure 3C) over time, reaching the lowest levels at 100 days postpartum.

AST and ALT concentrations followed a similar pattern, increasing progressively over the evaluation period ($P < 0.05$; Figures 6A and 6B). GGT levels ($P < 0.05$) peaked shortly after calving and gradually declined over time (Figure 6C).

4. Discussion

In this study, we observed no effect of calf sex and/or creep-feeding supplementation on maternal performance. Instead, the performance of multiparous Nellore cows under grazing conditions may vary according to their nutritional requirements and can be influenced by physiological changes during gestation (Gionbelli et al., 2024) and lactation (Lopes et al., 2022), as well as by seasonal variations in pasture availability and quality (Lopes et al., 2023).

These findings indicate the presence of maternal regulatory physiological mechanisms in Nellore cows that persist until calf weaning. Voluntary feed intake is one of the mechanisms that regulates maternal physiology and, consequently, can enhance or limit animal performance (NASEM, 2016). Studies indicate that Nellore cows exhibit reduced feed intake in late gestation (Carcco et al., 2023; Gionbelli et al., 2024; David et al., 2024), a pattern that extends into early lactation (Lopes et al., 2023; David et al., 2024), resulting in variations in BW and BCS. In this study, the dynamics of maternal BW and BCS during the transition period indicate increased energy demands in late gestation due to limited feed intake and fetal growth. This reflects a greater reliance on intermediate metabolism, which may affect performance over time.

Maternal metabolism undergoes progressive adaptation from gestation to weaning in response to the physiological demands of each stage. Linking cow performance to metabolic dynamics, the catabolism of body reserves enhances the oxidation of skeletal muscle and adipose tissue, enabling cows to meet nutritional requirements while regulating voluntary intake, which ultimately results in temporal changes in BW and BCS.

However, the blood concentration of energy metabolites, such as glucose, NEFA, and β-OHB, undergoes modulatory variations throughout gestation and lactation, particularly in response to the oxidation of body reserves (Ferreira et al., 2021; Vital et al., 2024).

Glucose, the primary metabolic biomarker, is tightly regulated by homeostatic mechanisms (Herdt, 2000; Leblanc, 2010) and may serve as the sole energy source for specific tissues, even as the catabolism of body reserves alters the availability of alternative substrates.

Furthermore, hormonal dynamics and the nutritional status of beef cows influence the synthesis and phosphorylation of muscle glycogen (Astessiano et al., 2014; Kolnes et al., 2015), favoring glucose storage and/or release into circulation to meet the energy demands of other tissues, thus reflecting shifts in nutritional needs during pre- and postpartum periods.

Some authors have noted that tropical grazing beef cows typically show baseline blood glucose concentrations postpartum (Moura et al., 2020; Ferreira et al., 2021; Saraiva et al., 2024). As a result, the energy requirements of beef cows during the late gestation and early lactation lead to increased blood glucose due to the energy demands of fetal development, calving, and milk synthesis (Bell and Bauman, 1997).

Consistent with these dynamics, increased NEFA levels were observed at calving, stabilizing after 30 days postpartum. This mechanism has been documented in *Bos indicus* beef cows raised on tropical pastures (Moura et al., 2020; Ferreira et al., 2021; Saraiva et al., 2024) and in grazing *Bos taurus* cows (Moriel et al., 2012; Briano et al., 2024). It is explained by fat oxidation and low dry matter intake (Adewuyi et al. 2005), resulting in reduced maternal BCS. This process involves lipolysis of fat reserves, triggered by catecholamines and insulin, which activate hormone-sensitive lipases (Stich and Berlan, 2004) at calving, and by reduced feed intake, facilitating fatty acid mobilization and NEFA metabolism.

Similar to dairy cows, Nellore cows exhibited intense negative energy balance (NEB) at the end of gestation and early lactation, mobilizing body reserves to support basal and productive metabolism (Leblanc, 2010; Orquera-Arguero et al., 2023), according to their metabolic status. Elevated blood concentration of β-OHB indicates increased energy demands in maternal tissues, including skeletal muscle, nervous system (Berg et al., 2023), and mammary gland (Singh et al., 2023), likely supporting colostrum fat synthesis while preserving glucose for fetal development.

The relationship between β-OHB levels and BCS indicates that maternal energy demands contributed to the mobilization of body reserves, supporting ketone body synthesis and BCS reduction, which prolonged oxidative stress during the peripartum period.

Concurrent reductions in BW and BCS reflect the mobilization of fat and muscle reserves and the resulting caloric deficit. These metabolic adjustments highlight how maternal energy demands alter both body condition and body total mass. Energy metabolism in beef cows is further influenced by nutrition (Moriel et al., 2013; Ferreira et al., 2020; Gionbelli et al., 2024), hormonal (Hort and Jorgensen, 1992; Cappellozza et al., 2014), genetic (Lopes et al., 2020), and parity order (Ferreira et al., 2021). To date, calf supplementation or sex appears not to affect maternal metabolic profiles.

Therefore, supplementation the calf using a creep-feeding system, when combined with maternal milk and forage intake, has an additive effect on the total nutrient intake of beef calves between 100 at weaning, enhancing their growth and performance (Lopes et al., 2023), as observed in the present study.

This effect is particularly relevant for male calves, which have higher nutritional requirements due to greater body development (Carvalho et al., 2019). Several authors have reported that calf supplementation does not reduce milk intake (Lopes et al., 2016; Almeida et al., 2018), and MY naturally declines toward weaning due to lactation physiology (Lopes et al., 2023).

Lopes et al. (2016), when reporting isolated effects and their interactions, also found that creep-feeding supplementation of the calf sex did not affect MY in crossbred Zebu and Nellore beef cows. Therefore, in cow-calf systems under tropical conditions, these factors do not impair maternal performance, which is consistent with previous findings.

At this stage, calves undergo intense allometric growth (Owens et al., 1993) regardless of sex (Lopes et al., 2014; Carvalho et al., 2019; Paixão et al., 2024), which does not impair maternal performance, as observed in the present study. Several studies report that creep-feeding supplementation improves calf weaning performance in tropical regions, regardless of sex. Thus, we observed that both creep-feeding and progeny sex affect calf growth without compromising maternal performance.

Variations in BW, BCS, and MY may be linked to a maternal biological predisposition for energy dynamics across different tissues during pregnancy and lactation, independent of calf sex or supplementation. Development of the gravid uterus, fetal membranes, mammary gland, and retention of triglycerides and amino acids in adipose and muscle tissue, respectively, all play fundamental roles in this process.

The decline in MY throughout lactation contributes to the maintenance of cow BW and BCS, since this period coincides with reduced maternal nutritional demands and greater forage availability compared to early lactation, and these changes are also associated with modifications in the cow’s energy and protein metabolism.

Energy and protein metabolism should be considered as dependent processes, particularly in the context of gestation and lactation in beef cows, as their integration contributes to the variation in maternal performance observed in the present study. Total protein, albumin, and BUN are reliable blood protein biomarkers for understanding homeorhetic mechanisms in cows during pre- and postpartum periods (Bertoni and Trevisi, 2013; Ferreira et al., 2020; Calderaro et al., 2024).

Blood total proteins consist mainly of albumin and globulins (Waterlow, 2006). Albumin is associated with transporting energy and protein molecules, particularly NEFA (Rothschild et al., 1973; Tennant and Center, 2008), preserving their biological integrity for oxidation in the liver, and transporting glucose precursors for milk synthesis in early lactation (Waterlow, 2006).

As observed in the present study, maternal albumin metabolism was modulated throughout the pre- and postpartum periods in association with performance, with a marked increase at weaning. This pattern may reflect hepatic activity, since albumin is synthesized in the liver and secreted into the blood (Rothschild et al., 1973).

During late gestation and early lactation, hepatic metabolism prioritizes NEFA oxidation (Bobbo et al., 2017), which can partially explain the reduced serum albumin concentration from -30 to 100 days relative to calving. Considering that albumin is composed of amino acid (AA) residues (Rothschild et al., 1973; Wu, 2013), this reduction may indicate that these AA residues were utilized by the maternal metabolism during the NEB. Conversely, the improved availability of AA near weaning may enhance hepatic albumin secretion into blood, contributing to the variation in maternal performance at this stage.

Regarding protein metabolism, BUN variations reflect protein intake, hepatic function, and muscular activity according to maternal physiology. This Effect may be attributed to maternal supplementation and homeostatic regulation during the study period, with variations in maternal performance from prepartum to weaning reflecting this underlying metabolic mechanism.

Therefore, BUN levels are influenced by protein intake (Schimk, 1962), and the urea formation mainly takes place through typical nitrogen excretion by the liver (Molefe and Mulunda, 2025). Therefore, higher BUN levels in prepartum suggest maternal supplementation with protein-energy. This effect can be explained by the CP concentration of the supplement (362.8 g CP/kg of DM), which promoted an increase in circulating urea. In contrast, the reduction in BUN concentrations observed from the postpartum period to weaning may reflect the lack of supplementation during this phase, highlighting the direct influence of dietary protein supply on maternal nitrogen metabolism.

This also relates to reduced BW and BCS resulting from decreased DMI, intensifying protein catabolism, and skeletal muscle loss as an adaptive mechanism to maintain maternal nutrient requirements. Over time, DMI stabilizes, and forage protein quality improves, resulting in a positive protein balance. However, the highest BUN concentrations occurred prepartum, and supplementation or calf sex did not affect the performance or health of beef cows.

Liver metabolism is also affected by physiological changes during the peripartum period compared to weaning. Thus, the enzymatic biomarkers in blood indicate enhanced hepatic activity toward specific metabolic pathways (Rafia et al., 2012), impairing metabolism and maternal health.

Mobilization of fatty acids and AAs from body reserves increases oxidative stress, potentially damaging the active liver tissue (González et al., 2011; Van Saun, 2023), as evidenced by increased ALT, AST, and GGT (Fiore et al., 2021) during pre- and postpartum periods in Nellore cows, indicating hepatic homeorhesis. Variations in blood ALT and AST concentration are related to AA metabolism (Maeda, 2022) via transaminase activity (Tennant and Center, 2008).

Thus, our findings suggest that, during late gestation and early lactation, maternal physiology is primarily regulated by energy metabolism, which may explain the lower blood concentrations of ALT and AST observed during this period. In contrast, at weaning, different physiological conditions were associated with higher levels of these blood biomarkers, coinciding with variations in maternal performance.

Elevated GGT activity at parturition suggests intense oxidative stress. This mechanism may protect hepatocytes against oxidative damage (Tennant and Center, 2008; Zhang and Forman, 2009), preserving liver health and function, as high NEFA levels in the liver activate glutamine metabolism (Jean et al., 2002), supporting glutathione synthesis to inhibit reactive oxygen species from β-oxidation and prevent hepatocyte apoptosis (Zhang and Forman, 2009). Thus, the dynamics of hepatic, energy, and protein biomarkers vary throughout gestation and lactation in beef cows, allowing synergistic maternal metabolic homeorhesis.

In summary, the nutritional status and metabolic adaptation of grazing Nellore cows are modulated by the physiological stages from late gestation to weaning. However, calf sex and/or creep-feeding supplementation do not impair maternal performance and metabolic profile.

Authors contributions

Pedro Henrique Borba Pereira: *investigation, methodology, formal analyses, data curation, writing, and editing.* **Luiz Jardel Müller Motta:** *formal analysis.* **José Augusto Moura Godinho:** *formal analysis.* **Sidnei Antonio Lopes:** *writing-review.* **Edenio Detmann:** *data curation, methodology, validation, writing-review, and supervision.* **Cláudia Batista Sampaio:** *conceptualization, data curation, funding acquisition, methodology, validation, writing-review, supervision, and approved the final version to be published. All authors have read and agreed to the published version of the manuscript.*

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that all animal care and handling procedures were approved by the Ethics Committee on the Use of Production Animals of Federal University of Viçosa (Protocol number 02/2023).

Data Availability Statement

The data are available from the corresponding author by reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence assisted technologies in the writing process.

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Declaration of interest

None.

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Tables

Table 1.

Ingredients and composition of the supplement provided to the cow-calf during an experimental period

Item	Maternal supplement	Calf supplement
Ingredients (%; as-fed basis)		
Cron	35.5	53.0
Soybean meal	27.7	41.0
Wheat bran	30.0	-
Urea	3.4	1.0
Ammonium sulfate	0.4	-
Mineral mix [a]	3.0	5.0
Chemical composition (g/kg of DM)		
OM [b]	948.6	937.0
CP [c]	362.8	266.6
apNDF [d]	164.8	68.80
iNDF [e]	23.30	16.60

[a] Mineral mix = 500g dicalcium phosphate, 472g sodium chloride, 15g zinc sulfate, 7g copper sulfate, 0.5g cobalt sulfate, 0.5g potassium iodate, 5g sulfate of manganese.

[b] OM = organic matter.

[c] CP = crude protein.

[d] apNDF = neutral detergent fiber corrected for ash and protein residue.

[e] iNDF = indigestible neutral detergent fiber.

Table 2.

The chemical composition of the forage (*Uruchloa decumbens*) according to the experimental sampling period

Item [a]	Sample period								
	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May.
DM (g/kg)	708.6	177.8	247.8	246.3	263.8	235.2	269.6	283.7	285.5
OM (g/kg, DM)	944.4	981.3	974.4	974.7	975.0	978.9	975.5	974.9	973.3
CP (g/kg, DM)	44.6	166.8	125.2	112.0	91.2	116.0	109.2	107.7	93.7
apNDF (g/kg, DM)	744.7	554.4	549.3	637.0	568.9	509.0	555.4	542.0	552.7
iNDF (g/kg, DM)	365.5	158.7	158.5	180.8	225.7	196.2	234.4	217.3	226.0
Herbage mass (kg DM/ha)	3234	2113	1908	2537	2725	2808	2932	2436	2687

[a] DM, dry matter; OM, organic matter; CP, crude protein; apNDF, neutral detergent fiber corrected for ash and protein; iNDF, indigestible neutral detergent fiber.

Table 3.

