UNIVERSIDADE ESTADUAL PAULISTA - UNESP
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CAMPUS DE JABOTICABAL

EFEITO DA OFERTA DE FORRAGEM DURANTE TERÇO MÉDIO E FINAL DA GESTAÇÃO DE VACAS NELORE SOBRE O METABOLISMO, DESEMPENHO E QUALIDADE DA CARNE DA PROGÊNIE MACHO DURANTE RECRIA E TERMINAÇÃO

Iorrano Andrade Cidrini
Zootecnista

2024

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Iorrano Andrade Cidrini

Orientador: Prof. Dr. Flávio Dutra de Resende

Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – UNESP, Campus de Jaboticabal, como parte das exigências para a obtenção do Título de Doutor em Zootecnia (Nutrição e Produção Animal).

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IMPACTO POTENCIAL DESTA PESQUISA

Esta pesquisa demonstra como ajustes de manejo na atividade de cria melhoram a produtividade de vacas Nelore gestantes, impactando todo o ciclo produtivo, do nascimento ao abate. Isso se deve ao efeito da programação fetal, onde uma melhor nutrição do feto na barriga da mãe modula seu potencial pós-natal. Destacando a relevância da pecuária nacional, evidencia-se que ações simples, como o manejo do pasto, podem gerar benefícios econômicos, sociais e promover a sustentabilidade de forma indireta, ao aumentar a eficiência na produção de alimentos com menor demanda de terras.

POTENCIAL IMPACT OF THIS RESEARCH

This research demonstrates how management adjustments in cow/calf operations improve the productivity of pregnant Nellore beef cows, impacting the entire production cycle from birth to slaughter. This is due to the fetal programming effect, where better fetal nutrition in the mother's uterus modulates its postnatal potential. Highlighting the relevance of national livestock farming, it is evident that simple actions, such as pasture management, can generate economic and social benefits and indirectly promote sustainability by increasing efficiency in food production with lower land demand.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE:

EFEITO DA OFERTA DE FORRAGEM DURANTE TERÇO MÉDIO E FINAL DA GESTAÇÃO DE VACAS NELORE SOBRE O METABOLISMO, DESEMPENHO E QUALIDADE DA CARNE DA PROGÊNIE MACHO DURANTE RECRIA E TERMINAÇÃO

AUTOR: IORRANO ANDRADE CIDRINI

ORIENTADOR: FLÁVIO DUTRA DE RESENDE

Aprovado como parte das exigências para obtenção do Título de Doutor em Zootecnia, pela Comissão Examinadora:

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Jaboticabal, 26 de janeiro de 2024

DADOS CURRICULARES DO AUTOR

Iorrano Andrade Cidrini, nascido em 26 de junho de 1992, Muriaé, Minas Gerais. Filho de Míria Lacerda de Andrade e Antônio Ferdinando de Oliveira Cidrini, professora e produtor rural, respectivamente. Possui graduação em Zootecnia (2012 - 2016), pelo Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Gerais, Campus Rio Pomba. Atuando no grupo SEZOO, sob orientação do professor Dr. Valdir Botega Tavares (2013 - 2016) e em iniciações científicas sob orientação dos professores Dr. Onofre Barroca de Almeida Neto (2013 - 2015) e Dr. Arnaldo Prata Neiva Júnior (2016). Em 2016 desenvolveu o estágio curricular na Embrapa Gado de Leite, Campo Experimental Coronel Pacheco - Minas Gerais, sob orientação dos professores Dr. Cristiano Gonzaga Jayme e Dr. Luiz Gustavo Pereira Ribeiro. Em fevereiro de 2017 ingressou no mestrado pela Faculdade de Ciências Agrárias e Veterinárias - UNESP, Jaboticabal São Paulo, no programa de pós-graduação em Zootecnia sob orientação do Dr. Flávio Dutra de Resende. Onde participou por seis meses, com início em novembro de 2018, de um intercâmbio (BEPE), atuando como pesquisador visitante no Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), sob orientação da Dra. Karen Beauchemin. Foi membro do Grupo de Estudos em Produção de Ruminantes – GEPROR; grupo que integra os responsáveis pela condução dos projetos científicos em gado de corte do Polo Regional da APTA em Colina, SP. No ano de 2019 seguiu no doutorado, sendo Presidente do 3º Beefday - A ciência a favor do campo. Realizou o doutorado sanduíche no período de agosto de 2022 a julho de 2023, na Montana State University, auxiliando na condução de projetos científicos sob orientação do Dr. Rodrigo Marques no Programa de Animal & Range Sciences, Bozeman, Montana, EUA.

"I was poor because I didn't have anything. I had no money, I had no things, we had no TV, we had no fridge, we had nothing as kids. But I was rich because I had a dream."

Arnold Schwarzenegger

"I don't like being comfortable. Once you get used to it, it's hard to give up. I'd rather stay hungry."

*Also, **Arnold Schwarzenegger***

Dedico...

A Deus, aos companheiros de estrada

e à minha família!

Aos meus orientadores, pelos ensinamentos e inspiração.

Às grandes e valorosas amizades conquistadas ao longo dessa trajetória, foram fundamentais...

...a vocês, **ofereço!**

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Aos meus orientadores, professores Flávio Dutra de Resende e Gustavo Rezende Siqueira, que não apenas foram exemplos, mas também estenderam a mão e ofereceram tudo o que fosse necessário para o meu desenvolvimento. Minha profunda gratidão a vocês!

À Universidade Estadual Paulista (UNESP) por proporcionar as condições necessárias para o desenvolvimento do Doutorado, e à Agência Paulista de Tecnologia dos Agronegócios (APTA), que disponibilizou toda a estrutura essencial para o progresso do projeto de pesquisa. Ao CNPQ, pela bolsa de estudos concedida.

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Aos pós-doutores que contribuíram significativamente para a concepção, realização e interpretação dos resultados experimentais, Laura, Ivanna, Yury e Abmael. Estendo esse agradecimento a meu grande amigo German Dario, que sempre contribui muito em nossos trabalhos e nos apoia.

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durante todas as fases do experimento. Gostaria também de expressar minha gratidão à Juliana Torrecilhas, pela contribuição nas análises de carne e, através dela, ao grupo do Professor Chardulo.

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Além desses, estendo meu agradecimento ao Professor Rodrigo Marques pela recepção, paciência e ensinamentos. Foi um período de intenso aprendizado! Muito obrigado pela oportunidade!

SUMÁRIO

CERTIFICADO Nº 0004/2020 - CEUA

Certificamos que o projeto apresentado dia 24 de agosto de 2020 intitulado: "**IMPACTOS DA OFERTA DE FORRAGEM NOS TERÇOS MÉDIO E FINAL DA GESTAÇÃO DE VACAS NELORE SOBRE OS ASPECTOS PRODUTIVOS DA SUA PROLE**" registrado com o **Protocolo nº 0004/2020**, sob a responsabilidade do Pesquisador Científico **Flávio Dutra de Resende**, que envolve a produção, a manutenção e a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da lei nº 11.794, de oito de outubro de 2008; do decreto nº 6.899, de 15 de julho de 2009; e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi **APROVADO** pela **Comissão de Ética no Uso de Animais do Departamento de Descentralização do Desenvolvimento (CEUA-DDD)**, em reunião extraordinária realizada no dia **27 de agosto de 2020**.

Finalidade: (X) Pesquisa científica – ( ) Ensino	
Vigência da autorização	01/01/2021 à 31/08/2023
Espécie/linhagem e/ou raça	Bovinos Nelore
Nº de animais	288
Sexo	F (216) / M (72)
Localização	Polo Regional Alta Mogiana - Colina / DDD / APTA / SAA - SP
Responsável Técnico	Dr. Gustavo Rezende Siqueira

Maria Izabel Merino de Medeiros
Coordenadora CEUA-DDD

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Secretaria de Agricultura e Abastecimento
Agência Paulista de Tecnologia dos Agronegócios

EFEITO DA OFERTA DE FORRAGEM DURANTE TERÇO MÉDIO E FINAL DA GESTAÇÃO DE VACAS NELORE SOBRE O METABOLISMO, DESEMPENHO E QUALIDADE DA CARNE DA PROGÊNIE MACHO DURANTE RECRIA E TERMINAÇÃO

RESUMO

Este estudo avaliou a influência de diferentes níveis de oferta de forragem (OF) desde a metade da gestação até o parto no desempenho e qualidade da carne de tourinhos Nelore criados em um sistema de produção intensivo. Foram testados dois níveis de OF, baixa (BOF; 2,81 kg de matéria seca de forragem por kg de peso corporal) e alta [AOF; 7,58 kg de matéria seca de forragem por kg de peso corporal (PC)], em 72 vacas Nelore multíparas, lactantes e prenhes de bezerros machos no dia -151. As vacas foram estratificadas por PC corporal inicial (444 ± 42 kg) e escore de condição corporal (3,66 ± 0,28) e alocadas aleatoriamente em 12 piquetes de *Urochloa brizantha* cv. Marandu. Após o parto, as vacas e seus bezerros foram manejados juntos até o desmame. No dia 252, os bezerros foram colocados em 12 piquetes de um hectare e receberam suplementação (1% do PC). Após a fase de recria, os piquetes foram transferidos para baias de confinamento por um período de 101 dias e posteriormente abatidos no dia 562. A prole das vacas do tratamento AOF apresentou uma concentração de IGF-1 13,1% maior (P = 0,034) e um nível de glicose 5,1 mg/dL menor (P = 0,043) em comparação com a prole das vacas do tratamento BOF. A expressão de mTOR também foi superior para AOF (P < 0,001). Em relação ao desempenho, a prole de vacas AOF apresentaram melhores conversão e eficiência alimentar (P ≤ 0,047) no confinamento e foram 17 kg, 21 kg, 23 kg e 37 kg mais pesados nos dias 252, 336, 462 e 563, respectivamente, em comparação com o tratamento BOF, além de um peso de carcaça quente 18 kg maior. A área de olho de lombo foi maior para o tratamento AOF em comparação com o BOF (83,38 vs. 79,20, P = 0,109), comportamento similar ao ratio, que foi superior para o tratamento AOF (0,57 vs. 0,54, P = 0,018). O marmoreio (3,1 pontos), espessura da gordura subcutânea (4,2 mm), espessura da gordura da garupa (6,4 mm) e pH (5,7) foram semelhantes entre os tratamentos maternos (P ≥ 0,257). Em relação à qualidade da carne, não houve interação entre os tratamentos maternos e o tempo de maturação da carne (1, 7 ou 14 dias) para nenhuma variável avaliada (P ≥ 0,102). Assim, uma maior oferta de forragem durante a metade e o final da gestação em vacas Nelore *B. indicus* influencia o crescimento pós-desmame sem alterar a qualidade da carne de tourinhos Nelore criados em um sistema de produção intensivo.