Probability value (*P* – value) of effects of supplementation on calf via creep-feeding (C), calf sex (S), over time in the experimental period (T), and their interactions on performance and metabolites of Nellore cows

Item	C	S	T	C x S	C x T	S x T	C x S x T
Cow-calf performance [a]							
Cow BW (kg)	0.38	0.71	<0.001	0.73	0.079	0.53	0.71
BCS (1 – 9)	0.93	0.77	<0.001	0.48	0.50	0.99	0.13
MY (kg/d)	0.36	0.73	<0.001	0.082	0.083	0.68	0.99
Calf BW (kg)	0.033	0.008	<0.001	0.11	<0.001	<0.001	0.080
Cows' metabolic profile [b]							
Glucose (mg/dL)	0.10	0.63	<0.001	0.65	0.53	0.65	0.26
β-HOH (mmol/L)	0.43	0.22	<0.001	0.15	0.79	0.73	0.31
NEFA (mmol/L)	0.072	0.16	<0.001	0.33	0.10	0.65	0.54
Total protein (mg/dL)	0.33	0.19	<0.001	0.84	0.32	0.18	0.61
Albumin (mg/dL)	0.62	0.88	<0.001	0.81	0.96	0.50	0.14
BUN (mg/dL)	0.85	0.66	<0.001	0.30	0.32	0.91	0.51
AST (U/L)	0.76	0.55	<0.001	0.45	0.052	0.19	0.51
ALT (U/L)	0.35	0.15	<0.001	0.87	0.45	0.83	0.86
GGT (U/L)	0.78	0.068	<0.001	0.45	0.84	0.24	0.75

[a] BW, body weight; BCS, body condition score; MY, milk yield.

[b] β-HOH, β-hydroxybutyrate; NEFA, non-esterified fatty acid; BUN, blood urea nitrogen; AST, enzyme aspartate amino-transferase; ALT, enzyme alanine amino-transferase; GGT, enzyme gamma-glutamyl transferase.

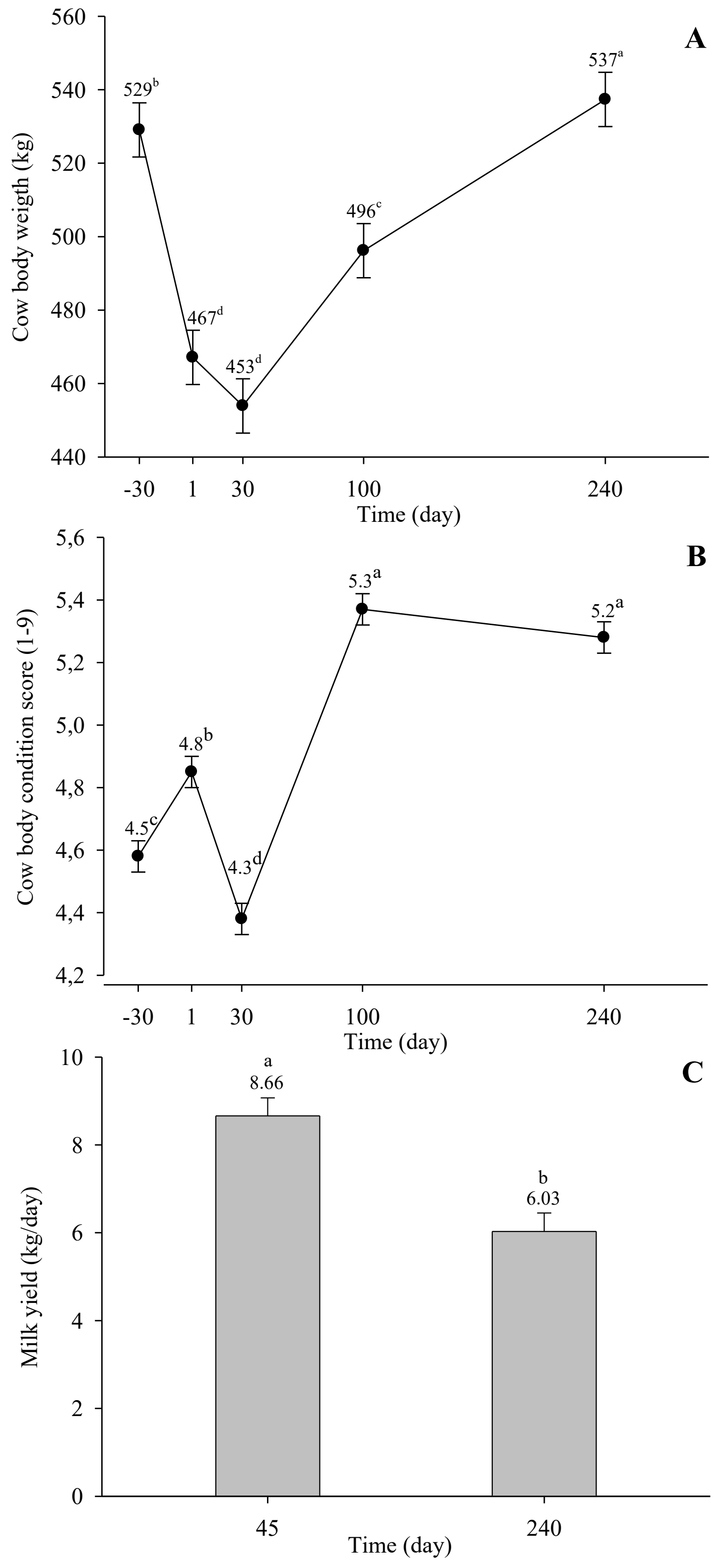

Figure 1. Cows' body weight (A), cows' body condition score (B), and milk yield (C) according to time (means followed by different superscripts differ at $P < 0.05$).

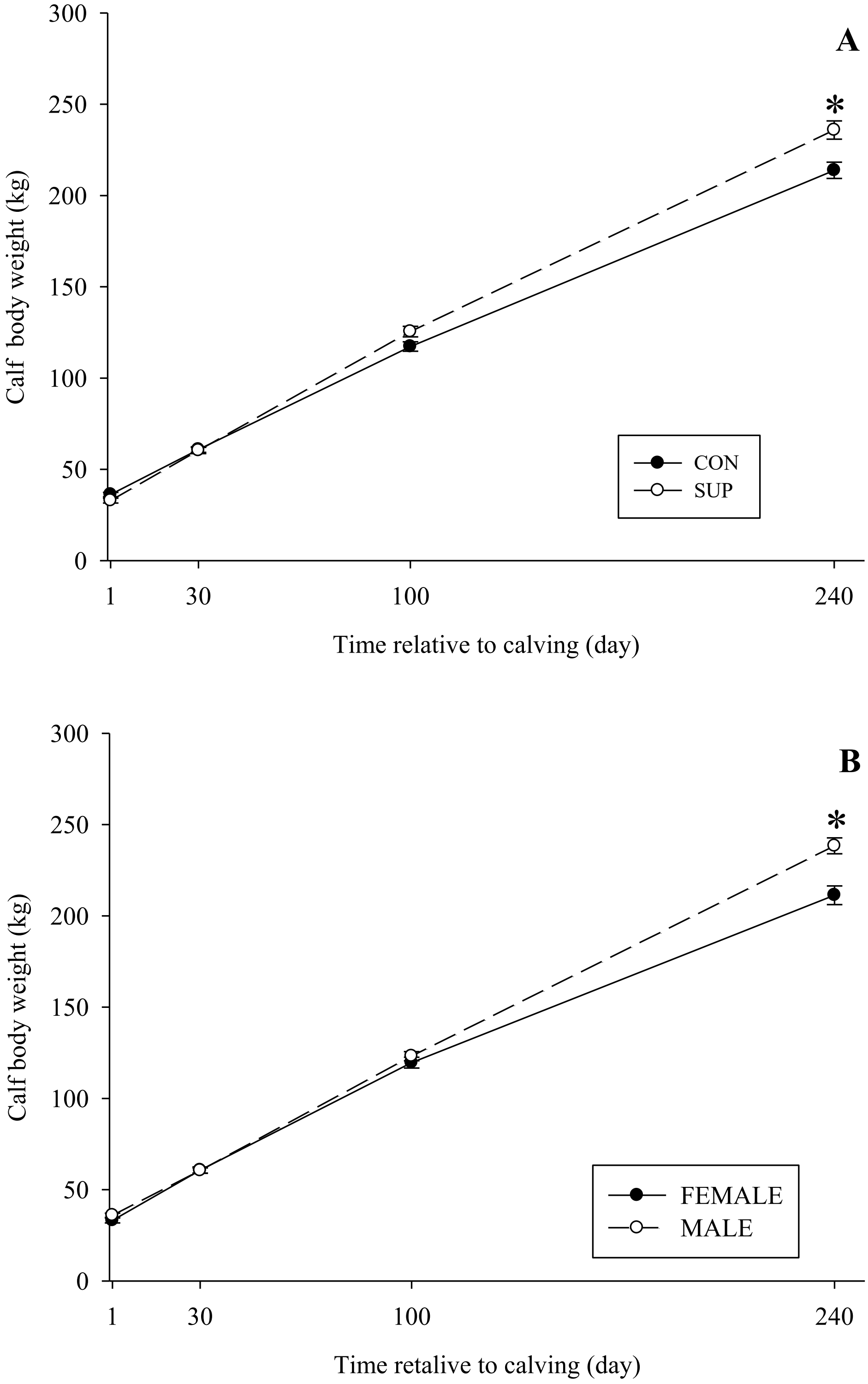

Figure 2. Variation of calf body weight according to time after calving and following the interaction with supplementation via creep-feeding (A) and with calf's sex (B). * Indicates differences within as weighing time (*P* < 0.05).

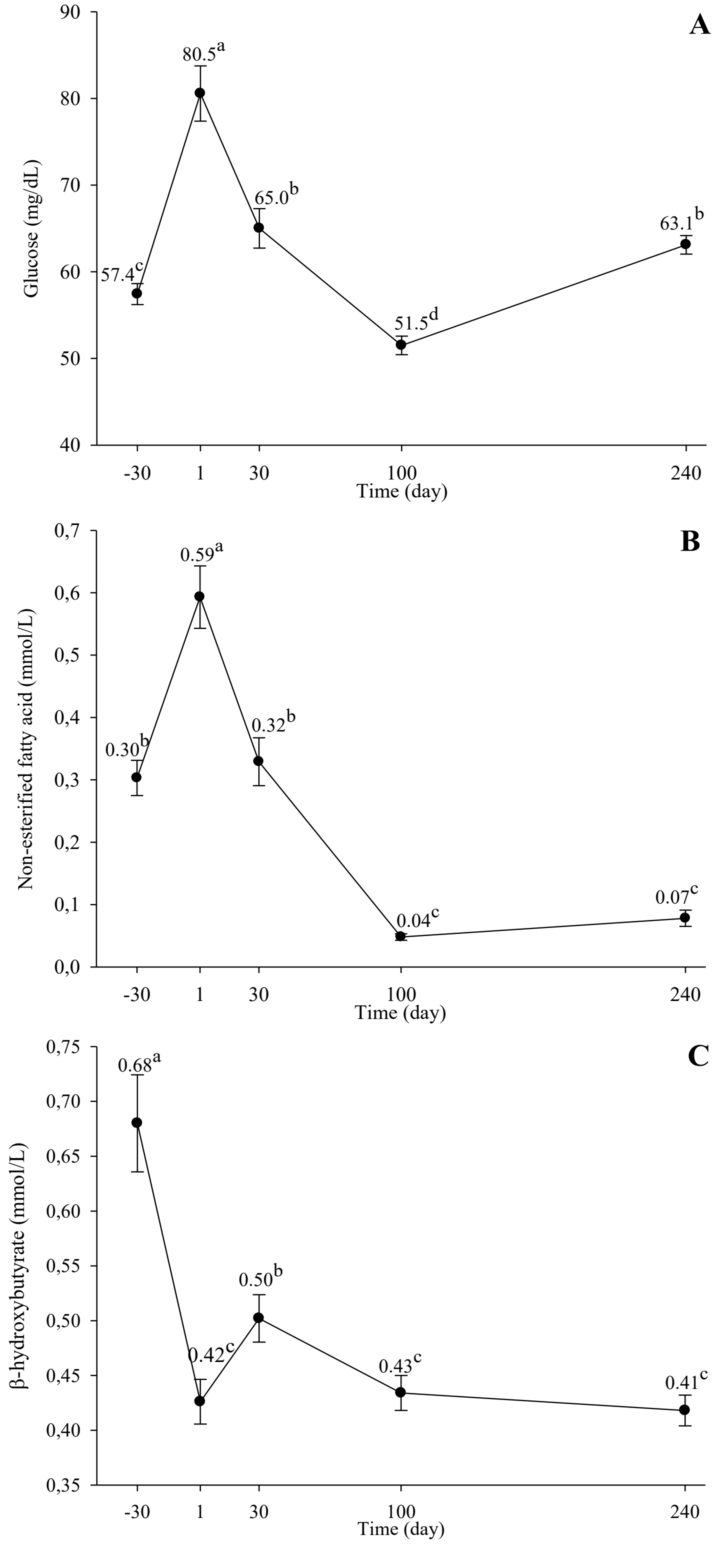

Figure 3. Blood concentrations of glucose (A), non-esterified fatty acid (B), and β-hydroxybutyrate (C) in cows according to time (means followed by different superscripts differ at $P < 0.05$).

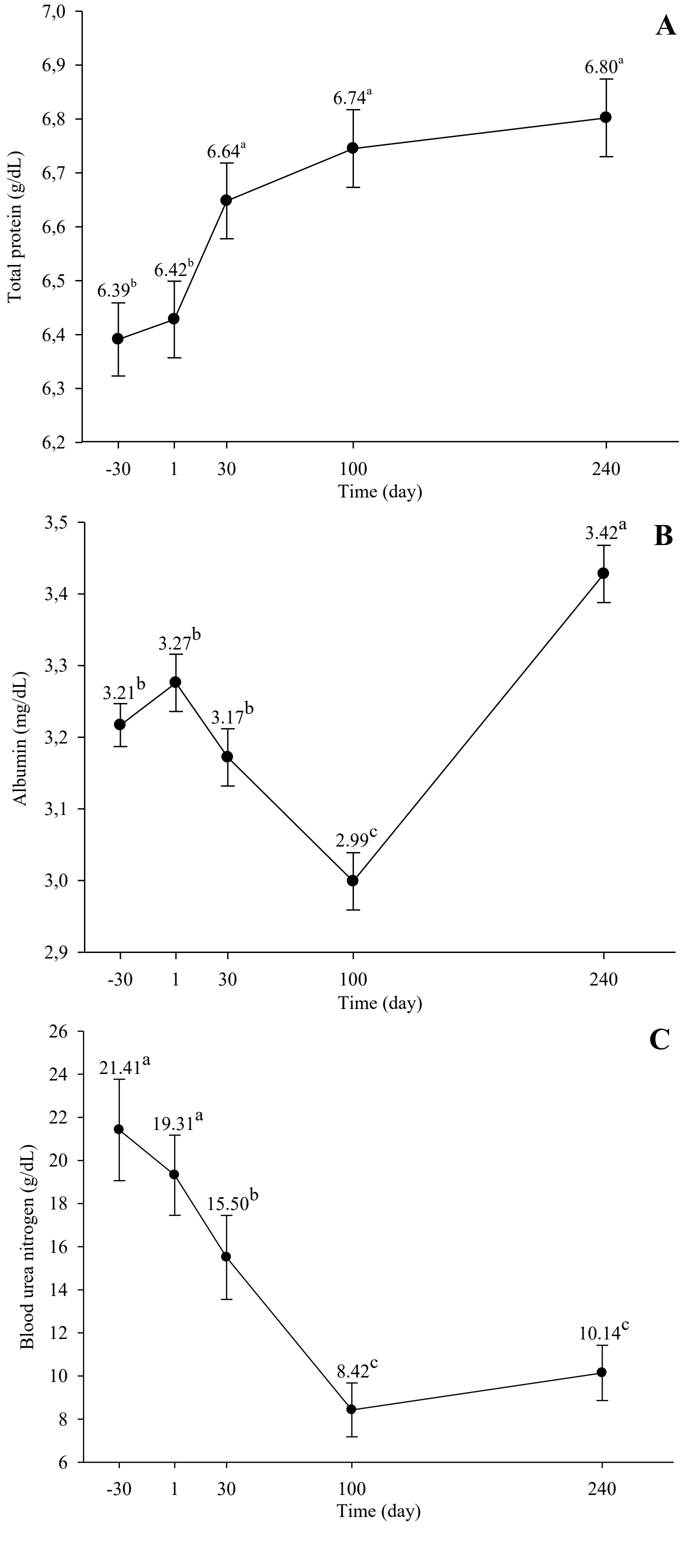

Figure 4. Blood concentrations of total protein (A), albumin (B), and (C) blood urea nitrogen in cows according to time (means followed by different superscripts differ at $P < 0.05$).

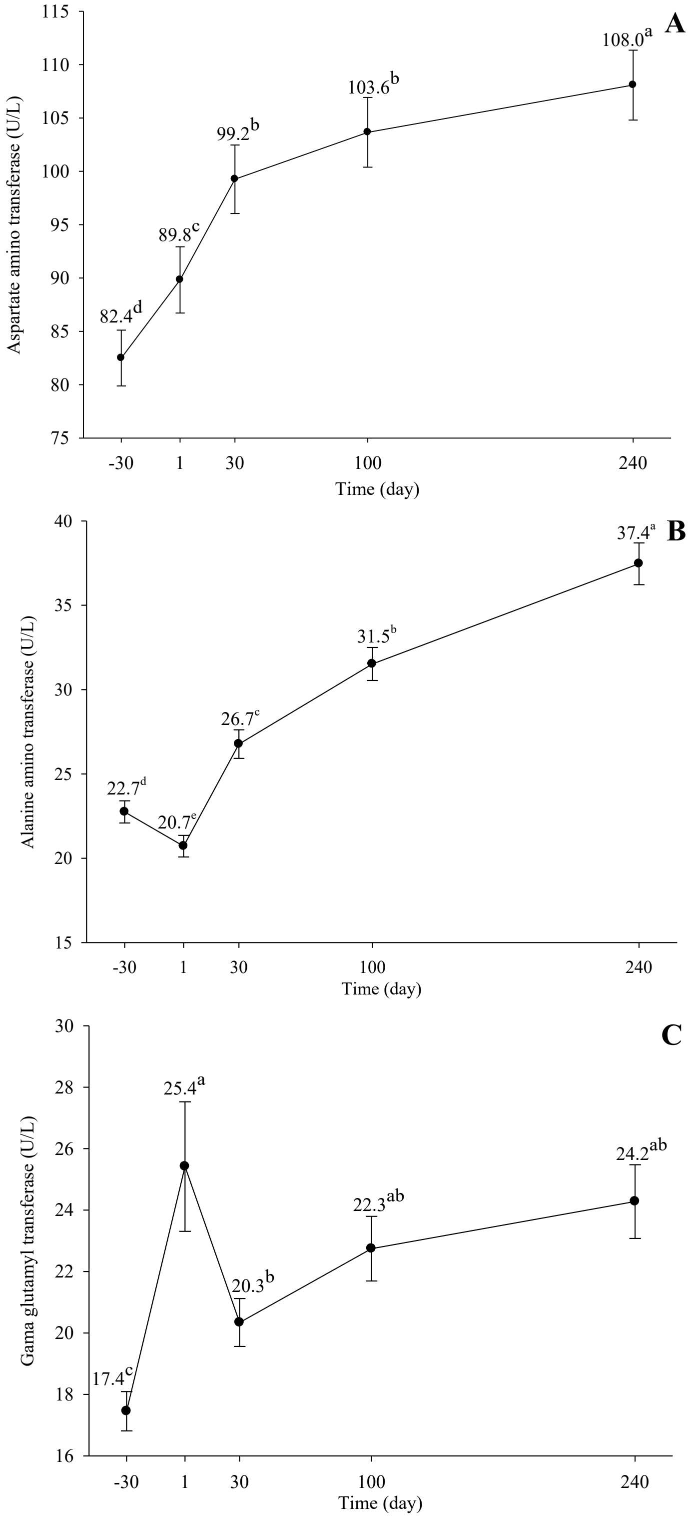

Figure 5. Blood concentrations of liver enzyme aspartate amino transferase (A), alanine amino transferase (B), and gamma-glutamyl transferase (C) in cows according to time (means followed by different superscripts differ at $P < 0.05$).

CHAPTER 2

Energy metabolism of beef cows during the peripartum period: insights from supplementation strategies

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Highlights

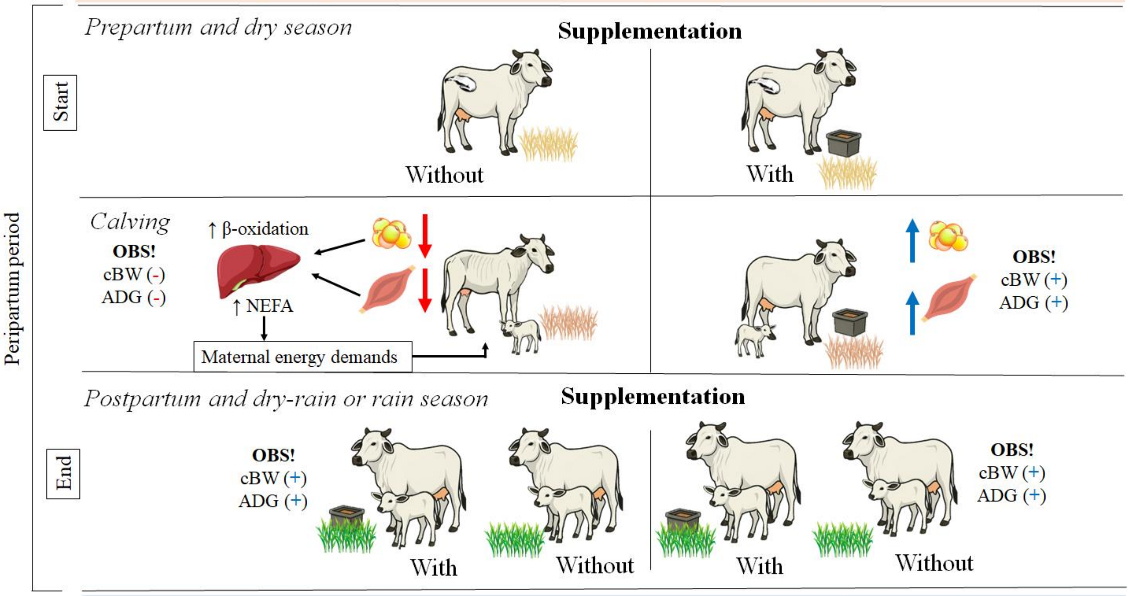
- Multiparous and primiparous Nellore cows without supplementation during late gestation show reduced body weight and average daily gain.
- Non-supplemented beef cows exhibit elevated blood NEFA concentration at calving.
- Postpartum supplementation does not impair maternal performance or energy metabolism in multiparous or primiparous Nellore cows.
- Blood energy biomarkers (glucose, NEFA, and β-OHB) during the peripartum period provide insights into the nutritional status of beef cows.

Abbreviations

ADG, average daily grain; BCS, body condition score; BEB, blood energetic biomarkers; BW, body weight; cBW, calving body weight; CP, crude protein; DM, dry matter; FSH, follicle-stimulating hormone; GLUC, glucose; GnRH, gonadotropin-releasing hormone; iNDF, indigestible neutral detergent fiber; LH, luteinizing hormone; NDF, neutral detergent fiber; NEFA, non-esterified fatty acids; POS, postpartum period; PRE, prepartum period; β-OHB, β-hydroxybutyrate.

Graphic Abstract

Dataset = 654 observation (n = 488 multiparous Nellore cows + n = 488 primiparous Nellore heifers)
Dams grazing *Urochoa decumbens* in the tropical system (July to December)



Conclusion:

1. Protein supplementation in Nellore cows fed tropical forage reduces circulating blood NEFA concentrations during late gestation.
2. Nutritional intervention does not modify overall energy metabolism or postpartum performance.
3. This effect reflects a maternal biological predisposition to sustain performance during late gestation and early lactation.

Abstract

Our objective was to evaluate the effects of maternal supplementation during the prepartum and/or postpartum period on performance and blood concentrations of glucose, non-esterified fatty acids (NEFA), and β-hydroxybutyrate (β-OHB) in Nellore cows grazing tropical pasture, using a meta-analytical approach. The dataset was compiled from 17 experiments conducted in Brazil between 2016 and 2025, totaling 80 treatment means. All studies included a basic model with fixed effects of prepartum and postpartum supplementation (no supplementation vs. supplementation), adjusted for animal category (multiparous cows or primiparous heifers). Prepartum supplementation significantly increased body weight at calving ($P < 0.001$), improved average daily gain ($P = 0.017$), and reduced blood concentration of NEFA ($P < 0.001$), compared to non-supplemented cows. Based on the results, the estimated optimal composition for prepartum supplementation is 309 g/kg of crude protein (CP), provided at 3.00 g/kg of body weight per day. No interaction between prepartum and postpartum supplementations ($P > 0.05$), and postpartum supplementation alone had no significant effect on postpartum body weight or blood concentrations of glucose, NEFA, and β-OHB ($P > 0.05$). In summary, prepartum protein supplementation modulates blood NEFA concentrations during late gestation, supporting maternal performance at calving, while postpartum supplementation does not affect maternal metabolism or performance, reflecting a biological predisposition to sustain peripartum performance.

Key-works Calving weight gain, Nellore cows, Non-esterified fatty acids, Protein supplement

1. Introduction

In tropical beef production systems, where cows are grazing, nutritional constraints especially, i.e. during the dry season, can compromise energy metabolism due to the limited availability and quality of forage (Lopes et al. 2020). This restriction is marked by increased concentrations of insoluble fiber and lignin, reducing digestibility, and decreased crude protein concentration in the dry period (Detmann et al. 2009; 2014a), which underscores the need for maternal protein supplementation (Nascimento et al. 2024).

Such nutrient restriction negatively impacts the metabolism of beef cows during the peripartum period (Wood et al. 2013; Bertoni and Trevisi, 2013; Lopes et al. 2020). In tropical systems without maternal supplementation during mid or late gestation, studies have reported impaired energy metabolism and performance in multiparous cows (Ferreira et al. 2020; Barcelos et al, 2022). These effects are even greater in primiparous beef heifers (Ferreira et al, 2021; Melo et al, 2024; Vital et al, 2024).

Thus, beef cows grazing tropical forages, when not supplemented during mid-to-late gestation (Barcelos et al., 2022) and/or during the breeding season, i.e., 30 to 60 days postpartum (Araújo et al., 2022), experience more pronounced reductions in body weight (BW) and body condition score (BCS), which are associated with impaired reproductive performance (Bohnert et al., 2013; Hill et al., 2018). Consequently, the premise is that Zebu cows with a low BCS, i.e., ≤ 5 on the 1-9 scale (Cooke et al., 2021) or between 2.0 and 2.5 on the 1-5 scale (Ayres et al., 2014), at the late gestation and extending into the breeding season, exhibit reduced fat reserves. This may impair the reproductive status of beef cows (Molefe and Mulunda, 2025). Consequently, the condition inhibits the hypothalamic-pituitary-gonadal axis, impairing gonadotropin-releasing hormone (GnRH) synthesis and disrupting luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion (Keisler and Lucy, 1996; Clément, 2016; D'Occhio et al., 2019), ultimately delaying follicular growth (Grimard et al., 1995) and extending postpartum anestrus (Ciccioli et al., 2003).

Prolonged anestrus is also observed in beef cows with low BW at calving (Barcelos et al., 2022), specifically between 300 and 400 kg and derived from crossbreeding *Bos indicus* and *Bos taurus* (Alforma et al., 2021). Thus, in addition to supplementation levels influencing cow BW variations, voluntary dry matter intake during the peripartum period must be considered (Gionbelli et al., 2024; David et al., 2024).

In this context, assessing the blood concentrations of energy biomarkers, such as glucose, non-esterified fatty acids (NEFA), and β-hydroxybutyrate (β-HOB), provides valuable insights into the maternal nutritional history, especially when combined with BW and BCS assessments (Mulliniks et al. 2013; Ferreira et al. 2020; Moreno et al., 2023).

Maternal nutritional strategies involving supplementation during late pregnancy and/or early lactation may reduce the intensity of muscle protein breakdown (Sadri et al. 2022), lipid mobilization, and β-oxidation during the peripartum period (Silva et al, 2017; Moura et al, 2020), potentially minimizing negative impacts on cow health and performance. However, inconsistencies remain in the literature regarding the effects of such interventions on energy metabolism.

Therefore, our objective was to evaluate the effects of maternal supplementation during the prepartum and/or postpartum period on performance and blood concentrations of glucose, NEFA, and β-OHB during the peripartum period in Nellore cows grazing tropical pasture, using a meta-analytical approach.

2. Material and methods

This study utilized previous data from published articles, M.S. Thesis, and Ph.D. Dissertations from the Department of Animal Science at the Federal University of Viçosa, thus not requiring approval from the institution's Animal Ethics Committee.

Data acquisition and experimental procedures

The dataset was collected from 17 studies conducted between 2017 and 2025 in Brazil, totaling 654 observations, comprising 488 cows (multiparous) and 166 heifers (primiparous), with 80 treatments (with an average of 4 treatments per study) in 150 experimental units, carried out according to completely randomized designs (Table 1). All studies were carried out at the Beef Cattle Research, Teaching, and Extension Unit of the Animal Science Department at the Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil.

The experiments involved Nellore cows or heifers grazing *Urochoa decumbens*, managed under continuous grazing, generally from July to December. Animals were divided into different groups (experimental units) according to their experimental treatments and allocated to paddocks provided with covered troughs and waterers. The experimental period covered the transition from the dry to the rainy season, during the prepartum, calving, and postpartum phases, respectively.