Palavras – chave: Características de carcaça, Desempenho, Expressão de genes, Metabolitos sanguíneos, Nelore, Programação fetal, Qualidade da carne.

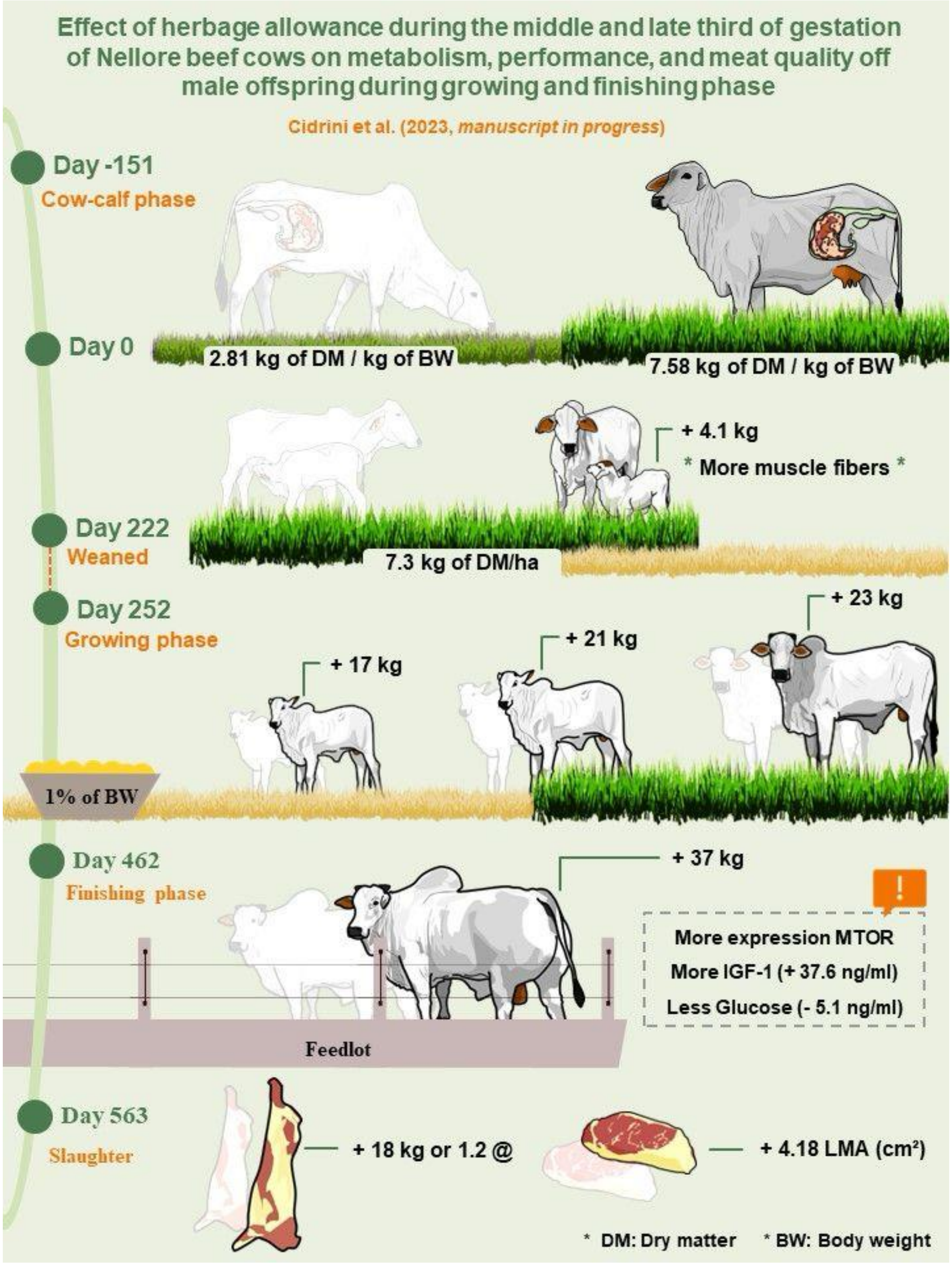
EFFECT OF HERBAGE ALLOWANCE DURING THE MIDDLE AND LATE THIRD OF GESTATION OF NELLORE BEEF COWS ON METABOLISM, PERFORMANCE, AND MEAT QUALITY OFF MALE OFFSPRING DURING GROWING AND FINISHING PHASE

ABSTRACT

This study evaluated the influence of different herbage allowance (HA) levels from mid-gestation to calving on the meat quality of young Nellore bulls raised within an intensive production system. Two HA levels, low [LHA; 2.81 kg of forage dry matter (DM) per kg body weight (BW)] and high (HHA; 7.58 kg of forage DM per kg BW), were tested on 72 multiparous, lactating, Nellore beef cows pregnant with male calves on day -151. The cows were stratified by initial body weight (BW = 444 ± 42 kg) and body condition score (3.66 ± 0.28) and randomly allocated to 12 paddocks of *Urochloa brizantha* cv. Marandu. After calving, the cow-calf pairs were managed together until weaning. On day 252, the offspring were placed in 12 one-hectare paddocks and fed a supplement (1% of BW). After growing phase, paddocks were relocated to feedlot pens for a 101-day period and subsequently slaughtered on day 562. Offspring from cows in the HHA treatment showed a 13.1% higher concentration of IGF-1 ($P = 0.034$) and a 5.1 mg/dL lower glucose level ($P = 0.043$) compared to those from cows in the LHA treatment. The mTOR expression was greater for HHA ($P < 0.001$). Concerning performance, offspring of HHA cows exhibited improved feed conversion and efficiency ($P \leq 0.047$) in the finishing period and were 17 kg, 21 kg, 23 kg, and 37 kg heavier on days 252, 336, 462, and 563, respectively, along with an 18 kg higher hot carcass weight. The *Longissimus* muscle area was greater for HHA compared to LHA (83.38 vs. 79.20, $P = 0.109$), the ratio also was higher for HHA (0.57 vs. 0.54, $P = 0.018$). Marbling (3.1 points), backfat thickness (4.2 mm), rump fat thickness (6.4 mm), and pH (5.7) were similar between maternal treatments ($P \geq 0.257$). Regarding meat quality, there was no interaction between maternal treatments and meat aging time (1, 7, or 14 days) for any assessed variable ($P \geq 0.102$). Thus, increased HA during the mid to late gestation in *B. indicus* Nellore cows improves post-weaning performance without alter meat quality in young Nellore bulls raised within an intensive production system.

Keywords: Blood metabolites, Carcass traits, Fetal programing, Gene expression, Meat quality, Nellore, Performance.

INFOGRAPHIC



Effect of herbage allowance during the middle and late third of gestation of Nellore beef cows on metabolism, performance, and meat quality off male offspring during growing and finishing phase

Cidrini et al. (2023, *manuscript in progress*)

CAPÍTULO 1 – CONSIDERAÇÕES GERAIS

1. Introdução

A pecuária de corte no Brasil ocorre predominantemente em sistemas baseados no uso de pastagens. Embora existam diferentes níveis tecnológicos entre as propriedades, o fenômeno da sazonalidade na produção leva a variações no crescimento das forragens ao longo do ano, sendo que as principais limitações quantitativas são observadas durante o período seco (REIS et al., 2011). Além disso, há uma redução no teor de proteína, um aumento na fibra indigestível e, consequentemente, uma queda na qualidade da forragem (ROTH et al., 2017).

Um aspecto importante durante o período seco é a redução na ingestão de nutrientes, o que afeta o desempenho de animais de diferentes categorias, incluindo vacas de corte. Considerando os sistemas de produção da fase de cria tipicamente desenvolvidos no Brasil Central, ocorre uma concentração de nascimentos de bezerros entre o final do período seco e o início do período chuvoso (LEMOS et al., 2012). Portanto, os protocolos reprodutivos são realizados com pastagens em condições favoráveis para as vacas. No entanto, para as vacas que ficarem prenhas nessas condições coincidirá o aumento na demanda nutricional devido ao desenvolvimento fetal (terços médio e final da gestação), com o período de menor disponibilidade de forragem (ROBINSON et al., 1977).

Além disso, deve-se considerar uma redução na capacidade de ingestão da vaca durante esse período de gestação (terços médio e final da gestação; GIONBELLI et al., 2015), aumentando a probabilidade de esses animais enfrentarem algum tipo de restrição nutricional. Além de impactar diretamente na eficiência do sistema, reduzindo as taxas de prenhez na próxima estação de monta, a deficiência de nutrientes durante a gestação afeta negativamente o desempenho da progênie ao longo de sua vida (FUNSTON et al., 2010; DU et al., 2013). Estudos focados na programação fetal buscam entender e avaliar o estímulo ou insulto materno durante um período fetal específico que pode afetar significativamente o crescimento e a qualidade da carne da progênie (FUNSTON et al., 2010).

Diversos estudos avaliando raças taurinas (*Bos Taurus*) em relação à nutrição materna durante a gestação mostraram seus impactos no desempenho futuro e na qualidade da carne da progênie (STALKER et al. 2006; UNDERWOOD et al., 2010; GREENWOOD et al., 2009; CAFE et al., 2009). No entanto, ainda há poucos estudos avaliando raças zebuínas (*Bos Indicus*), o que representa uma oportunidade para novas pesquisas relacionadas à programação fetal (Moriel et al., 2021). Essa lacuna do conhecimento se dá porque as respostas fisiológicas a eventos relacionados à nutrição diferem entre os grupos genéticos (Cooke et al., 2020).

Diante disso, é relevante conduzir estudos com o objetivo de compreender os mecanismos fisiológicos, o metabolismo e o crescimento da progênie de vacas que sofreram ou não restrição nutricional durante os terços médio e final da gestação em condições tropicais e genética zebuína, especialmente seus efeitos a longo prazo sobre a progênie e em condições que representem o sistema de produção brasileiro, ou seja, vacas mantidas em pastagens. Por tanto, o objetivo foi avaliar a influência de diferentes ofertas de forragem (baixa vs. alta) durante o terço médio e final de gestação no desempenho pós-desmame, parâmetros sanguíneos e características da carcaça de tourinhos Nelore criados em um sistema de produção intensivo.

1.1. Exigências nutricionais de vacas de corte durante a gestação e a oferta de forragem

Aproximadamente 75% do crescimento fetal do bezerro ocorre durante os últimos 2 meses de gestação (ROBINSON et al., 1977), o que implica em um aumento das exigências nutricionais das vacas. De acordo com as equações propostas para a predição das exigências de nutrientes para vacas de corte, segundo o método fatorial utilizado pelo BR-CORTE e NASEM (VALADARES FILHO et al., 2016; NASEM, 2016), as demandas nutricionais das vacas gestantes podem ser divididas em requisitos para manutenção, lactação, gestação e ganho de peso. Portanto, ao final da gestação, além de considerar as exigências nutricionais para a vaca, é fundamental levar em conta as necessidades para o desenvolvimento fetal, a manutenção dos tecidos gestacionais e o desenvolvimento do úbere (GIONBELLI et al., 2016).

O escore de condição corporal (ECC) é uma ferramenta importante para monitorar o estado nutricional de vacas, especialmente durante a gestação, uma vez que não depende do peso corporal (PC), tamanho do animal ou estado fisiológico (EDMONSON et al., 1989). Para vacas de corte, pode ser utilizada uma escala de 1 a 5, em que 1 representa um animal muito magro e 5 representa um animal muito obeso. Vacas com ECC alto no pré-parto (ECC > 3.25) apresentam menor consumo de matéria seca (MS) e maior risco de desenvolverem distúrbios metabólicos no pós-parto. Por outro lado, vacas com ECC baixo (ECC < 2.5) apresentam menor pico de produção de leite e menor produção total ao longo da lactação, o que pode aumentar o intervalo entre partos devido a um período prolongado de anestro (NASEM, 2016).

Tabela 1. Exigências nutricionais de vacas de corte ao longo dos meses após o parto.

Item	**Meses após o parto**											
	1	2	3	4	5	6	7	8	9	10	11	12
Peso corporal, kg	533	533	534	534	536	537	540	545	552	562	577	597
Produção de leite, kg/d	6,7	8,0	7,2	5,8	4,3	3,1	0,0	0,0	0,0	0,0	0,0	0,0
Energia líquida, Mcal/d	15,03	15,99	15,43	14,41	13,42	12,64	8,87	9,18	9,72	10,62	11,98	13,91
Proteína metabolizável, g/d	770	840	799	724	651	591	436	449	471	510	573	672
Cálcio, g/d	33	36	34	31	27	24	16	16	16	29	29	29
Fósforo, g/d	22	24	23	21	19	17	13	13	13	18	18	18

Exemplo adaptado da Tabela 9-7 do Capítulo 9 da 7ª edição do NRC (2000). O peso corporal apresentado é a soma do peso em jejum e peso do concepto. As exigências totais apresentadas são o somatório das demandas de manutenção, crescimento, lactação e gestação de vacas Angus, considerando 8 kg de produção de leite, 533 kg de peso à maturidade e 40 kg de peso ao nascimento para o bezerro.

Na Tabela 1, encontram-se as exigências nutricionais de vacas de corte do parto até o parto subsequente. Observa-se um aumento geral nas exigências imediatamente após o parto, atingindo os níveis mais elevados durante o pico de lactação. Os valores mais baixos de exigência são registrados logo após o desmame, momento propício para a recuperação do escore corporal das vacas de corte. Nesse período, é mais fácil atender às exigências de manutenção, permitindo excedentes de nutrientes para ganho de peso. Após esse intervalo, ocorre novamente um aumento nas exigências, devido à demanda de nutrientes para o desenvolvimento fetal.

Nesse contexto, é essencial dar atenção especial às vacas gestantes que são exclusivamente mantidas em pasto (CHILIBROSTE et al., 2012; GIONBELLI et al., 2015). A quantidade de forragem disponível, conhecida como oferta de forragem (OF), é determinada pela massa de forragem em kg de matéria seca por kg de peso corporal (SOLLENBERGER et al., 1995). A OF desempenha um papel crucial na determinação da disponibilidade de nutrientes para as vacas e está fortemente correlacionada com o desempenho animal, incluindo ganho de peso por animal e por área, juntamente com a composição morfológica e estrutural da pastagem (Santos e Corrêa, 2009).

A intensidade de pastejo dos animais é controlada pela OF, que provoca alterações na massa e altura da forrageira (Soares et al., 2003). Essas alterações, por sua vez, podem influenciar a ingestão de energia (Chapman et al., 2007). Isso ocorre por meio de modificações no peso corporal (PC) (Soares et al., 2003) e na própria atividade de pastejo (Brosh, 2007), impactando o equilíbrio energético das vacas. Portanto, a OF desempenha um papel crucial na determinação da qualidade da alimentação e no atendimento adequado das necessidades nutricionais das vacas gestantes em pasto.

Para melhor compreensão, é preciso compreender que animais em pastejo dividem seu tempo ao longo do dia em cerca de 1/3 do tempo destinado ao pastejo, 1/3 para ócio e 1/3 para ruminação. Assim, aproximadamente 8 horas diárias são destinadas ao consumo de pasto. Adicionalmente, o consumo diário de pasto pode ser expresso pela fórmula conceitual: consumo diário de pasto = massa de bocado × frequência de bocado × tempo de pastejo. Com isso em mente, o tempo médio de pastejo dos animais gira em torno de 8 horas por dia, sendo o restante basicamente dividido entre ruminação e ócio. Os componentes da equação apresentam valores flexíveis para que o consumo dos animais seja mantido. Por exemplo, em uma condição de alta OF em pastos com elevada proporção de folhas e sem alongamento de colmos, a massa de bocado é elevada, e os animais não necessitam de uma alta taxa de bocado ou tempo de pastejo para atingirem sua demanda diária de ingestão.

Em situações de baixa OF, sua estrutura está comprometida, apresentando elevada proporção de colmo e baixa participação de folha na massa de forragem. Neste caso, a massa de bocado é baixa, e a alta proporção de colmos não permite que os animais elevem a taxa de bocado, sendo inevitável que os animais aumentem o tempo de pastejo. Entretanto, o tempo de pastejo não ultrapassa o limite de 10 a 12 horas diárias, ou seja, o consumo é comprometido pelo fator estrutural ou morfológico do pasto. Esses fatores, somados à supressão do rúmen pelo feto e tecidos gestacionais no final da gestação (GIONBELLI et al., 2024) e à baixa qualidade do pasto durante a seca, representam uma limitação no consumo das vacas, o que provoca restrição nutricional.

Um estudo realizado durante dois anos com vacas de corte gestantes (Hereford, Angus e F1 - Hereford × Angus), submetidas a duas OF (4,9 e 2,9 kg de MS/kg de PC), conduzido por Do Carmo et al. (2018), observou, no primeiro ano, que bezerros filhos de mães submetidas a uma alta oferta de forragem apresentaram maior peso ao desmame (120 vs. 102 kg; 102 dias) e maior ganho de peso médio diário (GMD; 0,82 vs. 0,62 kg) em comparação com os bezerros filhos de mães submetidas a uma baixa oferta. No segundo ano, o mesmo padrão foi observado, com maior peso ao desmame (134 vs. 124 kg; 105 dias) e maior GMD (0,80 vs. 0,69 kg) para os animais filhos de vacas submetidas a uma alta oferta de forragem durante a gestação.

O efeito da melhor condição do pasto durante a gestação sobre a progênie pode ser observado a longo prazo, como demonstrado por Underwood et al. (2010), que, durante o terço médio da gestação (60 dias), ofereceram a um grupo de vacas acesso a pasto cultivado com 11% de proteína bruta, enquanto o outro grupo permaneceu em pastagem nativa com 6% de proteína bruta; durante todas as outras fases, o manejo alimentar foi semelhante. Eles observaram maior peso ao desmame (242 vs. 256 kg), além de um maior peso de carcaça quente (329 vs. 348 kg) para os animais provenientes de vacas que tiveram acesso a pastagem de melhor qualidade durante a gestação.

1.2. Programação fetal e seus efeitos no desenvolvimento de tecido muscular, conectivo e adiposo

A programação fetal, também conhecida como programação do desenvolvimento, foi concebida como um conceito pelo Dr. David Barker e seus colaboradores (BARKER, 1990; BARKER, 2004). A partir de estudos epidemiológicos em humanos, foi demonstrado que um baixo peso ao nascimento e outros insultos no desenvolvimento do indivíduo estão fortemente associados ao risco de desenvolver uma variedade de condições patológicas durante a vida adulta (BARKER, 1990; GODFREY; BARKER, 2001). No caso de animais de produção, os primeiros estudos foram realizados em ruminantes durante as décadas de 1950 e 1960, com o objetivo de entender como a programação fetal poderia afetar o crescimento e a produtividade dos rebanhos (SHORT, 1955; TAPLIN; EVERITT, 1964).

Durante a organogênese, órgãos como o cérebro, coração e fígado têm prioridade na alocação de nutrientes provenientes da circulação placentária, devido à sua grande importância para a sobrevivência do indivíduo (ZHU et al., 2006). Por outro lado, o músculo esquelético, embora seja um dos tecidos mais abundantes do corpo, é o mais susceptível à restrição nutricional, pois é um dos últimos na fila para receber nutrientes.

A restrição nutricional materna durante a gestação pode modular uma série de fatores de crescimento que afetam o desenvolvimento dos tecidos fetais, sendo a intensidade dessa restrição um fator determinante. Em condições de restrição nutricional severa, a gliconeogênese fetal é induzida, com aminoácidos como o principal substrato presumido. O resultado é uma redução na síntese proteica dos tecidos fetais e um desaceleramento no crescimento fetal (BELL e EHRHARDT, 2002).

Em termos de regulação nutricional, o fator de crescimento semelhante à insulina I (IGF-I) desempenha um papel fundamental. Portanto, durante a segunda metade da gestação, a restrição nutricional leva a uma diminuição nas concentrações circulantes de IGF-I, ativando uma cascata de fatores miogênicos, principalmente a miostatina (JEANPLONG et al., 2003). A ativação precoce da miostatina regula negativamente a proliferação de células musculares (REBBAPRAGADA et al., 2003).