The treatments included a control group (receiving only forage and mineral mixture) and one or more supplemented groups during the prepartum period (approximately 68±17 days before calving) and/or postpartum period (approximately 42±23 days after calving). In some studies, supplementation was provided in both periods (Table 1).

In all experiments, the dams were fed daily at 11h00 (Cardenas, 2017; Silva, et al. 2017; Trece, 2017; Marquez, et al. 2019; Moura et al., 2020; Ferreira et al., 2020; Abreu, 2020; Santos, 2021; Ferreira et al., 2021; Oliveira, 2022; Moreno et al., 2023; Melo et al., 2024; Calderado et al., 2024; Cabral et al., 2025) or infrequent (Moura et al., 2020; Motta, 2023; Vital et al., 2024; Borba Pereira, 2025). Supplement was provided in a linear trough at ±0.70 m per cow to ensure uniform intake. Overall, the cows in the control group received a mineral mixture *ad libitum*, while the supplemented group received 2.00 to 4.50 g/kg BW of supplements, with a crude protein (CP) concentrate averaging 302.4±74.7 g/kg in dry matter (Table 2).

For the BW assessment of the cows, all experiments standardized the weighing procedure at 08h00. Pregnant cows were weighed at the beginning of the experiment, i.e., approximately 68±17 days to the predicted calving date, as close as possible to the predicted calving date, at 12h00 post-calving, and 42±23 days postpartum. Fixed-time artificial insemination was performed on all animals during the postpartum period across all experiments.

Blood samples were collected via jugular venipuncture using a vacuum tube with clot activator and gel separation (BD Vacutainer SST II Plus 9 mL, São Paulo, Brazil), and sodium fluoride/EDTA (BD Vacutainer Flourinate/EDTA 5 mL, São Paulo, Brazil) before the predicted calving date (at 08h00), 12 hours post-calving, and at postpartum period (at 08h00), to quantify blood concentrations of glucose, NEFA, and β-OHB. After collection, they were centrifuged at 3,600 x g for 15 minutes (Centribio®, Model 80-2B, 15 mL, radius 10 cm, Mumbai, India). Serum and plasma samples were stored in microtubes at -20 °C for further analysis.

Glucose concentration was determined using an enzymatic glucose oxidase-peroxidase method (Bioclin®, K082). NEFA and β-OHB concentrations were analyzed using the Randox® enzymatic method (RB1008) and the Randox® colorimetric method (FA115β-OHB), respectively. All analyses were conducted using an automatic biochemical analyzer (BS200E, Mindray, China) at the Animal Physiology Laboratory, Department of Animal Science, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil.

To reduce heterogeneity among animals, experimental year, treatments, category (primiparous and multiparous), and time sample, the following equations were expressed:

(1)

$$ADGpre = \frac{\Delta BW}{\Delta DAY}$$

(2)

$$BWc = BWpre + (ADGpre + DTC)$$

(3)

$$ABEM = \frac{\sum_{i-1}^{n} BEMi}{n}$$

where *ADGpre* is the average daily gain in the prepartum (g/day), ΔBW is the variation of the last and the first body weight measurements in the prepartum, ΔDAY is the variation of fist and the last days to weighing in the prepartum, *BWc* is the body weight at calving (kg), *BWpre* is the lats prepartum body weight measurement (kg), *DTC* is the number of days from the last prepartum weighing to the day of calving (day), *ABEM* is the average concentration of blood energy metabolites (glucose, mg/dL; NEFA, mmol/L; β-OHB, mmol/L) in prepartum or postpartum, *BEMi* is the individual blood energy metabolites concentration at each sampling in the period considered, and *n* is the total number of samples in the period considered.

Statistical analysis

Data were analyzed according to a meta-analytical approach (St-Pierre, 2021) via the GLIMMIX Procedure of SAS 9.4. The basic model included the fixed effects of prepartum and postpartum (with or without supplementation) and their interactions. The data were adjusted for the animal category, i.e., multiparous cows or primiparous heifers. Random effects were included in the model to account for between-experiment variability.

The best (co)variance structure was evaluated using the corrected Akaike Information Criterion (AICC) with correction. All variance components were estimated using the restricted maximum likelihood method. Significance was declared at $P < 0.05$.

3. Results

The dataset used to evaluate the nutritional profile of forage and supplementation during the prepartum and postpartum periods is summarized in Table 2. It includes the nutritional composition of *Urochloa decumbens* in August, September, and October, representing the prepartum period during the dry season (forage CP and NDF ranged from 38 g/kg to 99 g/kg and 547 g/kg to 698 g/kg in DM, respectively), and in November and December, representing the postpartum period during the dry-to-rainy transition (forage CP and NDF ranged from 68 g/kg to 135 g/kg and 445 g/kg to 689 g/kg in DM, respectively).

In this context, the nitrogen limitation of forage during the dry season justified the use of maternal protein supplementation (supplement CP ranged from 195 g/kg to 435 g/kg in DM). Maternal supplementation during the prepartum period affected BW at calving ($P < 0.001$), ADG ($P = 0.017$), and blood NEFA concentrations ($P < 0.001$) in Nellore cows (Table 3). A difference of approximately 33 kg in BW at calving was observed between supplemented and non-supplemented beef cows during the prepartum period, resulting in a negative ADG in the non-supplemented group due to limited forage quality during this stage (Table 2).

In the prepartum period, beef cows with supplementation exhibited a 0.10 mmol/L decrease in blood NEFA concentrations ($P > 0.001$), comparing the beef cows without supplementation. Maternal supplementation did not influence ($P > 0.05$) glucose and β-OHB concentrations during the prepartum period and at calving, nor did it affect NEFA concentration on the day of calving.

There was no interaction between prepartum and postpartum supplementation ($P > 0.05$) for BW, blood glucose, NEFA, or β-OHB concentrations (Table 4), indicating that prepartum nutritional strategies did not influence the metabolic energetic profile of cows in the postpartum period. Furthermore, postpartum supplementations exclusively did not affect ($P > 0.05$) any of the studied variables (Table 4).

4. Discussion

Nellore cows raised under tropical grazing systems often experience nutritional deficiencies during late gestation. In these systems, where forage is the primary energy nutrient source, it is possible that its low nutritional quality during most of the prepartum period may limit the fulfillment of nutritional requirements. This nutritional deficiency is commonly managed through protein supplementation during the dry season when tropical forage shows a marked decrease in CP content (Detmann et al., 2014). As such, supplements based on CP, rumen-degradable protein, rumen-undegradable protein, on every rumen-protected functional amino acid are widely used to promote positive ADG in late gestation (Barcelos et al., 2022).

The improvement in maternal performance is attributed to increased nitrogen availability for ruminal microbial growth, which enhances fibrous degradation in low-quality forage. This process increases the production of volatile fatty acids and microbial protein, thereby improving energy extraction from fibrous carbohydrates during the dry season (Detmann et al., 2009, 2014, 2024), which coincides with the final trimester of gestation in beef cows in tropical regions.

Despite this knowledge, the lack of strategic supplementation during late gestation is still frequently observed in practice. This results in reduced BW and BCS in calving (Moreno et al., 2023), impairing the milk (Calderaro et al., 2024), reproductive performance (Bohnert et al., 2013), and ultimately shortening the productive lifespan of grazing beef cows. In our study, under controlled conditions, non-supplemented cows exhibited negative ADG and reduced BW at calving, reflecting the limited use of forage energy along with adaptive mechanisms of maternal energy metabolism during the prepartum period. This response reflects the limited use of forage energy, reducing VFA and MP synthesis (Detmann et al., 2009), as well as maternal physiology in late gestation to maintain homeorhesis (Bauman and Currie, 1998). These nutritional and physiological effects directly compromise the net energy and protein requirements for maternal maintenance and productivity (Gionbelli et al., 2023).

To maintain homeorhesis of basal metabolism, maternal metabolism in late gestation activates alternative energy-supplying pathways. This response increases blood concentrations of NEFA, thereby balancing maternal energy demands. Consequently, nutrient availability below nutritional requirements results in decreased BW, BCS, and reproductive cyclic throughout the year (NASEM, 2016). A positive relationship between

BW and BCS in beef cows has been proposed, with 46 kg corresponding to one BCS unit on a 1-to-9 scale (CSIRO, 2007), relative to mature body weight.

When associated with β-oxidation and free fat acid or ketone body synthesis (Hall, 1961; Emery et al., 1992; Mulliniks et al., 2013), cows without supplements in the prepartum period reduced BW and increased adipose tissue mobilization, as nutrient intake and digestion are limited. Similar to dairy cows, nutritional restriction during late gestation increases fatty acid oxidation through lipolysis, driven by negative energy balance in the peripartum period (Nguyeb et al., 2008). This may lead to elevated blood concentrations of acetate, β-OHB, and NEFA, known to suppress LH pulse frequency and amplitude (DiCostanzo et al., 1999).

If prepartum lipolysis persists postpartum, primarily due to limited nutrient intake, the hepatic synthesis of insulin growth factor-1 (IGF-1) and growth hormone (GH), as well as glucagon activity (Konigsson et al., 2007; Lüttgenau et al., 2016; Baddela et al., 2020), further compromises reproductive metabolic status (Pushpakumara et al., 2003). Thus, beef cows with low BW and reduced fat reserves tend to exhibit impaired hypothalamic-pituitary-ovarian axis activity, which negatively affects estrous cycles and fertility in the postpartum period (Yavas and Walton, 1999; D'Occhio et al., 2019; Barcelos et al., 2022). Conversely, strategic prepartum supplementations improve BW, BCS, colostrum, and milk yield, and support endocrine control of postpartum reproduction, especially in primiparous heifers (D'Occhio et al., 2019).

Therefore, it is well established that nutritional restriction during the final trimester of gestation compromises conception rates in beef cows (Mulliniks et al., 2013), whereas cows with positive ADG and moderate BCS are more likely to conceive successfully (Morrison et al., 1999; NASEM, 2016). Overall, protein supplementation during late gestation is recommended for Nellore cows and heifers raised under tropical grazing systems to enhance energy reserve availability and support reproductive function in the postpartum period.

In the postpartum period, there is a notable lack of studies investigating protein-energy supplementation strategies for beef cows and heifers (Table 2). Our findings indicate that BW and energy dynamics are generally not significantly influenced by supplementation (Table 4). This may also reflect the physiological stage, in which maternal nutritional requirements peak due to increased energy demand from milk production.

However, in tropical beef grazing systems, the high-quality forage available during the rainy season supports a positive energy balance in animal metabolism and satisfactory performance. Under these conditions, additional supplementation is often unnecessary, which reduces feed costs (Santos et al., 2019).

Previous studies demonstrated that protein-energy supplementation provided during both prepartum and postpartum (Soleto et al., 2018; Moura et al., 2020; Melo et al., 2024) or exclusively during the postpartum (Moreno et al., 2024) does not compromise maternal BW or energy metabolism. This effect is attributed to the metabolic modulation induced by the nutrients present in high-quality forage-based diets, which support maternal performance.

Furthermore, the reproductive season in tropical systems typically coincides with the dry-to-rainy transition or occurs during the rainy season itself, i.e., spring and summer, when forage availability and quality are highest (Saraiva et al., 2024; Baruselli et al., 2025). In such cases, even postpartum-only supplementation has been shown to improve milk yield and reproductive indices (Adjorlolo et al., 2019; Almeida et al., 2020). These benefits are attributed to the stimulation of the hypothalamic-pituitary-ovarian axis and the resumption of natural estrus cycles in beef cows (Dunn and Kaltenbach, 1980), resulting from improved nutritional status. Consequently, this mechanism directly enhances reproductive performance and, therefore, the efficiency of beef calf production systems.

During the dry-to-rainy transition, tropical forages exhibit superior nutritional quality compared to those found during the dry season, particularly in CP and organic matter digestibility (Figueiras et al., 2015). However, the increased nitrogen content and improved fiber digestibility may create an energy imbalance, reducing the efficiency of metabolizable energy use (Detmann et al., 2014b), and reinforcing the limited need for additional protein supplementations in postpartum Nellore cows.

Thus, the maternal BW and blood concentrations of glucose, NEFA, and β-OHB observed during postpartum beef cows suggest that supplementation at this stage does not negatively affect metabolic dynamics, nor the nutritional demands from the onset of lactations to reproductive resumption. These findings suggest that the metabolic status of beef cows becomes favorable during the postpartum period, likely driven by improved utilization of nutrient from tropical forage of high-quality during the dry-to-rainy or rainy transition period, due to the associative action of prepartum supplementation and multiple factors (e.g., voluntary fed intake, metabolic hormone, and homeorhetic adaptation for the peak lactation period) related to maternal metabolism at the onset of lactation.

5. Conclusion

Protein supplementation in Nellore cows fed tropical forage reduces circulating blood NEFA concentrations during late gestation, thereby supporting maternal performance at calving. Nevertheless, this nutritional intervention does not modify overall energy metabolism or postpartum performance. Rather, it reflects a maternal biological predisposition to sustain performance during late gestation and early lactation.

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Cláudia B. Sampaio: Writing – review & editing, Conceptualization, Supervision, Project administration.

Conflict of interest

The authors declared that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this paper.

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Tables

Table 1.

Summary of the Nellore cow category and pre-postpartum treatments in the different studies

Study	References [a]	Animals [b]			Treatments	
		Category	*n*	N	Prepartum	Postpartum
1	Cardenas (2017)*	Multiparous	8	40	2	4
2	Silva et al. (2017)	Multiparous	4	35	4	-
3	Trece (2017)*	Multiparous	4	44	2	4
4	Marquez et al. (2019)	Multiparous	3	28	3	3
5	Moura et al. (2020)	Primiparous	6	23	3	3
6	Ferreira et al. (2020)	Multiparous	8	38	2	-
7	Abreu (2020)*	Multiparous	8	42	2	2
8	Ferreira et al. (2021)	Multiparous + Primiparous	6	38	3	3
9	Santos (2021)*	Multiparous	4	37	2	-
10	Oliveira (2022)**	Multiparous + Primiparous	4	40	-	4
11	Moreno et al. (2023)	Multiparous	4	48	2	4
12	Motta (2023)*	Primiparous	8	35	2	2
13	Calderaro et al. (2024)	Multiparous	8	44	4	-
14	Melo et al. (2024)	Primiparous	10	35	5	5
15	Vital et al. (2024)	Primiparous	7	38	2	2
16	Cabral et al. (2025)	Multiparous	8	39	4	-
17	Borba Pereira (2025)**	Multiparous	8	50	-	2

[a] References: * M.Sc. Dissertation; ** Ph.D. Thesis.

[b] Animals: *n*, experimental units; N, number of animals per study.

Table 2.

Descriptive statistics of the nutritional profile of *Urocloa decumbens* and supplements used in the prepartum and postpartum period

Item [a]	Descriptive statistic [b]				
	Mean	*Minimum*	*Maximum*	*s*	N
Prepartum					
Forage CP, g/kg DM	63.0	38.6	99.5	18.0	17
Forage NDF, g/kg DM	639	547	698	47.6	17
Forage iNDF, g/kg DM	301	249	349	33.1	17
Forage mass, t DM/ha	3.51	1.31	4.94	1.13	15
Supplement, g/kg BW	3.00	2.00	4.50	0.80	17
Supplement CP, g/kg DM	309	195	435	76.1	17
Postpartum					
Forage CP, g/kg	85.8	42.9	135.6	20.1	15
Forage NDF, g/kg	593	507	689	62.5	15
Forage iNDF, g/kg	207	154	265	32.0	15
Forage mass, t DM/ha	3.86	1.85	8.94	1.91	11
Supplement, g/kg BW	2.45	2.00	3.25	0.60	5
Supplement CP, g/kg DM	319	209	435	90.1	5

[a] CP, crude protein; DM, dry matter; NDF, neutral detergent fiber; iNDF, indigestible neutral detergent fiber; BW, body weight.

[b] Descriptive statistic: *s*, standard deviation; N, number of studies.

Table 3.

Descriptive level of probability (P-value) for the fixed effect of maternal supplementation in prepartum on the performance and energetic blood profile of Nellore cows in the prepartum and calving period

Item [a]	Supplementation		P–value
	Without	With	
cBW, kg	487±6.1	520±10.1	<0.001
ADG, g	-74±85.9	258±136.9	0.017
preGLUC, mg/dL	60.2±1.02	60.2±1.61	0.96
cGLUC, mg/dL	79.6±2.70	80.9±2.97	0.72
preNEFA, mmol/L	0.46±0.02	0.36±0.02	<0.001
cNEFA, mmol/L	0.41±0.01	0.42±0.03	0.45
preβ-HOB, mmol/L	0.49±0.02	0.46±0.02	0.36
cβ-HOB, mmol/L	0.41±0.01	0.42±0.03	0.78

[a] cBW, calving body weight; ADG, average daily gain; preGLUC, prepartum glucose blood concentration; cGLUC, calving glucose blood concentration; preNEFA, prepartum non-esterification fatty acid blood concentration; cNEFA, calving non-esterification fatty acid blood concentration.; preβ-OHB, prepartum β-hydroxide-butyrate blood concentration; cβ-OHB, calving β-hydroxide-butyrate blood concentration at calving.

Table 4.

Descriptive levels of probability (P-value) for the fixed effects of the maternal supplementation in prepartum (PRE), postpartum (POS), and the interaction (PRE x POS) on performance and metabolism of Nellore cows in the postpartum period

Item [a]	Supplementation				P-value		
	PRE (-)		PRE (+)				
	POS (-)	POS (+)	POS (-)	POS (+)	PRE	POS	PRE x POS
BW, kg	448±6.80	459±10.7	456±6.67	457±8.8	0.65	0.52	0.50
GLUC, mg/dL	61.85±1.25	60.77±2.38	59.49±1.70	59.29±2.30	0.31	0.75	0.78
NEFA, mmol/L	0.28±0.03	0.33±0.06	0.32±0.05	0.35±0.06	0.51	0.50	0.52
β-OHB, mmol/L	0.42±0.01	0.40±0.03	0.44±0.03	0.39±0.03	0.80	0.24	0.79

[a] BW, body weight; GLUC, glucose; NEFA, non-esterification fatty acid; β-OHB, β-hydroxide-butyrate.

CHAPTER 3

Exploring Vitamin A Supplementation in Beef Cattle: Implications for cow-calf systems

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Abstract

Vitamin A is listed in the fat-soluble vitamins group, whose absorption, transport, and storage are similar to those of lipids. However, the vitamin A functions go beyond those reported in the ocular cells' metabolism, being also described in reproductive functions, growth, and specific tissue maintenance through gene regulation. In ruminants, vitamin A supplementation can be done in different ways. Orally with carotenoids, while β-carotene is the main representative, and intramuscularly by injectable applications of synthetic vitamin A. In cows, it is common to perform oral supplementation with β-carotene in the middle and/or final third of gestation to increase plasma retinol concentrations, benefiting the fetus, colostrum synthesis, and reproductive indices. In newborn calves, vitamin A administered intramuscularly might favor cell differentiation for lipogenesis, an increase in muscle tissue, indicating a higher intramuscular fat degree, and resulting in a future improvement in meat quality. Thus, this review aimed to address the vitamin A supplementation dynamic on the effect on gestational/maternal and neonatal metabolism in beef cattle.

Keywords: antioxidant, immunoglobulin, intramuscular fat, pregnancy, retinol

1. Introduction

The micronutrient requirements related to minerals and vitamins must be considered in ruminant nutrition. Given that these nutrients have essential functions in animal organisms acting as cofactors and enzymes, they are related to improving animal metabolism, and subsequently increasing growth and performance (Harris et al., 2018). Regarding fat-soluble vitamins, vitamin A supplementation in the diet can be done by providing plant-originated provitamin A molecules, or by intramuscular injections of molecules artificially synthesized (Maciel et al., 2022).

Until now, cases of vitamin A deficiency in beef cattle produced in pasture systems are rare, once tropical pastures contain adequate β-carotene concentrations (Factor et al., 2024), and its storage in liver tissue ensures long-term use (D'Ambrosio et al., 2011). A reduction in *β-carotene* concentrations can be observed in pastures during the dry periods of the year, reinforcing the importance of animal supplementation with vitamin A, or its precursors (Reynoso et al., 2004).

Vitamin A recommendations for beef cattle are reported considering the animal physiology and category. According to the NASEM (2016) system for beef cattle, vitamin A requirements can be expressed per international unit in kilograms of dry matter per day (IU/kg DM) or Retinol Equivalents.

Ruminal degradation of vitamin A precursors can affect its bioavailability for animal metabolism. In general, microbial vitamin requirements are limited to water-soluble vitamins, e.g., those of the B complex and vitamin C, to stimulate their cell growth and are associated with biochemical reactions as enzymatic cofactors in microbial metabolism (Nagaraja et al., 1997).

However, in vitro studies conducted by Hino et al. (1993) demonstrated that supplementation with β-carotene and α-tocopherol, whether administered individually or in combination, stimulates the growth of ruminal microorganisms and enhances the digestion of the fibrous fraction of the diet. Therefore, the vitamin A metabolism mechanisms in the rumen are still unclear (Zeoula and Geron, 2006).

Vitamin A supplementation is valid when there is an increase in animals' requirements which depends on the animal's physiological phase, or when the food quality sources that provide carotenes are poor. Supplementation should be provided for pregnant beef cows, in which the need for macro and micronutrients increases in the peripartum period (Jo et al., 2020).

In this case, β-carotene supplementation is indicated and has a positive effect on oxidative stress (Kamiloglu et al., 2005), fetal growth (Jo et al., 2020; Marceau et al., 2007), colostrum synthesis (Nishijima et al., 2017; Ishida et al., 2018), ovulation (Hidalgo et al., 2005; Ay et al., 2012a) and embryonic adherence (Agarwal et al., 2006; Gouvêa et al., 2018). In addition, the vitamin A supplementation in beef cows at late gestation enhances intramuscular adipogenesis in offspring (Dean et al., 2024).

Furthermore, when dealing with the supplementation effect of injectable synthetic vitamin A in newborn calves, it is common to observe a gene action in cell regulation and differentiation through hyperplasia and hypertrophy in intramuscular fat (Harris et al., 2018; Campos et al., 2020; Peng et al., 2020; Maciel et al., 2022; Scapol et al., 2023). These are related to ensuring higher weaning weight (Jo et al., 2020; Peng et al., 2020), and intramuscular fat deposition (Peng, et al., 2020; Maciel, et al., 2022; Scapol, et al., 2023) via lipogenesis, providing a higher meat marbling degree during the slaughter period in beef cattle (Daniel et al., 2009; Wellman et al., 2020; Scapol et al., 2023). Thus, this literature review aimed to analyze the effects of vitamin A supplementation on gestational, maternal, and neonatal metabolism in beef cattle.

2. Vitamin A characterization

Micronutrients, although required in smaller quantities compared to macronutrients, play a crucial role in maintaining health and the proper functioning of the body (Andrès, et al., 2024). Among micronutrients**,** vitamins stand out for their variations and specific functions, which are grouped into two main categories: water-soluble vitamins, including ascorbic acid (vitamin C) and the B-complex vitamins, and fat-soluble vitamins, which include retinol (vitamin A), as well as cholecalciferol (vitamin D), tocopherol/tocotrienol (vitamin E), and phylloquinone/menaquinone (vitamin K) (Andrès, et al., 2024).

Vitamin A exhibits a specific affinity for hepatic tissue, where it is primarily stored, although smaller amounts can also be found in the adipose tissue of animals (Peng et al., 2021). In the body, it may bind to plasma transport proteins. Furthermore, vitamin A is present in plant pigments in the form of carotenoids, such as β-carotene, which are generally considered to be abundantly available. Despite structural differences, these carotenoids share an unsaturated isoprenoid chain and perform similar physiological functions in the organism (Carazo et al., 2021).

In grasses and legumes, the reflected light spectrum that results in the green color of the plants indicates proper levels of provitamin A, particularly represented by β-carotene. However, there is a significant decrease in the biodisponibility of β-carotene as the forage matures or undergoes drying or preservation processes (Elgersma et al., 2013; NASEM, 2016; NASEM, 2021). In such conditions, vitamin A supplementation becomes necessary to ensure animals receive an adequate amount of this nutrient.

Vitamin A is biologically active in the forms of retinol, retinal, or retinoic acid, which contain an alcohol group, an aldehyde group, and a carboxylic acid group, respectively (Figure 1) (Polcz and Barbul, 2019). Understanding the different bioforms of vitamin A and its metabolism is crucial for comprehending its role in cellular growth and tissue differentiation in animals.

Although vitamin A is not directly involved in coenzyme-catalyzed reactions, it plays hormonal and regulatory functions in gene expression (Balmer and Blomhoff, 2002). However, both its deficiency and excess can compromise animal metabolism (González and Silva, 2019).

2.1.Bioavailability and functions

The bioavailability of micronutrients refers to the fraction of the ingested nutrient that effectively contributes to meeting the physiological demand of the target tissue (Jackson, 1997). In the case of vitamin A, this availability is essential for the performance of vital functions, such as the regulation of reproductive function, growth, tissue maintenance (Ambrósio et al., 2006), and gene regulation (Balmer and Blomhoff, 2002).

The degradation of vitamin A begins in the rumen, where retinol esters are cleaved by microorganisms, releasing free retinol (Rode et al., 1990), which can be converted into retinal and retinoic acid (Srinivasan and Buys, 2019). In the small intestine, vitamin A esters are hydrolyzed by pancreatic lipases, releasing retinol, which is absorbed and re-esterified in enterocytes, being incorporated into chylomicrons. These chylomicrons are transported to the liver, where vitamin A is esterified and stored in hepatic stellate cells (Grumet et al., 2016). Retinol-binding protein (RBP) carries retinol in circulation, being absorbed by cells expressing Stimulated by Retinoic Acid-6 (STRA6) (Kelly and von Lintig, 2015).

Intracellularly, vitamin A is converted into its active form, retinoic acid, which binds to nuclear receptors such as retinoic acid receptors (RAR) and retinoid X receptors (RXR), regulating the transcription of genes involved in cell differentiation and metabolism (Huang et al., 2014). One of its primary biological roles is to promote cellular differentiation, a process essential for the development and specialization of tissues and organs from embryogenesis to adulthood (Barber et al., 2014). In early life stages, particularly during fetal development, the suckling phase, retinoic acid influences the commitment of mesenchymal stem cells toward the adipogenic lineage, favoring the development of intramuscular adipocytes (Harris et al., 2018). Adequate vitamin A status during these phases, either via maternal colostrum, milk, or forage, is thus critical for proper adipose tissue programming.

Interestingly, the role of vitamin A appears to shift in later stages of production. During the finishing phase, for instance, vitamin A restriction has been associated with increased intramuscular fat deposition or marbling (Kruk et al., 2018). This paradoxical effect may stem from the reduced inhibitory action of retinoic acid on adipocyte differentiation at this stage. Mechanistically, retinoic acid acts as a ligand for RAR, forming heterodimers with RXR (Chawla et al., 2001; de Thé et al., 1990), which bind to retinoic acid response elements in the genome, modulating the expression of key adipogenic and lipogenic genes such as PPARγ (Lefterova et al., 2009). Moreover, there is evidence that retinoic acid influences skeletal muscle metabolism by enhancing fatty acid oxidation, potentially limiting lipid accumulation in muscle tissue (Amengual et al., 2018). Collectively, these findings highlight the stage-specific effects of vitamin A on adipogenesis and energy metabolism, emphasizing the need for targeted nutritional strategies across different phases of cattle development.

2.2.Deficiency and toxicity

In pasture-based production systems, particularly under tropical conditions, clinical cases of vitamin A deficiency or toxicity in beef cattle are uncommon. This is largely attributed to the high availability of β-carotene in fresh green forages, which is efficiently converted into retinol and stored in the liver, ensuring sufficient reserves to maintain vitamin A homeostasis over extended periods (Pickworth et al., 2012).

Fresh tropical forages have been reported to contain vitamin A equivalents ranging from 39,000 to 59,600 IU per kilogram of dry matter (DM), while conserved forages, such as good-quality hay, typically contain much lower concentrations, between 1,400 and 3,600 IU/kg DM. These values are highly susceptible to oxidative degradation during storage, particularly under inadequate drying and preservation conditions (Pickworth et al., 2012). Vitamin A requirements vary according to animal category and physiological status.

According to NASEM (2016) and Valadares Filho et al. (2023), beef feedlot cattle require approximately 2,200 IU/kg of dry feed, pregnant heifers around 2,800 IU/kg, and lactating cows or breeding bulls up to 3,900 IU/kg. Under well-managed grazing conditions, cattle typically meet these requirements through voluntary intake of β-carotene from fresh forages. In contrast, animals consuming primarily conserved or low-quality forages are at increased risk of deficiency and may require dietary supplementation to ensure adequate intake and hepatic storage of vitamin A.