O desenvolvimento dos tecidos que compõem o músculo esquelético ocorre em três estágios ao longo da vida fetal (Figura 1). Durante o primeiro terço da gestação, são formadas as fibras musculares primárias, que são de metabolismo oxidativo e têm poucos requisitos para o seu desenvolvimento (DU et al., 2010). No segundo terço, a partir das fibras musculares primárias, que servem como "andaime", começa uma segunda onda de formação de fibras musculares secundárias, geralmente do tipo fibras rápidas tipo II (SWATLAND, 1973; RUSSELL e OTERUELO, 1981; DU et al., 2010; GAGNIÈRE et al., 1999). Além disso, nesta fase, ocorre o desenvolvimento do tecido adiposo subcutâneo e, em menor proporção, a adipogênese intramuscular (DU et al., 2017). É importante destacar que a miogênese secundária é responsável pela maior formação de fibras que compõem o músculo esquelético e que permanecem ao longo da vida do animal (GREENWOOD et al., 2000; DU et al., 2010). No último terço da gestação, o desenvolvimento muscular esquelético muda de um crescimento por hiperplasia para hipertrofia, ou seja, ocorre um aumento na massa muscular devido ao aumento no diâmetro das fibras (DU et al., 2010). Além disso, neste último trimestre da fase fetal, a formação do tecido adiposo intramuscular se intensifica (DU et al., 2010).

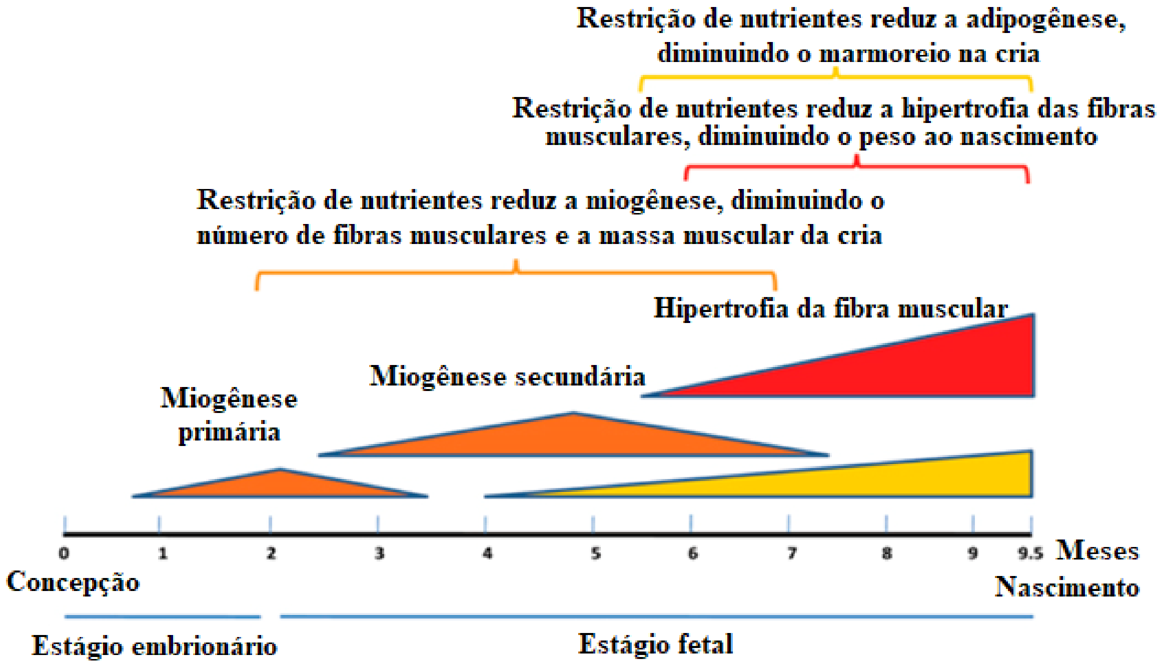


Figura 1. Efeito da nutrição materna no desenvolvimento do músculo esquelético fetal em bovinos de corte. Fonte: Adaptado de Du et al. (2010).

A adipogênese em bovinos de corte começa em fases precoces do desenvolvimento fetal, intensificando-se inicialmente nos depósitos viscerais, seguidos pelos depósitos intermusculares, subcutâneos e, por último, intramusculares. Vale ressaltar que os tecidos subcutâneos e intramusculares continuam a crescer por hiperplasia até os 180 e 250 dias após o nascimento, respectivamente (DU et al., 2013; DU et al., 2017). É importante salientar que as células adipogênicas e fibrogênicas compartilham a mesma linhagem de células progenitoras. Portanto, a adipogênese intramuscular e a fibrogênese são processos competitivos. Dessa forma, a expressão de fatores de transcrição que favoreçam a diferenciação adipogênica e reduzam a diferenciação fibrogênica das células progenitoras aumentará tanto a marmorização quanto a maciez da carne (DU et al., 2013).

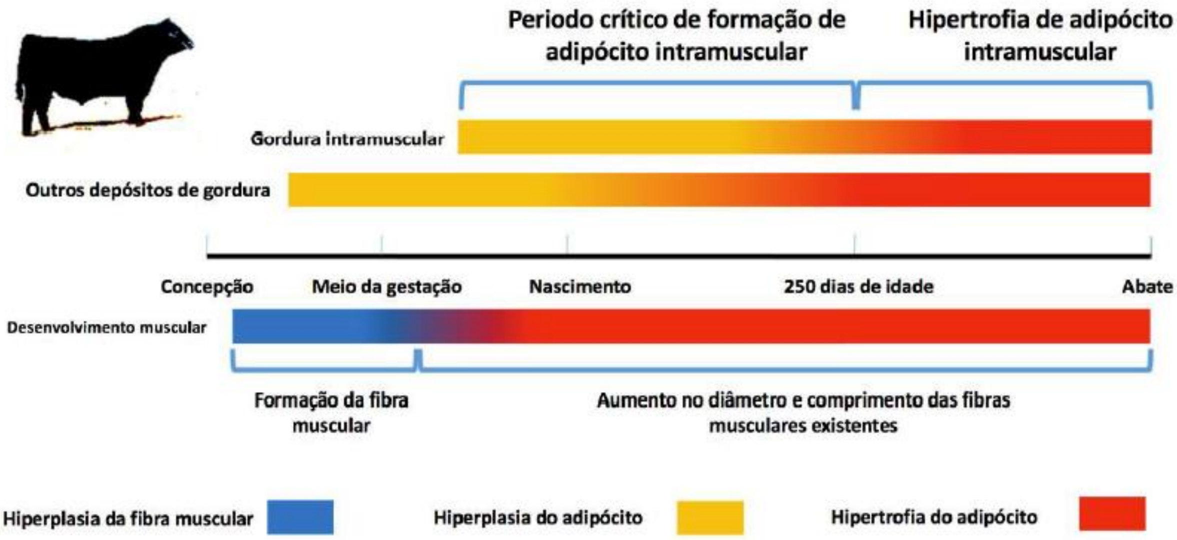


Figura 2. Cronogramas para o desenvolvimento do músculo esquelético e do tecido adiposo em bovinos de corte. Os tempos para hiperplasia e hipertrofia das fibras musculares e hiperplasia e hipertrofia dos adipócitos são aproximados.

A nutrição materna desempenha um papel fundamental nos eventos de programação do desenvolvimento e, consequentemente, afeta a qualidade da carne (Alvarenga et al., 2016; Webb et al., 2019). Por exemplo, a restrição materna de nutrientes durante janelas críticas de desenvolvimento (Caton e Hess, 2010; Reynolds e Caton, 2012) pode influenciar na força de cisalhamento e no perfil de ácidos graxos da progênie no momento do abate, como demonstrado por Alvarenga et al. (2016). Além disso, Webb et al. (2019) mostram que a restrição materna de proteína durante o segundo e terceiro terços da gestação resulta em perda de desempenho, aumento na força de cisalhamento e alterações no perfil de ácidos graxos no músculo da progênie.

Apesar do grande número de estudos sobre nutrição materna em bovinos de corte, ainda não há consenso sobre estratégias de alimentação durante a gestação para garantir os benefícios da programação fetal. No entanto, a compreensão dos complexos mecanismos de controle da miogênese e adipogênese torna possível a manipulação da diferenciação das células progenitoras, visando a maximização do desempenho e da qualidade da carne bovina (Du et al., 2013).

1.3. Epigenética

Os mecanismos epigenéticos são responsáveis por promover modificações nas funções genéticas hereditárias sem alterar a sequência de DNA do indivíduo. Essas mudanças na função de genes e genomas são transmitidas por meio da mitose e, provavelmente, da meiose. Esses mecanismos são essenciais durante a gametogênese, o desenvolvimento embrionário inicial e a subsequente diferenciação celular, reforçando assim as decisões celulares de se comprometerem com diferentes destinos e mantendo a expressão de conjuntos diferentes de genes, tornando essa decisão irreversível (Chavatte-Palmer et al., 2018).

A epigenética torna possível manter todas as linhagens celulares compartilhando um único genoma em um único indivíduo. Além de seu papel na diferenciação celular normal, as marcas epigenéticas podem ser modificadas por fatores ambientais, como a nutrição, e podem ser consideradas como a memória da célula. Esse é o pilar do conceito de programação fetal e influencia diretamente na construção do fenótipo animal.

Podemos citar como exemplo de mecanismos epigenéticos a metilação do DNA, as modificações pós-traducionais de histonas (PTMs) e os RNAs não-codificantes pequenos e grandes, mas eles não se limitam somente a esses (Jammes et al., 2011). Coletivamente, esses mecanismos fornecem remodelagem específica da cromatina e selecionam as informações genômicas que serão transcritas em atributos funcionais.

Adicionalmente, as alterações na expressão gênica como resultado das mudanças epigenéticas respondem de maneira rápida às adaptações sob pressão de seleção ambiental, e seus efeitos podem persistir ao longo de várias gerações (RAVELLI et al., 1998; REYNOLDS et al., 2019). Portanto, a exploração da programação fetal visa minimizar os efeitos adversos do ambiente, a fim de melhorar a eficiência do animal no final do ciclo produtivo (WU et al., 2006; DU et al., 2015).

1.4. Aspectos relacionados à qualidade da carne em bovinos

A qualidade da carne é um conceito que envolve diversos aspectos que se inter-relacionam e é influenciada por fatores que vão desde o modo de preparo da carne até mesmo antes do nascimento. Por isso, podemos dividir os fatores que afetam a qualidade da carne em *antemortem* e *postmortem* (Bridi, 2004; Guerrero et al., 2013).