If hepatic retinol stores decline, clinical signs of vitamin A deficiency may emerge (NASEM, 2016). According to Speer et al., 2022, the deficiency is characterized by low plasma retinol concentrations (< 300 ng/mL) and deficient liver retinol reserves (< 300 µg/g DM). Clinically, affected cattle exhibit stunted growth, especially in bone development; impaired immunity, marked by reduced phagocytic activity in macrophages and neutrophils, leading to increased susceptibility to infections and, in severe cases, mortality; and reproductive disorders. In females, common manifestations include prolonged estrous cycles, anovulation, reduced conception rates, abortion, and retained placenta. In males, testicular atrophy, degeneration of accessory sex glands, poor sperm quality, and disrupted spermatogenesis have been reported (Zeoula and Geron, 2006).

Regarding hypervitaminosis/toxicity from vitamin A, toxicity in cattle has not been reported under grazing conditions. This may be explained by the ruminal fermentative metabolism and the degradation of carotenoids by the microbiota, which prevents excessive absorption of vitamin A by the intestinal epithelium, ensuring adequate plasma levels for the animal's metabolic homeostasis (NASEM, 2016; NASEM, 2021; Valadares Filho, 2023).

3. Vitamin A metabolism

Such any other nutrient, vitamin A performs specific functions in cellular metabolism. As a result, there is a mechanism for the activation of this biomolecule at both the cytoplasmic and nuclear levels, optimizing the absorption, storage, and transport processes and its use in target tissues (Polcz and Barbul, 2019).

The functionality of this regulatory system depends on the lipoproteins association, and proteins and molecular complexes transport, which provide the dynamic for the activation and storage of this molecule for cellular metabolism (Polcz and Barbul, 2019).

3.1.Absorption, storage, and transport

The absorption of vitamin A is directly related to the intake of its precursors, known as provitamin A, represented by carotenoids (D’Ambrosio et al., 2011). The main carotenoids, such as α-carotene, β-carotene, and β-cryptoxanthin (Conaway et al., 2013), are found in the foods provided to animals, meeting the nutritional requirements of this vitamin. After ingestion, these compounds are emulsified in the intestine, forming a micellar aggregate that includes fatty acids, monoacylglycerol, phospholipids, cholesterol, bile salts, and free retinol (Blaner et al., 2016).

This emulsification process prepares the carotenoids for hydrolysis, which is carried out by enzymes such as pancreatic lipases. Hydrolysis releases free retinol, which is absorbed by intestinal cells (enterocytes) through facilitated diffusion (Soprano et al., 1994; Bennekum et al., 2000; Reboul et al., 2006).

Since retinol is insoluble in water, it must bind to transport proteins, such as retinol-binding protein I (RBP I) and retinol-binding protein II (RBP II), which facilitate the transport of retinol through intestinal cells (Ong and Chytil, 1978; Conaway, 2013; Blaner et al., 2020). Retinol derived from β-carotene can be converted into all-trans retinoic acid (ATRA), also known as tretinoin, or it can remain in the form of retinol. This conversion occurs not only in enterocytes but also in hepatocytes and other cells, where ATRA can be converted back into retinol and released into the bloodstream for use by the body (Carazo et al., 2021).

Retinol-binding proteins are involved in the formation of chylomicrons through the reesterification of compounds absorbed during the digestion of long-chain fatty acids, a process mediated by the enzyme retinyl acyltransferase (Harrison, 2012; Polcz and Barbul, 2019). This results in the formation of a complex that includes retinal esters, carotenoids, and provitamin A, which are esterified with fatty acids, incorporated into chylomicrons, and transported via the lymphatic system for later storage in the liver (Polcz and Barbul, 2019).

In this process, as the absorption and transport of retinol depend on the presence of fat, approximately 50% of the consumed provitamin A is absorbed intact by the intestinal mucosa and directed to the portal system, while the other 50% undergo oxidation to be converted into retinol (Conaway et al., 2013). Thus, fat intake is essential to ensure the effective absorption of vitamin A, a fat-soluble compound (Blomhoff et al., 1990). Additionally, some carotenoids, such as β-carotene, are absorbed by enterocytes with the help of membrane proteins, such as the scavenger receptor class B1 (SCARB1) and cluster of differentiation 36 (CD36) (Blomhoff et al., 1990).

After intestinal absorption, vitamin A is incorporated into chylomicrons and transported via the lymphatic system to the liver. In hepatocytes, chylomicron remnants are internalized through receptor-mediated endocytosis, a process involving apolipoprotein E and lipoprotein lipases, which hydrolyze retinyl esters to release free retinol (Harrison, 2012; Cooper, 1997). Once in the liver, retinol may be re-esterified by hepatic cells, primarily as retinyl palmitate, and stored for future use in the absence of immediate metabolic demand (Conaway et al., 2013).

It is estimated that approximately 90% of the body’s vitamin A is stored in the liver, with around 40% utilized in metabolic processes and the remainder retained in hepatic reserves. When needed, retinol is mobilized by the action of retinyl ester hydrolase and released into circulation bound to plasma retinol-binding protein (RBP-II) or as complexes with cytoplasmic RBP. These complexes are then transported to storage sites, such as hepatic and adipose tissues (Ambrósio et al., 2006).

The transport of vitamin A in plasma occurs through the esterification of retinyl esters in liver cells, which can be converted into retinol. Retinol then binds to RBP, forming the retinol-RBP complex, which is transported through the bloodstream with the help of membrane transporters (Soprano and Blaner, 1994). In plasma, the retinol-RBP complex requires a carrier protein, which may be transthyretin (TTR), prealbumin, or albumin, to ensure proper transport (Carazo et al., 2021). In addition to transporting the retinol–RBP complex, TTR also carries the thyroid hormones (T3 and T4). Thus, the complex formed by retinyl esters and/or retinol-RBP with TTR helps prevent the excretion of vitamin A through renal filtration (Carazo et al., 2021), protecting circulating vitamin A precursors from depletion or oxidation until they are utilized by specific tissues (Figure 1).

3.2.Molecular Mechanisms of Retinoid Signaling in Target Tissues

As previously mentioned, the absorption and transport of vitamin A precursors intended for storage depend on specific proteins that convert retinoic acid into retinol, which is subsequently retained in the liver. A portion of this retinol is then mobilized as retinyl esters and converted back into retinoic acid. Thus, when specific tissues require it for metabolism, target cells activate mechanisms to capture these precursors in the bloodstream through membrane receptors. This process directs the precursors to the cell nucleus, where gene expression occurs to benefit cellular metabolism (Figure 2).

When the ATRA complex is available with RBP, or by plasmatic albumin in low concentrations, peripheral cells absorb this compound (Conoway et al., 2013) through the transmembrane receptor Strat6, encoded by retinoic acid 6 stimuli (Kawaguchi et al., 2007; Conoway et al., 2013; Carazo et al., 2021).

In this way, the target tissues' cellular metabolism for vitamin A precursors, especially retinol, depends on binding proteins and receptors for their signaling pathways, beginning with the retinol oxidation into *all-trans* retinal acid by cytosolic alcohol dehydrogenase (ADH), being complexed with the cellular retinol receptor protein (CRBP), passing through the oxidative action of the enzyme retinal dehydrogenase (RALDH), providing the active vitamin A metabolite, ATRA, being bound to a cellular retinoic acid receptor protein (CRABP; Conoway, 2013, Carazo et al., 2021) at the cytosol level.

Despite the cell nucleus, vitamin A and its precursors are an important factor for gene expression (Balmer and Blomhoff, 2002; Bohn, 2017) in which nuclear receptors interact with transcription, modulating the target gene expression (Carazo et al., 2021). Furthermore, nuclear hormone receptors are responsible for enabling the transport of vitamin A precursors + binding proteins present in the cytosol, namely retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Therefore, RAR receptors form heterodimers with RXR receptors and RXR receptors (*e.g.*, isolated in homodimer form) have transcription factor functions, leaving retinoic acid response elements (RAREs) active in target genes (Bastien and Rochett-Egly, 2004; Conoway et al., 2013; Carazo et al., 2021).

Retinoic acid exerts its biological effects primarily through its interaction with nuclear receptors, particularly the retinoic acid receptors (RARs), which include the isoforms RARα, RARβ, and RARγ (Giguere et al., 1987). Among these, RARα is the most widely expressed isoform in the body, whereas RARβ is predominantly localized in the liver, kidneys, and nervous tissue. The interaction between vitamin A and gene expression is mediated by all-trans retinoic acid (ATRA), which binds to RARs and activates signaling pathways involved in key physiological processes such as cell differentiation and tissue development (Bastien and Rochette-Egly, 2004).

Once vitamin A precursors are mobilized from hepatic stores into the circulation, complexed with specific binding proteins, they become available to intracellular receptors. RARs preferentially bind to ATRA, while retinoid X receptors (RXRs) exhibit higher affinity for the 9-cis-retinoic acid isomer (Germain et al., 2002).

The functional divergence between RAR and RXR is largely attributed to differences in their ligand-binding domains (LBDs), which define their ligand specificity and determine the nature of the receptor-ligand complex that translocates from the cytosol to the nucleus, ultimately regulating the transcription of target genes (Carazo et al., 2021).

At the molecular level, the inactivated RAR form occurs when there is no binding to retinoids, remaining deprived in the cell nucleus, composing the co-repressor complex (Carazo et al., 2021). In this way, there is a structural molecule rearrangement with the specific ligand for the co-activator RAR, generating the co-repressor activation (Chebaro et al., 2017).

The transcription machinery of cellular genetic material must be accomplished by the RAR heterodimerization with RXR to carry out transcription, providing the co-activator ligand heterodimer complex for DNA sequences recognition, binding to the promoter region of target genes (*e.g.*, retinoic acid responsive elements, RARE) which affect the genes transcriptional regulation (Carazo et al. 2021).

The RARs present in the cell nucleus also have a binding action to the CRABPII (0) complexes, transporting ATRA to the nucleus (Conaway et al., 2013), which is another active form of vitamin A precursor transport for gene regulation. Another nuclear receptor with a hormonal nature forms a heterodimer with RXR and is called peroxisome proliferative activated receptors (PPARs) with isomers α, β, γ, and δ.

The PPAR synthesis might be affected by the cellular retinal concentrations in adipose tissue with antagonistic PPAR activity effect, inhibiting adipogenesis and increasing the cell insulin sensitivity (Ziouzenkova et al., 2007). This RXR-PPAR complex, called a heterodimer, for example, has transcription factor functions activating specific gene expression responses (Conaway et al., 2013; Carazo, 2021).

Additionally, to the transporters aforementioned, retinoid transporters might complex with orphan transporters (RORs), which do not form dimers and might regulate gene expression in monomers by binding to RARE (Conoway et al., 2013; Carazo, 2021), with α, β, and γ isoforms.

Thus, with the retinol oxidation reducing *ATRA*, genomic function modifications are regulated by the action of nuclear transporters/receptors related to vitamin A precursors (PPGRs, RARs, RXRs, and RORs) for gene expression or target genes regulation. Considering this, the transcription may not occur when no ligands are available to be associated with the transport complexes present in the nucleus through cellular retinoic acid-binding proteins (*CRABP*) or fatty acid-binding proteins (*FABP*); otherwise, transcription is not directed (Carazo et al., 2021).

4. Vitamin A utilization

The vitamin A requirement for beef cattle is expressed in international units (IU) or retinol equivalents (RE) relative to body weight. For ruminants, β-carotene is the recommended precursor of vitamin A to be used in diets and supplements. According to NASEM (2016), the vitamin A requirement varies in accordance to beef cattle category and physiological state, in which at 2,200 IU/kg dry feed for beef feedlot cattle, 2,800 IU/kg dry feed for pregnant beef heifers and 3,900 IU/kg dry feed for lactating cows and breeding bulls.

Regarding the vitamin A requirements for pregnant beef females (e.g., heifers and cows) and pre-weaned calves, there are limitations in data validation for these categories (NASEM, 2016). This makes it essential to understand the role of vitamin A supplementation for performance and metabolism during these specific physiological stages.

4.1.Supplementation forms

The dietary supply of vitamin A in ruminants is influenced by both the type of diet and the rate of ruminal fermentation. Various carotenoid isoforms, such as β-carotene, α-carotene, γ-carotene, and cryptoxanthin, are present in forages and grains and serve as vitamin A precursors (NASEM, 2016).

However, the availability of these compounds can fluctuate significantly depending on forage quality, particularly in pasture-based systems. During dry seasons, when forage carotenoid content is reduced, supplementation with provitamin A sources is often required to meet nutritional demands (Pickworth et al., 2012; NASEM, 2016).

Although the precise requirements of vitamin A for pregnant ruminants and growing calves have not yet been fully defined, cases of hypervitaminosis A are rare. This low incidence is mainly due to the extensive microbial degradation of vitamin A and its precursors in the rumen, which limits their intestinal absorption and systemic accumulation (Casals and Calsamiglia, 2012; NASEM, 2016; Valadares Filho, 2023).

In practice, dietary vitamin A supplementation is commonly achieved through the inclusion of β-carotene-rich feedstuffs, vitamin–mineral premixes, or, in some cases, parenteral administration. Injectable vitamin A is typically used in strategic phases such as early life, weaning, or gestation, particularly in situations where dietary intake may be insufficient (Harris et al., 2018; Campos et al., 2020; Peng et al., 2020; Wellmann et al., 2020; Song et al., 2023; Scapol et al., 2023; Dean et al., 2024).

While both supplementation routes are effective in increasing vitamin A status, injectable forms tend to yield more immediate and consistent increases in plasma and hepatic retinol concentrations (Knight and Death 1999), particularly in neonates and periparturient cows, where gastrointestinal absorption may be limited or dietary intake insufficient.

Conversely, long-term maintenance of adequate vitamin A status is more efficiently sustained through dietary supplementation, which better supports gradual accumulation and storage in hepatic tissue (Knight and Death 1999). Therefore, the choice of supplementation method should consider the animal's physiological stage, nutritional status, and production system.

4.2.Effects on pregnant beef cows

Metabolic alterations associated with physiological transitions in the pre- and postpartum periods are well-documented in mammals (Redmer et al., 2004). This transition is often characterized by a negative energy balance due to the sudden increase in energy demands for fetal development, parturition, and the onset of lactation. Such an imbalance leads to the mobilization of body reserves, which consequently increases oxidative stress in peripheral tissues as a result of elevated reactive oxygen species (ROS) production at the cellular level (Sordillo and Aitken, 2009). The accumulation of free radicals can disrupt systemic homeostasis (Castillo et al., 2005; Sordillo, 2005), diverting energy toward milk production for the calf and thereby reducing antibody synthesis and reproductive hormonal activity, ultimately compromising maternal health (Tufarelli et al., 2023).

Evaluations of the vitamin A supplementation effects in beef cows at the end of gestation are scarce (Jo et al., 2020; Dean et al., 2024), but hypotheses might be generated by adopting analogies to other ruminant production systems, such as dairy cows or lambs. Jo et al. (2020) identified the vitamin A oral supply interaction for pregnant beef heifers at the end of gestation. It was found that maternal supplementation in the period from 225 days of gestation until the day of calving, with 78,000 IU/day of vitamin A in the total diet leads to a decrease in vitamin A serum levels after calving due to its use for fetal growth, and the female nutritional needs increase during the lactation period. These findings indicate that the supplementation level used should be higher than those suggested by the nutritional requirements of beef cattle nowadays.

It is well established that placental morphology plays a crucial role in the maternal–fetal transfer of nutrients (Fowden et al., 2009). Ruminants, including cattle, possess an epitheliochorial placenta, which consists of multiple cellular layers separating maternal and fetal blood supplies, and therefore significantly limits the passive transfer of vitamin A from the dam to the fetus (Donoghue et al., 1985). Despite this anatomical limitation, vitamin A and its derivatives (retinoids) are critical for embryonic development and fetal tissue differentiation, particularly during late gestation, when adipogenesis is actively occurring.

Recent studies have shown that vitamin A supplementation during late gestation enhances intramuscular adipogenesis in the offspring. Dean et al. (2024) reported increased mRNA expression of retinoic acid receptor β (RARβ) and greater protein abundance of adipogenic markers (DLK1 and PPARγ) in the offspring of beef cows supplemented with vitamin A, which was associated with increased intramuscular fat deposition throughout postnatal development. These findings suggest that maternal retinoid status during late gestation can modulate fetal adipocyte differentiation via retinoid signaling pathways, despite the restrictive placental environment.

In addition to developmental effects, vitamin A and its precursors, such as β-carotene, have recognized antioxidant functions, particularly important during the peripartum period when oxidative stress is heightened due to increased metabolic demands and hormonal fluctuations. Carotenoids can neutralize reactive oxygen species (ROS) through mechanisms involving electron transfer and hydrogen abstraction, contributing to maternal redox balance (Zubova et al., 2021; Mitsuishi and Yayota, 2024). Elevated cortisol levels during labor can exacerbate oxidative stress and modulate immune responses; thus, maintaining adequate antioxidant status through dietary carotenoids may help mitigate these effects (Oliveira, 2014).

Although the transfer of vitamin A across the placenta is limited in ruminants due to the epitheliochorial barrier, studies suggest that neonates are born with detectable hepatic vitamin A stores (Ross and Gardener, 1994; Collins et al., 1994). This indicates that, despite its restrictiveness, the placenta is capable of a regulated and selective retinoid transport. According to Marceau et al. (2007), placental tissues express retinoic acid receptors (RARs) and cellular retinol-binding proteins (CRBPs), which enable the intracellular uptake and trafficking of maternal retinol, carried in the blood bound to retinol-binding protein (RBP), into placental cells and eventually into the fetal circulation.

Notably, during early organogenesis, the concentration of retinoids in placental tissue can be up to eight times higher than in the embryo, suggesting that the placenta may function as a transient storage site for retinoids. As gestation advances, particularly in the final trimester, fetal retinoid levels gradually surpass those of the placenta, indicating a regulated increase in retinol transfer to support tissue differentiation and accelerated growth (Marceau et al., 2007). However, in ruminants, this transfer remains quantitatively limited due to the structural characteristics of the epitheliochorial placenta, which imposes a greater barrier to fat-soluble vitamin diffusion compared to species with hemochorial placentation (Soares et al., 2018).

Given these physiological constraints, postnatal vitamin A sources become essential to sustain adequate neonatal status. Colostrum, rich in retinol and retinyl esters, plays a crucial role in this regard (Nozière et al., 2006). Nonetheless, in scenarios of poor colostrum intake or maternal deficiency, strategic neonatal supplementation, such as intramuscular vitamin A injection shortly after birth, can be beneficial, promoting immune competence and supporting optimal early development (Harris et al., 2018; Wang et al., 2018).

Jo et al. (2020) observed a marked decline in maternal plasma retinol concentrations during the first week postpartum, attributing this decrease to the high nutritional demands of the fetus during late gestation and the onset of lactation. This finding is supported by other studies in cattle that demonstrate significant prepartum redistribution of vitamin A into fetal and colostral compartments. For instance, Kankofer and Albera (2008) reported that retinol concentrations were significantly higher in the fetal portion of the placenta compared to the maternal portion, and that colostral retinol levels increased substantially within the first 24 hours after birth.

Similarly, Speer et al. (2024) estimated that approximately 60% of the vitamin A content in colostrum originates directly from the maternal diet in late gestation, while the remaining 40% is mobilized from hepatic stores. These findings indicate that both dietary intake and liver reserves are strategically mobilized to support colostrum formation at parturition.

Newborn calves typically have reduced hepatic vitamin A stores at birth, and are therefore considered functionally deficient in vitamin A and β-carotene at this stage

(Puvogel et al., 2008). Thus, colostrum represents the primary and immediate source of retinol necessary to normalize neonatal vitamin A status.

Considering that fat-soluble vitamins, such as vitamin A, do not cross the bovine placenta efficiently, neonatal blood and liver retinol concentrations at birth do not directly reflect maternal levels. Recent data indicate that many calves exhibit low hepatic vitamin A stores at birth, even when their dams are vitamin A sufficient.

Taken together, these findings highlight a functional maternal–neonatal axis of vitamin A metabolism in cattle, in which maternal mobilization during late gestation, particularly toward the placenta and colostrum, plays a central role in establishing the neonatal vitamin A status. In this context, it can be concluded that plasma vitamin A concentrations in newborn calves are directly influenced by maternal supplementation during late gestation.

This results in elevated vitamin A levels (IU/dL) at birth, even prior to colostrum intake, thereby supporting the hypothesis that placental transfer of vitamin A does occur in beef cattle. In a study with beef cows, Jo et al. (2020) observed that maternal supplementation with 78,000 IU/day of vitamin A during the final third of gestation led to significantly higher plasma retinol concentrations (±120.3 IU/dL) compared to non-supplemented controls (±83.7 IU/dL).

Complementing these findings, Lotfollahzadeh and Golchin (2016) reported that calves born to dams supplemented with 2,000,000 IU of vitamin A via intramuscular injection exhibited pre-colostrum plasma retinol concentrations ranging from 90.6 to 93.1 µg/dL, compared to only 35.5 µg/dL in the control group, representing a more than 2.5-fold increase. Additionally, Kumagai et al. (1994) demonstrated that plasma vitamin A concentrations in calves more than double between birth and five days of age, underscoring the importance of colostrum as a critical source of vitamin A during the neonatal period.

Together, these findings confirm that, although placental transfer of fat-soluble vitamins in ruminants is generally limited (NASEM, 2021), maternal supplementation in late gestation can promote biologically and statistically significant increases in neonatal vitamin A status. Further research is needed to determine the specific vitamin A requirements for pregnant cows, especially considering the increasing fetal demand in late gestation (Jo et al., 2020), and to better understand how maternal intake and hepatic reserves influence both fetal supply and early calf development.

As described and indicated previously, few considerations are available about vitamin A or its precursors supplementation for beef cows, however, studies conducted with dairy cows indicate a colostrum quality improvement related to higher immunoglobulins (IgG) and fats concentrations when supplementation is performed in prepartum (Nishijima et al., 2017; Ishida et al., 2018). Considering the digestive physiology, gestational physiology, and colostrum production physiology analogies of dairy cattle and beef cattle, we can relate studies carried out with dairy cattle to understand the vitamin A or its precursors' supplementation effects in colostrum quality.

Colostrum synthesis is influenced by the blood flow and nutrients circulating in the mammary gland, which may alter its composition (Foley and Otterby, 1978). Other factors might alter this synthesis, such as immunity and cow breed. Thus, when cows are supplemented with β-carotene in the final phase of gestation, they provide higher IgG1 and IgA colostral concentrations (Nishijima et al., 2017; Ishida et al., 2018) or not (Kaewlamun et al., 2011; Prom et al., 2022), requiring further investigations into mammary metabolism for colostrum synthesis and immunological quality.

As previously reported, the vitamin A antioxidant action and β-carotene protect the cells from free radicals degenerative effects, produced during oxidative metabolism, in the peripartum period and attenuate oxidative stress by unbalancing the free radical number, also known as reactive oxygen species (ROS) characterized by glutathione, glutathione peroxidase, vitamin A, vitamin E, β-carotene and others (Kamiloglu et al., 2005), and antioxidants.

Considering a temporal scale, during the peripartum period in cattle, the free radicals are intense due to the metabolic increase resulting from oxidative stress. It is common that in intensive beef cattle production systems, after 60 days of calving, reproductive management occurs with the dams through artificial insemination (whether at a fixed time or not). However, with the higher metabolic rate causing oxidative stress, failures related to estrus may be noted, compromising ovulation (Ay et al., 2012a).

In this sense, it is necessary to observe prepartum supplementation with vitamin A or β-carotene, reinforcing the liver storage in the retinoid form idea, or the intramuscular application of commercial products containing vitamin A and β-carotene might ensure adequate blood levels granting benefits in the cows' reproductive phase in the postpartum period (Mitsuishi and Yayota 2024).

Thus, the lack of antioxidant activity in the presence of synergistic interactions between free radicals and ROS may impair essential processes such as cell division, primordial cell differentiation, and embryonic development (Agarwal et al., 2006). In addition, oxidative stress can damage the membrane structure of luteal cells, leading to reduced progesterone production and impaired expression of luteinizing hormone receptors, which may ultimately inhibit ovulation (Agarwal et al., 2006).

In this context, in front of situations with a low β-carotene serum concentration in the reproductive period, immediately after parturition, we can witness cases of long estrous periods, low ovulation, and low conception rates. If there is an ideal β-carotene circulation, positive effects are observed regarding a reduction in the uterine involution time, greater progesterone production by the corpus luteum, and a shorter service period (Rakes et al., 1985; Arikan and Rodway, 2000).

Another point that should be addressed regarding the vitamin A effect on the cows' reproductive period is related to the corpus luteum (CL) metabolism. In general, adequate β-carotene plasma concentrations increase the follicles and CL metabolic activity relative to the estrous cycle and gestation stages (Oliveira, 2014). Regarding CL, when supplementation with β-carotene is used, the follicular fluid and CL become a vitamin A deposit, observing a positive correlation for the CL weight and diameter, proving its functionality in the reproductive period (Haliloglu et al., 2002).

Findings indicate that β-carotene supplementation in the periods preceding the cows' reproductive phase can positively affect ovarian activity with greater development of mature oocytes (Hidalgo et al., 2005), and can be used as a nutritional strategy for higher pregnancy rates with artificial insemination.

Regarding β-carotene plasmatic levels in cows undergoing reproduction, Michael et al. (1994) suggest a threshold between 300 and 1200 µg/dL, and for vitamin A values between 25 and 80 µg/dL, which should be associated with the pregnant beef cows or heifers' requirements, equivalent to 2,800 IU/kg of vitamin A in a DM basis (NASEM, 2016; Valadares Filho et al., 2023).

Aguiar-Zalzano et al. (2022) compared dairy cows supplemented with β-carotene via two different routes: a single intramuscular injection administered on day 30 postpartum, and continuous oral supplementation starting 30 days before calving and continuing until 150 days postpartum. Both groups showed similar β-carotene concentrations, all exceeding the reference range (+300 µg/dL). However, intramuscular administration resulted in higher plasma β-carotene levels over shorter time periods following application.

Therefore, to optimize the artificial insemination management of beef cows in intensive production systems, the carotenes or vitamin A application via the intramuscular route can improve ovulation and CL metabolism, as mentioned above. Using this strategy associated with artificial insemination protocols in dairy cows, it was reported that there is an increase in serum β-carotene, causing greater CL functionality and increasing the pregnancy rate (Ay et al., 2012a; Ay et al., 2012b).

Observing the blastocyst interaction after fertilization, the maternal immune system might damage the cell division and differentiation initial phase, due to the production of free radicals, compromising embryonic and fetal development. However, the damage might be prevented by the vitamin A action, with the increase in antioxidant production inactivating the ROS action (Kamiloglu et al., 2005), thus reducing cell degeneration and affecting embryonic development.

Associated with this, supplementation with β-carotene with fat-soluble vitamins (e.g., more specifically vitamins A and E) ensures an increase in the pregnancy rate in beef cows raised on pasture, when they were aroused to the first breeding season using the fixed-time artificial insemination technique (Gouvêa et al., 2018), ensuring more developed calves due to better embryonic cells damage prevention and granting its development (Olson and Seidel, 2000; Gouvêa et al., 2018). This action can be explained by the vitamin A association with β-carotene allowing antioxidant effectiveness ensuring low lipid peroxidation action of embryonic cell membranes, which affects the healthy embryo development.

Studies in the reproduction area have clearly demonstrated the adequate vitamin A or β-carotene applicability to optimize reproductive indices in dairy cows, possibly due to its higher oxidative stress in the peripartum period, reducing the effects of free radicals on ovarian and hormonal action (Khemarach et al., 2021). When compared with beef cows there is little reported about the use of this strategy to optimize reproductive indices, and further research is needed into vitamin A and its precursors use as supplements, when necessary.

4.3.Effects on beef calves

Vitamin A supplementation in neonatal calves has been widely studied from the perspective of muscle development and intramuscular fat accumulation, particularly in early-finishing production systems (Cheryl and Fabianne, 2016; Harris et al., 2018; Jo et al., 2020; Peng et al., 2020; Maciel et al., 2022; Scapol et al., 2023).

However, the effects of this fat-soluble vitamin extend far beyond adipogenesis, influencing a range of fundamental physiological processes that begin during gestation and continue throughout the early stages of calf development (Figure 3). Vitamin A acts as an epigenetic and transcriptional modulator, primarily through retinoic acid, which binds to nuclear receptors (RAR and RXR), thereby regulating the expression of genes involved in cellular differentiation (Chawla et al., 2001).

In adipose tissue, this signaling pathway is crucial for the formation and proliferation of preadipocytes, with direct implications for intramuscular adipose tissue synthesis and, consequently, meat quality (Peng et al., 2021; Scapol et al., 2023). This process, however, does not occur in isolation. The action of vitamin A on progenitor cell differentiation is also evident in skeletal muscle tissue, promoting the development of muscle fibers and the expansion of satellite cell populations, key elements for muscle hypertrophy and the animal's future productive performance (Jo et al., 2020; Du et al., 2017).

Neonatal vitamin A supplementation has been associated with increased average daily gain, higher weaning weights, and improved early growth, particularly when administered within the first 24 hours of life and, in some protocols, reinforced at 30 days of age (Harris et al., 2018; Maciel et al., 2022). On the other hand, the administration of vitamin A during the growing and finishing phases promotes changes in lipid metabolism, stimulating pathways related to lipid oxidation and the reduction of lipogenesis (Daniel et al., 2009; Kruk et al., 2018; Campos et al., 2020; Wellmann et al., 2020).