Alguns exemplos de fatores *antemortem* incluem genética, nutrição materna, idade ao abate, nutrição, sexo e manejo. Quanto aos fatores *postmortem*, podemos citar estimulação elétrica, resfriamento, maturação e modo de preparo (Bridi, 2004). Os principais atributos da carne que são relevantes para a indústria são pH, cor, textura, capacidade de retenção de água e teor de gordura.

A carne nada mais é do que o tecido muscular dos animais que sofre ação de processos bioquímicos após o abate dos animais e consiste principalmente de fibras musculares, gordura subcutânea e intramuscular (gordura marmorizada) e tecidos conectivos. Após o abate, o suprimento de oxigênio é interrompido para as células e, devido à homeostase, o metabolismo celular continua funcionando até que as reservas de energia se esgotem, utilizando o ATP e o glicogênio como fonte (Guo e Greaser, 2017). Após o esgotamento de energia, ocorre a ligação irreversível de actina e miosina, o que resulta no encurtamento máximo do sarcômero, fase caracterizada como *rigor mortis* (England et al., 2017). Em um ambiente anaeróbico, o ácido pirúvico é convertido em ácido lático, o que consequentemente reduz o pH (England et al., 2017).

Na sequência do *rigor mortis*, ocorre a fase de resolução do rigor, caracterizada pelo amaciamento da carne devido à ação de enzimas proteolíticas. Duas isoformas importantes dessas enzimas são a µ-calpaína e a m-calpaína, que degradam a estrutura miofibrilar e influenciam diretamente no amaciamento da carne (Luchiari Filho, 2000). Por outro lado, a calpaína possui um inibidor, a calpastatina, e o aumento da quantidade de calpastatina pode diminuir a maciez da carne (Bridi, 2004). O fator raça também interfere nessa relação; por exemplo, genéticas zebuínas tendem a apresentar maiores concentrações de calpastatina, o que resulta em carne menos macia.

A curva de redução do pH adequada favorece a atividade de enzimas relacionadas à proteólise. Por outro lado, a resistência na queda do pH devido à redução das reservas de glicogênio pode causar a inibição dessa atividade e encurtamento excessivo das fibras, tornando a carne mais dura e com menor capacidade de retenção de água (Ertbjerg e Puolanne, 2017).

Animais mais reativos apresentam maior susceptibilidade ao estresse e, consequentemente, menor reserva de glicogênio, o que limita a produção de ácido lático e aumenta a resistência à queda do pH (Apaoblaza et al., 2017). O fator sexo pode interferir nesse ponto, onde animais inteiros mais predispostos ao estresse apresentam, em geral, carnes com pH mais elevado e, consequentemente, mais duras, com aparência escura e seca, devido a uma maior quantidade de água retida no interior das células, fenômeno conhecido como DFD (dark, firm e dry), um dos principais problemas da indústria brasileira de carne (Purslow, 2017). A carne com coloração escura não é desejada pelo consumidor, além de ser suscetível à proliferação de microrganismos indesejáveis.

A coloração da carne é um dos aspectos determinantes para a compra, sendo o primeiro atributo a ser avaliado pelo consumidor (Mancini e Hunt, 2005). A diferença na coloração da carne também é causada pela quantidade e pelo estado oxidativo da mioglobina, uma proteína presente no músculo responsável por sua oxigenação (Mancini e Hunt, 2005). A mudança na cor da carne é causada principalmente pela oxidação, redução ou desoxigenação que ocorre na mioglobina, dessa forma essa proteína pode estar presente em três formas: oximioglobina, metamioglobina e desoximioglobina (Mancini e Hunt, 2005). O tipo de fibra muscular também interfere na coloração da carne, isso acontece devido à maior atividade física e necessidade de oxigenação dos músculos, o que indica maiores concentrações de mioglobina (Vestergaard et al., 2000) (Purslow, 2017).

Outra característica importante para o consumido é a maciez da carne (Ornaghi et al., 2020) e está relacionada, entre outros fatores, ao comprimento do sarcômero (Battaglia et al., 2019), que é influenciado pela temperatura no resfriamento da carcaça. Adicionalmente, a concentração de colágeno e o grau de acabamento animal também afetam a maciez e se houver exploração das janelas de formação dos tecidos, a programação fetal pode afetar a concentração de colágeno e gordura da carcaça. Carcaças com um acabamento de carcaça com espessura mínima de 3 mm de gordura funcionam principalmente como proteção durante o resfriamento da carcaça, evitando o escurecimento da carcaça, perdas excessivas de água, encurtamento do sarcômero (cold shortening) e, consequentemente, diminuição da maciez (Joo et al., 2017), enquanto o marmoreio influencia na percepção de maciez e no sabor da carne, devido à composição de ácidos graxos (Nian et al., 2019).

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CHAPTER 2 – *The following article is in accordance with Translational Animal Science Journal's publication guidelines.*

IMPACT OF HERBAGE ALLOWANCE DURING MID AND LATE PREGNANCY OF *BOS INDICUS* NELLORE COWS ON POSTWEANING RESPONSES IN MALE OFFSPRING RAISED WITHIN AN INTENSIVE PRODUCTION SYSTEM

Abstract

This study evaluated the influence of different herbage allowance (HA) levels from mid-gestation to calving on post-weaning performance, blood parameters, and carcass traits of young Nellore bulls raised in an intensive production system. Two HA levels, low [LHA; 2.81 kg of forage dry matter (DM) per kg body weight (BW)] and high (HHA; 7.58 kg of forage DM per kg BW), were tested on 72 multiparous, lactating, Nellore beef cows pregnant with male calves on day -151. The cows were stratified by initial body weight (BW = 444 ± 42 kg) and body condition score (3.66 ± 0.28) and randomly allocated to 12 paddocks of *Urochloa brizantha* cv. Marandu. After calving, the cow-calf pairs were managed together until weaning. On day 252, the offspring were placed in 12 one-hectare paddocks and supplemented (1% of BW). Afterward, on day 462, the calves were placed in collective pens in a feedlot for 101 days, and at the end of this period, the animals were slaughtered. Offspring born to HHA cows had a 13.1% higher IGF-1 concentration ($P = 0.034$), while glucose levels were 5.1 mg/dL lower ($P = 0.043$) compared to calves born to LHA cows. The mTOR expression also was greater for HHA ($P < 0.001$). Regarding performance, offspring that received higher HA during gestation showed better feed conversion and efficiency at feedlot ($P \leq 0.047$), with a 17 kg, 21 kg, 23 kg, and 37 kg difference in BW on days 252, 336, 462, and 563, respectively, compared to the LHA treatment ($P < 0.018$). Additionally, there was an 18 kg enhancement in hot carcass weight ($P = 0.005$). Thus, greater HA during the mid and late gestation in Nellore *B. indicus* cows improves post-weaning growth of male offspring, with effects extending throughout the production cycle.

Keywords: Beef cattle, Feedlot, Fetal Programing, Gene expression, Nellore.

1. Introduction

The developing fetus depends exclusively on the dam for nutrient supply, which are delivered through the placenta (Bell and Ehrhardt et al., 2000; Bell et al., 2005). Consequently, maternal nutrition during pregnancy influences the developmental trajectory of the offspring (Martin et al., 2007; Bohnert et al., 2013; Moriel et al., 2020). Numerous studies have been conducted to investigate the impact of maternal gestational nutrition on offspring. However, most of these studies utilized *Bos taurus* beef cows (Moriel et al., 2021; Waldon et al., 2023), and the response to different changes in the environment, energy, nutrient supply, and the effects of nutrients on developmental outcomes differs between *B. taurus* and *B. indicus* breeds (Cooke et al., 2020).

In a recent literature review, Moriel et al. (2021) emphasized that although some recent studies have begun to address the research gap in fetal programming of *B. indicus* genetics, the limited existing research presents an opportunity for future investigations. Our research group recently investigated this topic by supplementing Nellore (*B. indicus*) beef cows during the second and third trimesters of gestation (Rodrigues et al., 2021), focusing on improving cow reproduction and progeny performance. Rodrigues et al. (2021) documented that calves born from supplemented cows exhibited greater birthweights and a higher density of muscle fibers compared to their cohorts from non-supplemented cows. However, no disparities in weaning weight were observed (Rodrigues et al., 2021), nor in slaughter weight (Ramirez-Zamudio et al., 2022). The authors attributed these outcomes to the low performance during rearing phase (Ramirez-Zamudio et al., 2022), compensatory changes in the intestine of offspring from non-supplemented cows (Cruz et al., 2019), and possibly favorable forage conditions that may have attenuated the effect of supplementation during gestation period (Rodrigues et al., 2021).

Nonetheless, grazing spring-calving beef cows are often subjected to nutrient restrictions during the second and third trimesters of gestation (Millen et al., 2011). Herbage allowance (HA) can significantly impact forage intake and productive performance (Chilibroste et al., 2012), potentially resulting in either nutrient surplus or restriction during pregnancy, which in turn affects both in-utero development and postnatal performance of the progeny. Thus, we hypothesized that a high HA during mid to late gestation of *B. indicus* Nellore cows will improve the postweaning development of male offspring compared to cohorts born from cows assigned to a low HA. To test this hypothesis, this experiment aimed to assess the influence of different HA (low vs. high) from mid-gestation to calving on the postweaning performance, blood parameters, and carcass traits of young Nellore bulls raised within an intensive production system.

2. Materials and Methods

The current study was conducted at the Experimental Farm of Agência Paulista de Tecnologia dos Agronegócios, Regional Center of Colina (APTA-Colina), Colina, SP, Brazil (20°43’5’’S and 48°32’38’’W) from June 2019 to June 2021. Procedures used in this study were approved by the Ethics Committee on Animal Use of Development Decentralization Department (CEUA-DDD, protocol no 0004/2020). This manuscript describes the postweaning responses of the male offspring reared within an intensive production system for slaughter. A companion manuscript (Souza, 2021, *manuscript in progress*) describes pre- and postpartum responses of cows, as well as offspring responses from birth through weaning.