In addition, to its roles in muscle and adipose tissue development, vitamin A plays a pivotal role in the maturation of the immune system. Newborn calves are born with limited hepatic retinol reserves due to the restricted placental transfer of this vitamin. As a result, they rely exclusively on colostrum as their initial source of vitamin A, making them particularly vulnerable to deficiency, especially when born to dams with insufficient vitamin A status. Studies have demonstrated that hypovitaminosis A in the early neonatal period impairs epithelial integrity, weakens mucosal immune responses, and compromises the functional capacity of T lymphocytes and dendritic cells, thereby increasing the incidence of respiratory disease and enteritis (McGill et al., 2019).

In this context, intramuscular administration of vitamin A at birth has emerged as a practical and effective strategy to enhance immune competence and reduce the susceptibility of neonatal calves to infectious agents during the critical period of postnatal adaptation (McGill et al., 2019).

It is equally important to recognize that the physiological influence of vitamin A is not confined to the neonatal stage. The maternal-fetal interface plays a central role in shaping the intrauterine environment and, consequently, in the developmental programming of the offspring.

Cows supplemented with vitamin A or its precursor β-carotene during the final third of gestation produce colostrum with higher retinol concentrations and are more effective at transferring this vitamin to their calves, resulting in improved immune function and overall performance through weaning (Scapol et al., 2023). This evidence highlights the importance of an integrated approach that accounts for both maternal nutritional management and direct postnatal supplementation to optimize neonatal development.

Within the metabolic endocrine axis, vitamin A also exerts relevant regulatory effects. The early deposition of adipose tissue, stimulated by neonatal vitamin A administration, may influence circulating levels of leptin, a peptide hormone primarily secreted by mature adipocytes (Tang and Jiang, 2024).

Leptin acts as a metabolic signal to the central nervous system, particularly the hypothalamus, modulating appetite, energy homeostasis, and the activation of the reproductive axis (Tang and Jiang, 2024). The differentiation of mesenchymal stem cells into preadipocytes, followed by their maturation into fully functional adipocytes, a process driven by retinoic acid, the bioactive form of vitamin A, leads to an increased pool of leptin-secreting cells. Consequently, early-life vitamin A supplementation may elevate leptin levels during the pre-weaning period.

Although the direct causal relationship between this intervention and puberty onset in cattle remains to be fully established, elevated leptin concentrations during early life have been associated with earlier puberty and first estrus in heifers (Fantuz et al., 2024). Furthermore, vitamin A deficiency in both sexes has long been linked to reproductive dysfunction, delayed sexual maturity, and reduced fertility (Shastak and Pelletier, 2024), reinforcing its essential role in gonadal development and neuroendocrine regulation of reproduction.

While the enhancement of intramuscular adipogenesis remains one of the primary advantages associated with neonatal vitamin A supplementation (Cianzio et al., 1985; Li et al., 2023), the systemic effects of this strategy are broad and physiologically interconnected. By modulating cellular differentiation, supporting immune and endocrine maturation, and maintaining epithelial integrity, vitamin A functions as a key regulatory element in early postnatal development. Recognizing the multifactorial roles of this micronutrient enables the design of more comprehensive supplementation protocols that address not only carcass quality but also calf health, productivity, and reproductive potential throughout the production cycle.

5. Final considerations and future implications

The scientific evidence presented in this review highlights the pivotal role of vitamin A and its precursor, β-carotene, in supporting reproductive performance, fetal development, neonatal growth, and meat quality in beef cattle systems. Although tropical pastures generally meet vitamin A requirements, critical physiological phases such as late gestation, early lactation, and neonatal development demand strategic supplementation to address increased metabolic and antioxidant needs. It is recommended that pregnant beef cows receive elevated levels of vitamin A or β-carotene, approximately 60,000 to 78,000 IU/day of vitamin A via oral supplementation, or 1,000,000 to 2,000,000 IU via a single intramuscular injection during the last 3 or 4 weeks of gestation, to enhance hepatic retinol stores, optimize colostrum synthesis, reduce oxidative stress, and support fetal tissue development. These levels exceed the standard recommendations of 2,800 IU/kg of dry matter and reflect the increased physiological demands during the peripartum period.

Similarly, a single intramuscular injection of 150,000 to 300,000 IU of vitamin A in newborn calves within the first 24 hours of life has shown potential to promote intramuscular adipogenesis, improve early growth, and enhance meat marbling in early-finishing systems.

Given the current gaps in precise vitamin A requirement values for pregnant beef cows and neonates, further studies are warranted to define optimal dosage, duration, and delivery methods for different production contexts. A better understanding of the long-term effects of maternal and neonatal supplementation on retinoid metabolism, immune competence, reproductive success, and carcass traits will contribute to the development of more efficient and sustainable beef production systems.

Therefore, the strategic use of vitamin A and β-carotene, particularly in key physiological windows, should be considered a valuable nutritional tool to enhance maternal and offspring performance, improve meat quality, and support animal health in modern beef cattle production.

6. References

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Figure

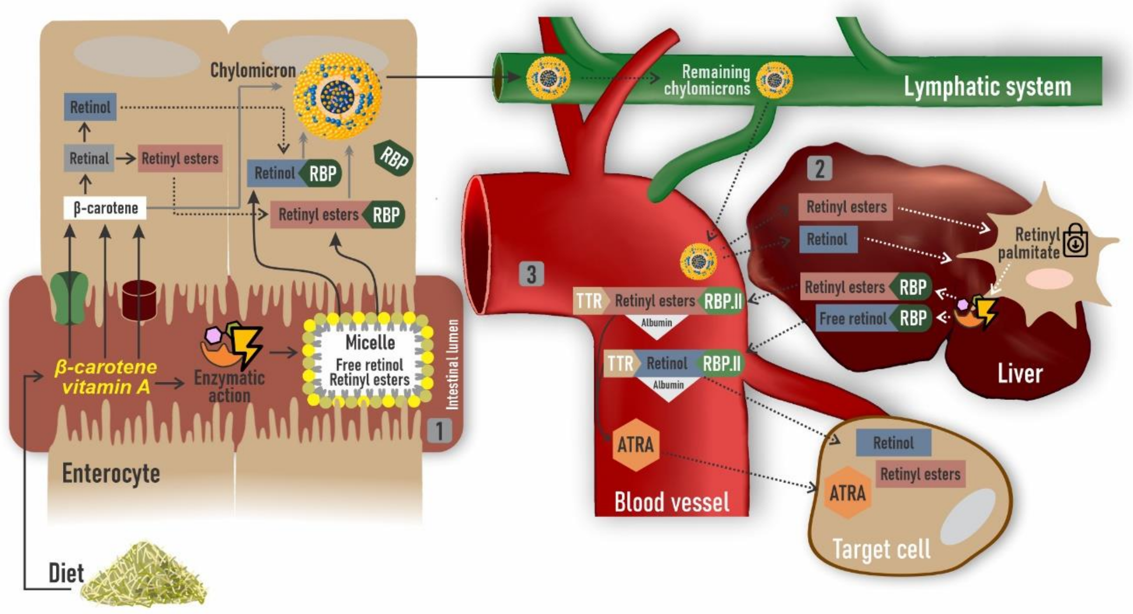

Figure 1. Schematic of the β-carotene and vitamin A precursors absorption, transport, and storage in the beef cattle intestine. **1)** part of the β-carotene contained in the diet, related to the forage content and/or in vitamin and mineral supplements, undergoes the enzymatic action of 15-15' β-carotene decarboxylase enzyme (from β-carotene to retinal) and retinaldehyde reductase (from retinal to retinyl and/or retinyl esters) to form the micelle for absorption by facilitated diffusion, cleaving the β-carotene molecule into free retinol and/or retinyl esters; some β-carotene fraction or other carotenoids can be absorbed by membrane receptors without necessarily undergoing enzymatic action. As in lipid digestion, vitamin A precursors are re-esterified and grouped into chylomicrons by the retinol transport proteins (RBPs) action. **2)** After re-esterification and chylomicron formation, the aggregate containing lipids, fat-soluble vitamins, and lipoproteins is directed to the lymphatic system, once this system caliber supports the transporting lipids and their aggregate's function, with the liver as the final destination in the remaining chylomicrons form. In the liver tissue, retinol and retinol esters are transported to the liver stellate cells, where they are stored in the retinyl palmitate form (the most stable vitamin A form). **3)** When the vitamin A need in tissue is triggered, the vitamin A stored in the liver is converted by enzymatic action into free retinol or retinol esters, which are transported to the hepatic portal system with the cytoplasmic RBPs help, and to the plasma system by type II RBPs in addition to albumin and transthyretin (TTR), which

direct the provitamin A molecules to the target tissue. Some of the provitamin A molecules are converted into all-trans retinoic acid (ATRA), being absorbed by the target cell.

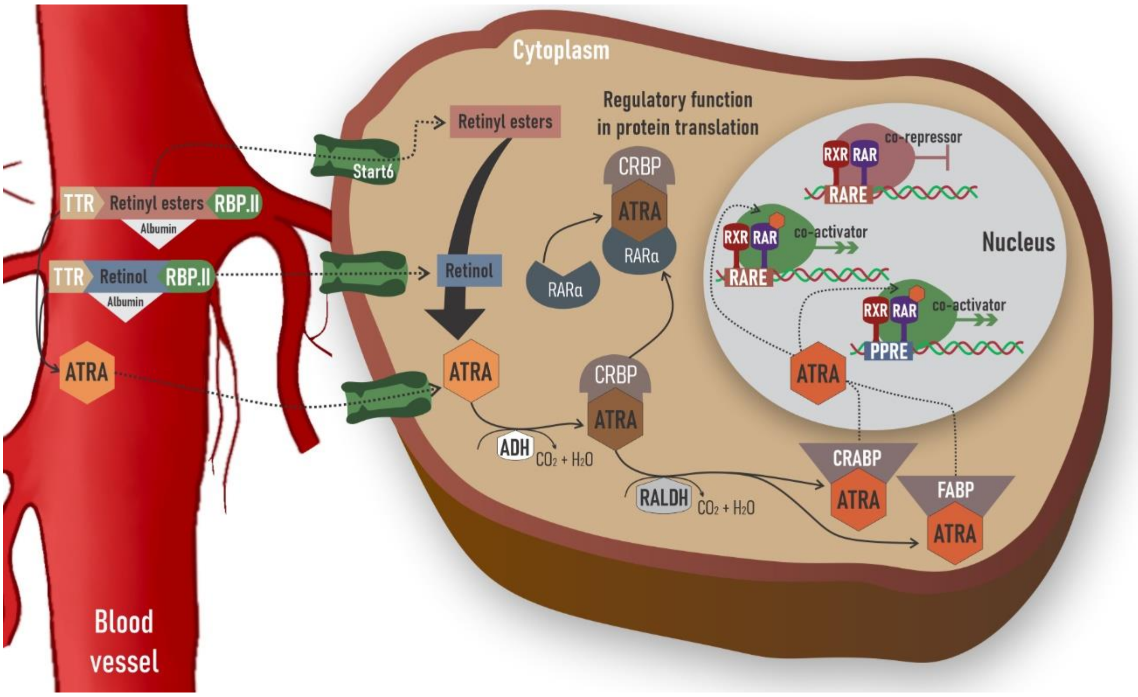

Figure 2. After transport to the target tissue, at the cellular level, membrane receptors allow the retinyl esters, retinol, and all-trans retinoid acid (ATRA) entry into the cytoplasm. This results in a retinyl ester and retinol to ATRA conversion, which will be oxidized by cytosolic alcohol dehydrogenase (ADH) and the enzyme retinal dehydrogenase (RALDH), enabling the molecule to have bioactivity as vitamin A. The ATRA + CRBP complex associates with RAR molecules, which have a regulatory function in protein translation in the cytoplasmic environment. Some transport proteins found in the cytosol have an affinity for ATRA and have the function of transferring the molecule to the cell nucleus, with this function being commonly performed by cellular retinoic acid-binding protein (CRABP) and fatty acid transport protein (FABP). Thus, in a nuclear environment when bioactivated, ATRA associates with the co-activators + nuclear receptors complex, represented by RAR, RXR, PPAR, and RARE, activating genomic functions through the target genes transcription or their repression, when necessary.

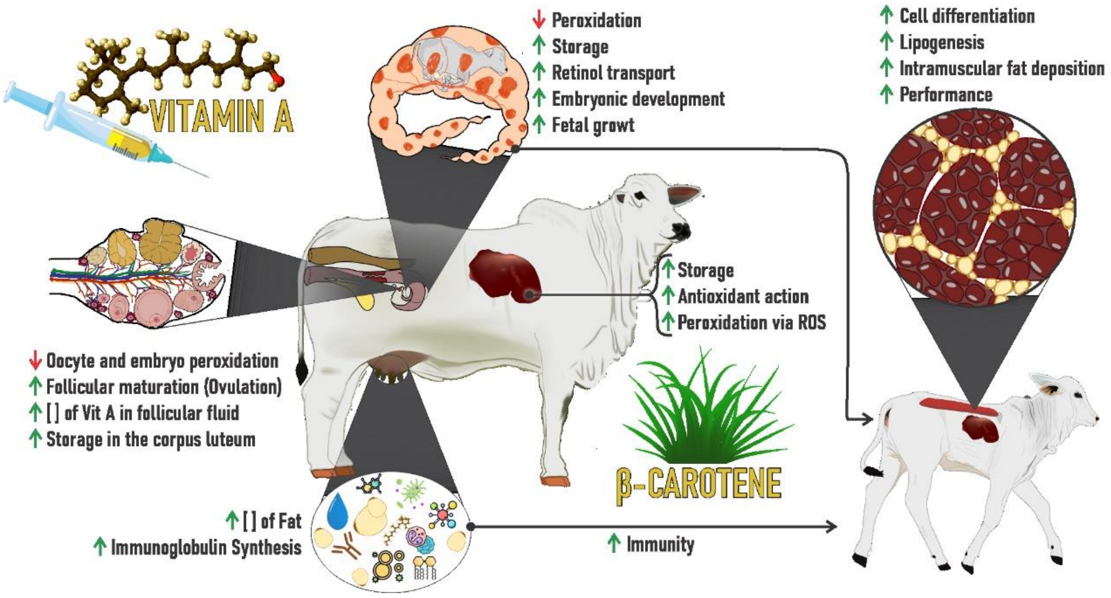

Figure 3. Dynamic effects of injectable β-carotene and vitamin A supplementation on the pregnant cows' metabolism, regarding the antioxidant action, metabolic responses, reproduction, and gestation; and newborn calves, regarding the metabolic responses in the immune system and cellular differentiation in skeletal muscle tissue.