2.1. Cow management and treatments

A full description of the management scheme and treatments applied to beef cows is provided in the companion manuscript (Souza, 2021, *manuscript in progress*). Briefly, on day 140 ± 15 of gestation (day -151 of the study), 72 multiparous (4.5 ± 1.5 yr of age) and lactating Nellore beef cows pregnant with male calves via artificial insemination (3 bulls) were stratified by initial body weight (BW = 444 ± 42 kg) and body condition score (BCS = 3.66 ± 0.28), and then randomly assigned to one of 12 *Urochloa brizantha* cv. Marandu paddocks (2 paddocks per block; 6 cows and 7.5 ha per paddock). Prior studies from our research group were used to define the treatments (Rodrigues et al., 2021), setting both a maximum and minimum HA levels. A lower HA was defined as a maximum of 3.50 kilograms of dry matter (DM) per kilogram of BW, while a higher HA was set as a minimum of 6.50 kilograms of DM per kilogram of BW, resulting in two treatments based on HA: low (LHA; 2.81 kg of forage DM per kg BW) and high (HHA; 7.58 kg of forage DM per kg BW; Sollenberger et al., 2005). The treatments were randomly assigned to paddocks within each block (6 paddocks per maternal treatment). In this phase, cows were managed under continuous grazing, with a fixed stocking rate to create differences in HA, resulting in two stocking rates: 3.4 and 1.7 AU/ha in LHA and HHA, respectively. The cows used for adjustment were of the same category and physiological condition.

Treatments were applied from day -151 until day 0 (calving and the end of treatment application). All cows received daily protein supplementation at 0.10% of BW from day -151 to 0 [Matsuda Winter Fós Boi Seca; Matsuda; 40% crude protein (CP), 34% non-protein nitrogen – protein equivalent, 33% total digestible nutrients, 3.5% Ca, 1.8% P, 11.5% Na, 1.5% S, 2.000 mg/kg Mg, 45 mg/kg Co, 350 mg/kg Cu, 25 mg/kg I, 260 mg/kg Mn, 6 mg/kg Se, 1.350 mg/kg

Zn, and 180 mg/kg F]. After calving, on day 0, all cow-calf pairs were placed under the same forage conditions (7332 kg of DM/ha and 5.9% CP) with free-choice access to mineral salt (Matsuda Fós 80 S; Matsuda; 16% Ca, 8% P, 10.7% Na, 1.2% S, 5.000 mg/kg Mg, 107 mg/kg Co, 1.300 mg/kg Cu, 70 mg/kg I, 1.000 mg/kg Mn, 18 mg/kg Se, 4.000 mg/kg Zn, and 800 mg/kg F) until day 221. On day 222, calves were transferred to be weaned to a single pen (25 × 25 m) for a 30-day preconditioning period with *ad libitum* access to Tifton 85 hay, water, and supplement (dry season supplement, Table 2). Due to eight unexpected sexing errors, four calf losses or refusals, one calf death, and three spontaneous abortions, only 56 cow-calf pairs were included in the statistical evaluation.

2.2. Postweaning offspring management

Growing phase

After experimental day 252 (postweaning evaluation), the offspring were moved to twelve 1-hectare paddocks of *Urochloa brizantha* cv. Marandu and equipped with water troughs and feeders, ensuring a minimum of 50 cm per animal. We chose to use the criterion of keeping the experimental units separate, which allows for assessments to be conducted in the individual experimental units. To prevent potential compensatory gains in subsequent stages, which might confound our hypothesis investigation, an intensive supplementation regimen was provided (1% of BW). This phase was subdivided into five 42-d periods to facilitate group management and adjustments in supplement supply. Additionally, the two groups (offspring born from cows subjected to low or high HA) within each block alternated between two paddocks every 21 days to maintain similar forage conditions. Two different supplements were formulated: one for dry season (two initial 42-d periods) and another for the wet season (subsequent three 42-d periods). Forage composition is in Table 1, and supplements are detailed in Table 2.

Finishing phase

On experimental day 462, the offspring was moved from the paddocks and allocated to 12 open-air feedlot pens with an area of 60 m² each (covered feed bunk of 4 m), equipped with individual water fountains with a capacity of 100 L, featuring high-flow valves. In this phase, we also kept the experimental units separated. During a 101-day feedlot period, three different total mixed ration (TMR) diets were offered to the animals once daily, as presented in Table 2. As animals were fed once a day, a 7-day transition between diets was implemented. This transition encompassed the average inclusion of each ingredient for both the current and upcoming diets. For the first 14 days, the acclimation diet was provided, followed by a mixture of the acclimation and growth diets (50:50) for the subsequent 7 days. From day 21 to day 42, the animals received the growth diet, followed by a mixture of the growth and finishing diets (50:50) for 7 days, and after day 49, the animals received the finishing diet exclusively. These diets were formulated to meet the nutrient requirements of Nellore bulls gaining 1.4 kg daily, following the recommendations of the National Academies of Sciences, Engineering, and Medicine (2016). The diets were offered daily at 0800 h *ad libitum* using a mixer wagon (RX-40 E; Casale, São Carlos, SP, Brazil) equipped with a scale. Daily feed orts were weighed, and the amount of feed offered was adjusted to maintain 3% to 5% orts and to measure dry matter intake (DMI).

Slaughter

On experimental day 562, all young bulls were transported to a commercial packing plant (Minerva Foods; Barretos, SP, Brazil), which was located approximately 20 km from the research facility. At the slaughterhouse, the animals were placed in resting pens for 18 hours with free access to water and then subjected to humane slaughter under Brazilian Federal Inspection. Following the slaughter, all carcasses were individually identified for subsequent analyses. All procedures adhered to the guidelines outlined in the 'Regulamento de Inspeção Sanitária e Industrial para Produtos de Origem Animal' (Brasil, 2000).

2.3. Samples collection

Forage samples were collected to assess the quantitative and structural components of the forage canopy every 42 days, from day 252 to 462 (growing phase), samples were collected at the average height of each paddock and divided into four fractions: green leaf, green stem, dead/senescent leaf, and dead/senescent stem. Hand-plucked samples were used to estimate the forage's nutritional value (Sollenberger and Cherney, 1995; De Vries, 1995). The average stocking rate was calculated by dividing the total weight of the animals by the paddock area in hectares, with one animal unit (AU) considered as 450 kg of BW. Samples of ingredients composing the supplements and TMR were collected weekly throughout the study and pooled monthly. Immediately after collection, all forage samples were dried at 55°C for 72 hours using a forced-air oven and then combined with the other collected samples. They were ground in a Wiley mill (Thomas Model 4, Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm mesh sieve before further analysis.

Blood samples (10 mL) were collected from the jugular vein of all animals into commercial tubes (BD Vacutainer® SST II Advance) containing 158 USP units of sodium-heparin on days 252, 462, and 561 to determine the plasma concentrations of glucose, urea, albumin, creatinine, total proteins, cholesterol, triglycerides, aspartate aminotransferase (AAT), gamma-glutamyl transferase (GGT), insulin, and insulin-like growth factor 1 (IGF-1). All blood samples were collected before morning supplementation, immediately placed on ice following collection, and then centrifuged at 3000 × g for 15 minutes at 4 °C. Plasma samples were stored frozen at −20 °C until laboratory analysis.

Liver sampling was performed on day 561 in three animals per experimental unit (randomly selected) via needle biopsy (Tru-Cut biopsy needle; Care Fusion Corporation, San Diego, CA, USA), following the procedure described by Mølgaard (Mølgaard et al., 2012). After local anesthesia with 5 mL of lidocaine, an incision was made between the 10th and 11th ribs, and tissue samples from the right hepatic lobe were collected. Liver samples (~45 mg of tissue) were placed in cryotubes, immediately cleaned of excess blood with sterile gauze, and stored at -80°C until further analysis of gene expression.

BW measurements were recorded after a 16-hour period without water and feed, representing shrunken BW, on days 252, 336, 462, and 563. The interval between days 252 and 336 represents the dry season, while between days 336 and 462 represents the wet season during the growth phase. Intermediate weighings without water and feed deprivation were performed every 42 days during the growing phase to adjust supplement supply.

Carcass ultrasound was performed on offspring on day 561 using a veterinary ultrasound machine, Piemedical—Scanner 200, with a linear probe (ASP-18) and a frequency of 3.5 MHz. Backfat thickness (BFT; mm), and marbling (points 1 to 10) was measured through ultrasound images. Images were taken between the 12th and 13th ribs, transverse to the *Longissimus thoracis* muscle. Thus, fat thickness was measured in the distal middle third of the rib-eye area. Vegetable oil was used as an acoustic coupling agent in all evaluations.

The average daily gain (ADG) was calculated using the initial BW and final BW of each time interval divided by the number of days. At the slaughterhouse, all carcasses were individually weighed to determine hot carcass weight (HCW), and dressing percentage (DP) was calculated by dividing HCW by final BW. Moreover, approximately 24 hours post-slaughter, after cold-chamber storage, all carcasses were weighed to determine cold carcass weight (CCW) and cooling loss (in kg and percentage). Concurrently with cooling loss determination, the pH was measured on the left side of the carcass in the *Longissimus* muscle between the 12th and 13th ribs (Cañeque and Sañudo, 2005), using a potentiometer with digital identification, a temperature compensation sensor, and a glass electrode suitable for deep pH analysis (model 1001-001, Sentron; Amsterdam, The Netherlands). For quantification of primal cuts of the carcass, the right half-carcass was separated into forequarter (between the 5th and 6th rib), thin flank and hindquarter according to the Brazilian Beef Cuts Standards.

2.4. Laboratorial Analysis

The forage and supplement/TMR ingredients were analyzed to evaluate dry matter (DM) content (method 934.01), mineral matter (MM; method 942.05), crude protein (CP; method 978.04), and ether extract (EE; method 920.39). These measurements were conducted following AOAC (1995) methods. Neutral detergent fiber (NDF) content was determined according to the procedure outlined by Robertson and Van Soest (1981) using a Tecnal® TE-149 fiber analyzer

(Piracicaba, São Paulo, Brazil). Cellulose was solubilized with 72% sulfuric acid, and lignin content was determined by the difference (Goering and Van Soest, 1970).

Plasma samples were analyzed in duplicate to assess the concentrations of glucose (K-082–3; Bioclin), urea (K-056; Bioclin), albumin (K-040; Bioclin), creatinine (K-222; Bioclin), total proteins (K-031; Bioclin), cholesterol (K-083; Bioclin), triglycerides (K-117; Bioclin), amino aspartate-transferase (K-048–6; Bioclin), and gamma-glutamyl transferase (K-080–2; Bioclin) using commercial kits with absorbances measured on a spectrophotometer (SBA 200, Celm). Additionally, commercial IGF-1 and insulin kits (IMMULITE® 1000) were used, and readings were performed using chemiluminescence obtained with the Dimension EXL 200 Integrated Biochemistry system (Siemens Healthcare Diagnostics, Munich, Germany). All blood assays were conducted at the Animal Biochemistry and Physiology Laboratory (LBFA) within the Department of Nutrition and Animal Production at the University of São Paulo, São Paulo, Brazil.

Total RNA was extracted using TRIzol Reagent (Invitrogen Co., Carlsbad, CA, USA), following the manufacturer's instructions. The concentration of RNA was determined using a NanoDrop OneC spectrophotometer (Thermo Scientific, Carlsbad, CA, USA), and integrity was assessed by examining the 28S/18S rRNA band pattern on a 1% agarose gel. The isolated RNA was treated with DNase I (Thermo Scientific, Carlsbad, CA, USA) to remove genomic DNA from the samples.

cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied BiosystemsTM, #4368814, Foster City, CA, USA) following the manufacturer's protocol. Primer pairs for the three target genes, including Peroxisome Proliferator Activated Receptor Gamma (PPARγ), Mechanistic Target of Rapamycin Kinase (mTOR), ribosomal protein S6 kinase A1 (RPS6KA1), and two reference genes, actin beta (ACTB) and glyceraldehyde-3-

phosphate dehydrogenase (GAPDH), were designed using the primer3Plus online software program (Untergasser et al., 2007). The specificity of the designed primers was evaluated using the PrimerBlast software in the NCBI database (Ye et al., 2012). The primer sequences used are listed in Table 3.

Quantitative analyses of mRNAs were carried out in a CFX96TM thermocycler (Bio-Rad) in 10 μL reactions containing 2x qPCRBIO SyGreen Mix (PCRBiosystems, London, UK), 1 μL cDNA, and forward and reverse primers at optimized concentrations determined for each gene (Table 3). The cycling parameters for ACTB, GAPDH, PPARγ, and mTOR were 2 min at 95 °C, followed by 40 cycles of 5 s at 95 °C and 30 s at 60 °C. Finally, the cycling conditions for the Rps6K gene were 2 min at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at 60 °C, and 10 s at 78 °C. All reactions were followed by melt curve analysis to confirm amplification of the single cDNA products. All samples were amplified at least twice from the same RNA preparation, and the mean values were considered. RT-PCR efficiency was assessed for each gene based on the slope of a linear regression model using a pool of cDNA samples as a PCR template in a range of 3-fold dilution series. PCR amplification efficiency was calculated using the following equation: E= 10(-1/slope) (Pfaffl, 2001).

2.5. Statistical analyses

Initially, the mathematical assumptions of data normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test) were tested. The data were analyzed as a complete block design using the MIXED procedure of SAS (version 9.4) with paddock as the experimental unit, while calf (paddock) was included as random effects in all statistical analyses. Offspring performance data and blood parameters were analyzed as repeated measures and tested for fixed effects of maternal treatments, day of the study, and all resulting interactions, using calf (paddock) as the subjects. The covariance structure was selected based on the lowest bayesian information criterion. Supplement intake on growing phase, dry matter intake on feedlot, gene expression, carcass ultrasound, and carcass traits were tested for fixed effects of maternal treatment using calf (paddock) as a random effect. For all variables, the initial BW of the cow was included as a covariate ($P \leq 0.05$). All results are reported as least-square means. Means were separated by PDIFF when a significant F-test was detected. A probability of $P \leq 0.05$ was considered significant, while tendencies were considered when $0.05 < P \leq 0.10$ for all tests.

3. Results

There was no interaction between maternal treatments × days for any of the blood parameters ($P \geq 0.164$, Table 4). Concerning the maternal treatments effects on blood parameters, offspring born to HHA cows exhibited a 13.1% higher IGF-1 concentration ($P = 0.034$), while glucose was 5.1 mg/dL lower ($P = 0.043$) compared to their cohorts born to LHA cows. The other blood parameters were not influenced by maternal treatments ($P \geq 0.201$). About collection day, there was a trend for insulin to increase over time ($P = 0.068$), while the other parameters were influenced by collection days ($P \leq 0.001$). Albumin, total protein, aspartate aminotransferase, gamma-glutamyl transferase, and glucose increased over the collection days ($P \leq 0.001$), while IGF-1 and triglycerides increased until day 462 and then decreased again ($P \leq 0.001$). Urea concentrations raised until day 336 (34.9 mg/dL), followed by a decline on day 462 (20.2 mg/dL), and a subsequent increase on day 563 (34.5 mg/dL, $P \leq 0.001$). Creatinine and cholesterol initially increased until day 462 and then exhibited a subsequent decrease ($P \leq 0.001$).

The relative gene expression in the liver samples is presented in Figure 1. Maternal treatments had a significant impact on relative mTOR expression, with higher values observed for HHA compared to LHA (1.62 vs. 0.90, $P < 0.001$). However, PPARγ and Rps6k were not influenced by maternal treatments ($P > 0.100$) and exhibited mean values of 1.24 and 1.05, respectively.

In relation to animal performance (Table 5), during the first 84 days of the growing phase (dry period), the HHA maternal treatment resulted in higher supplement intake (2.250 vs. 2.029 kg/day, $P = 0.012$) compared to LHA, while supplement conversion and efficiency were not influenced ($P \geq 0.253$), with mean values of 2.935 kg/kg of BW and 0.343 kg of BW/kg, respectively. During the 126 days of wet season, supplement intake tended to be higher for the HHA maternal treatment compared to LHA (3.244 vs. 2.981 kg/day, $P = 0.052$). However, supplement conversion (2.821 vs. 3.004 kg/kg of BW, $P = 0.028$) and efficiency (0.356 vs. 0.333 kg of BW/kg, $P = 0.031$) were better for LHA offspring than HHA. Dry matter intake tended to be higher for HHA compared to LHA during the finishing phase (9.036 vs. 8.488 kg/day, $P = 0.105$). Additionally, feed conversion and feed efficiency were better for the HHA maternal treatment than LHA ($P \leq 0.047$).

The offspring born to LHA cows were lighter than their HHA cohorts throughout the study ($P \leq 0.018$), with differences of 17 kg, 21 kg, 23 kg, and 37 kg on day 252, day 336, day 462, and day 563, respectively. The average daily gain during the growing phase was similar between maternal treatments ($P \geq 0.306$), with means of 0.723 kg/day during dry season (from day 252 to 336) and 1.069 kg/day during wet season (from day 336 to 462). However, there was a significant maternal treatment effect on offspring performance during the finishing phase ($P < 0.001$), where HHA induced a +0.144 kg/day gain compared to LHA (1.329 vs. 1.185 kg/day).

Regarding carcass traits, there was an effect of maternal treatment on HCW, where HHA presented carcasses 18 kg heavier than LHA (305 vs. 287 kg, $P = 0.005$). Additionally, carcass ADG was 0.065 kg/day higher for the HHA maternal treatment compared to LHA ($P = 0.039$). Carcass transfer, dressing percent, backfat thickness, and pH were similar between the treatments ($P = 0.257$). Cold carcass weight and cooling loss in kg were higher for HHA compared to LHA ($P \leq 0.033$), while there was no difference between maternal treatments for cooling loss in % ($P = 0.937$). With respect to carcass primal cuts, there was an effect of maternal treatment on forequarter and hindquarter in kg, which were greater for HHA compared to LHA ($P \leq 0.017$). Thin flank in kg was similar between the treatments ($P = 0.234$). However, forequarter, hindquarter, or thin flank were not influenced by maternal treatment when compared as a proportion of the cold carcass ($P \geq 0.131$).

4. Discussion

A comprehensive presentation of pre- and postpartum outcomes in cows, as well as offspring from birth through weaning, can be found in the companion manuscript (Souza, 2021, *manuscript in progress*). In summary, the estimated intake near calving was 10.28% lower for LHA cows compared to HHA (7.83 vs. 7.10 kg of DM/day). This resulted in meeting 73.1% and 79.7% of the CP/day requirements for LHA and HHA, respectively. Consequently, these differences led to lower BW (-43 kg), ADG (-0.315 kg/day), BCS (-0.4 points), and LMA (-5.4 cm^2) at calving for the LHA treatment due to reduced nutrient intake and tissue mobilization to meet maintenance and gestational tissue requirements.

Depending on the nutritional impact, there may be a restriction of nutrients reaching the fetus through the placenta, leading to metabolic changes with long-term consequences (Sibley et al., 2010). Considering the differences between *B. taurus* and *B. indicus* breeds in stimulus responses (Cooke et al., 2020), and the lack of studies evaluating the effects of maternal insults on *B. indicus* breeds (Moriel et al., 2021), the present study examines postweaning outcomes of male offspring subjected to in utero treatments based on different herbage allowances (HA) from mid-gestation to calving.

Concerning postweaning outcomes, the lower blood levels of insulin-like growth factor I (IGF-I) and higher glucose in the maternal treatment LHA indicate a long-term effect of fetal programming on offspring metabolism. As reported by Micke et al. (2010), reduced blood levels of IGF-I can be observed in the offspring of dams exposed to lower nutrient intake during gestation. IGF-I, an insulin-homologous protein crucial for organism growth and development (Nicholls and Holt, 2016), is primarily synthesized in the liver in response to growth hormone (GH) stimulation from the pituitary gland (Al-Samerria and Radovick, 2021). Additionally, various tissues have the capacity for local production of IGF-I. Among its actions in muscle tissue, it enhances glucose and amino acid uptake, stimulates protein synthesis, and promotes muscle fiber hypertrophy (Nicholls and Holt, 2016; Miller et al., 2022). According to Baker et al. (1993), mouses with IGF-1 deficiency exhibited a reduced growth rate and reached only 30% of their adult weight.

Thus, the metabolic alterations induced by fetal programming in the present study support improved growth potential in the HHA offspring. Their lower serum glucose concentration may have occurred in response to the higher IGF-1 level. Numerous studies have linked the use of IGF-1 as a treatment for insulin resistance, hyperglycemia, and obesity in humans (Froesch et al., 1996; Dunger and Acerini, 1997; Boni-Schnetzler et al., 1999).

These findings align with the higher gene expression of mTOR in the HHA offspring. The sequential activation of IGF-1R, IRS1-2, AKT, and mTOR forms a cascade of intracellular signaling events that integrate information about the cellular environment, including nutrient availability and the presence of growth factors (Yoshida and Delafontaine, 2020; Miller et al., 2022). IGF-I binds to its receptor (IGF-1R) on the cell membrane, triggering a signaling cascade. After IGF-1R activation, IRS1 and IRS2 proteins are phosphorylated, acting as bridges to transmit signals from the receptor to the next phase of the signaling cascade (Schiaffino et al., 2021). AKT is a kinase crucial for regulating cell growth, survival, and metabolism. It is activated through phosphorylation by IRS1-2, initiating a series of events promoting cell growth and proliferation (Al-Samerria and Radovick, 2021; Schiaffino et al., 2021). AKT directly activates mTOR through phosphorylation. mTOR activation leads to positive regulation of protein synthesis, cell growth, and the inhibition of autophagy (Saxton and Sabatini, 2017). Due to these characteristics, mTOR has been used as a marker to evaluate nutritional plans in cattle (Wang et al., 2014; Moriel et al., 2015).

The higher supplement intake during the growth phase in HHA offspring is related to their higher body weight (BW), as supplements were offered at fixed rates relative to BW. As described by Souza (2021, *manuscript in progress*), LHA offspring started the postweaning phase lighter, as the HHA maternal treatment increased BW by 4.2 kg, 13 kg, and 14 kg at birth, day 120, and weaning, respectively. However, the effects of maternal treatments on supplement conversion and efficiency may be related to forage quality. During the dry period, forage quality was low, with high levels of NDF and low CP, and thus, the main limiting factor of intake is physical fill.

In the wet season, the higher supplement intake by HHA offspring may have caused a substitutive effect on forage intake, as the forage had higher quality. In this case, a substitutive effect may have occurred due to the chemical limitation (hepatic oxidation), resulting in a similar total intake but lower forage intake. This is evidenced by the similar performance between treatments during this period. Thus, considering only the supplement intake, the conversion and efficiency were poorer for HHA offspring during the wet season. This approach is related to the hepatic oxidation theory of the control of feed intake proposed by Allen et al. (2009).

During the finishing phase, offspring from the LHA maternal treatment exhibited poorer performance and feed efficiency. This may be attributed to epigenetic effects, constraining their growth potential, as suggested by previously examined metabolic variables and gene expression. According to Du et al. (2017), muscle fiber development can be separated into prenatal and postnatal stages. Muscle fibers primarily develop during the second trimester of gestation, and by birth, virtually all fibers have already formed (Du et al., 2013). Subsequent postnatal muscle growth is characterized by an increase in both the diameter and length of pre-existing muscle fibers (Kuang et al., 2007).

The present study assessed the effects of fetal programming by manipulating HA during mid to late gestation, a critical period for muscle fiber formation. As reported by Souza (2021, *manuscript in progress*), offspring born to cows submitted to the HHA maternal treatment showed a 16% higher number of muscle fibers at birth (106 vs. 91 muscle cells). This, combined with the high nutrient intake during the finishing phase, contributed to greater performance and HCW in HHA offspring compared to LHA. Studies by Stalker et al. (2007) and Larson et al. (2009) support our results; in both works, offspring born to supplemented cows (taurine breeds) had higher performance at feedlot. However, offspring from Nellore cows (*B. indicus*) supplemented during gestation did not show improvement in postnatal performance (Rodrigues et al., 2021; Ramirez-Zamudio et al., 2022; Fernandes et al., 2023). The authors attribute the absence of effects to the high slaughter age (Ramirez-Zamudio et al., 2022) and to the low level of nutritional restriction, which could be mitigated by prioritizing nutrient partitioning by the dams (Rodrigues et al., 2021; Fernandes et al., 2023).

Regarding fat deposition, backfat thickness was not influenced by maternal treatments. The hierarchy of adipose tissue appearance in cattle follows the detection of adipocytes in visceral fat, followed by subcutaneous fat, and lastly, intramuscular fat (Du et al., 2013). The onset of subcutaneous fat occurs during the mid-third of gestation (Du et al., 2017). Although the period in which treatments were applied to cows in the present study favored subcutaneous fat adipogenesis, unlike muscle fibers, the number of adipocytes continues to emerge during early postnatal development, and the total number of adipocytes becomes fixed at adolescence (Spalding et al., 2008).

Moreover, the phenotype is determined by animal genetics and its interaction with the environment. Therefore, as emphasized by Ramos et al. (2024), *B. indicus* breeds have a lower genetic predisposition for fat deposition, and the genetic factor may have minimized the effects of fetal programming on backfat thickness in Nellore offspring. This aspect was potentiated by the physiological condition of the offspring, as intact males have a predisposition to deposit lean tissue (Silva et al., 2019). The study by Christofaro Fernandes et al. (2023) supports these conclusions, observing no effects of fetal programming on the backfat thickness of Nellore offspring. In contrast, the work of Underwood et al. (2010) demonstrated that improved forage conditions during the mid-third of gestation enhanced carcass fat deposition in offspring of taurine cows.

The other carcass traits, such as pH, cooling losses, and primal cuts ratio, were not influenced by maternal treatments. Carcass pH is mainly affected by pre-slaughter management and physiological condition (Mach et al., 2008). Cooling losses are influenced by carcass fat cover, efficiency of the cooling system, temperature, and relative humidity. Additionally, heavier carcasses generally exhibit greater cooling losses due to their larger mass (Savell et al., 2005). The heavier carcasses of HHA offspring presented higher cooling losses when assessed in absolute weight, although as a proportion of the carcass, there was no difference. Regarding primal cuts, maternal treatments showed no impact in the current study when assessed as a proportion of carcasses. However, primal cuts are influenced by various factors, such as fat deposition, sex, and feedlot length (Ferreira et al., 2023).

5. Conclusion

Overall, offspring born to HHA cows had lower blood glucose levels, higher IGF-1 concentrations, exhibited greater mTOR expression, and superior growth throughout the production cycle. Also, they were 37 kg of BW heavier at the end of finishing phase compared to their cohorts born to cows with LHA. HHA offspring showed a carcass ADG 0.065 kg/day higher, along with increased DMI and enhanced feed efficiency, resulting in a HCW 18 kg greater at slaughter. These outcomes suggest that a high HA during mid to late gestation in *B. indicus* Nellore cows improves the post-weaning growth of male offspring, with effects that extend throughout the entire production cycle.

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7. Conflict of Interest Statement

The authors declare no conflict of interest.

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Table 1. Mean of quantitative and qualitative characteristics of *Urochloa brizantha* cv. Marandu throughout the growing phase of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item	**Dry season**		**Wet season**	
Quantitative characteristics, whole canopy				
Height (cm)	22.43	±4.46	22.98	±1.75
Forage mass (kg DM/ha)	4467	±1845	3762	±1008
Green forage mass (kg DM/ha)	550	±349	3079	±1015
Herbage allowance (kg DM/kg BW)	4.26	±2.24	2.60	±1.97
Stocking rate (AU/ha)	2.57	±0.53	3.72	±0.75
Density (kg/m³)	2.08	±0.38	1.78	±0.82
Green leaf (g/kg DM)	76.17	±112.63	561.30	±83.80
Senescent leaf (g/kg DM)	258.91	±117.23	56.52	±44.80
Green stem (g/kg DM)	72.66	±40.80	270.88	±91.27
Senescent stem (g/kg DM)	592.26	±30.77	111.30	±62.75
Leaf:stem ratio	0.84	±1.20	2.72	±1.42
Qualitative characteristics (g/kg DM), hand-plucked samples				
Dry matter (DM)	631.39	±281.74	247.61	±17.19
Ash	65.72	±2.17	68.17	±1.18
Crude protein	63.73	±33.77	105.96	±21.44
Ether extract	13.77	±3.16	13.90	±6.22
Neutral detergent fiber	727.13	±66.02	603.77	±40.33
Acid detergent fiber	394.40	±30.68	310.59	±14.82
Lignin	58.57	±3.69	40.86	±4.56
Neutral detergent fiber-nitrogen (N)	367.99	±71.27	308.80	±69.15

Acid detergent fiber-N	198.94	±47.38	140.91	±24.38
In vitro DM digestibility	649.21	±46.19	803.58	±18.38

Animal unit (AU) = 450 kg of body weight. Means followed by standard deviation. 84 days of dry season and 126 days of wet season.

Table 2. Feed ingredients and chemical composition of the supplements and diets throughout the growing and finishing phase of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item, g/kg of DM	**Growing phase**		**Finishing phase**		
	Dry season	**Wet season**	**Diet A**	**Diet B**	**Diet C**
Ground corn	638	734	140	247	508
Soybean meal	314	221	140	120	85.0
Citrus pulp	-	-	140	247	140
Nutri gordura[1]	-	-	12.0	18.0	42.0
Corn silage	-	-	530	330	187
Urea	-	-	10.0	10.0	10.0
Mineral mix GP[2]	48.0	45.0	-	-	-
Mineral mix FP[3]	-	-	28.0	28.0	28.0
Crude protein	250	200	157	151	130
TDN	700	750	810	840	860
Type	SUP	SUP	TMR	TMR	TMR
Intake, % of BW	1.0	1.0	*Ad libitum*	*Ad libitum*	*Ad libitum*
NEm, Mcal/kg	-	-	1.65	1.70	1.74
NEg, Mcal/kg	-	-	0.95	1.05	1.18

TDN = total digestible nutrient; SUP = supplement for growing phase; TMR = total mixed ration; BW = body weight. Supplement offered 84 days during the dry season and 126 days during the wet season. For the first 14 days, the acclimation diet (diet A) was provided, followed by a mixture of the acclimation and growth diets (diet B; 50:50) for the subsequent 7 days. From day 21 to day 42, the animals received the growth diet, followed by a mixture of the growth and finishing diets (diet C; 50:50) for 7 days, and after day 49, the animals received the finishing diet exclusively.

[1] Nutri Gordura, calcium salts of FA from soybean oil (Nutricorp, Araras, SP, Brazil).

[2] Mineral mix GP = FOSBOVI® Confinamento N; DSM Tortuga; 84.3% non-protein nitrogen – protein, 12.2% Ca, 1.35% P, 2.15% S, 1.45% Mg, 2.15% K, 4.65% Na, 6.50 mg/kg Co, 465.00 mg/kg Cu, 5.80 mg/kg Cr, 23.50 mg/kg I, 915.00 mg/kg Mn, 5.80 mg/kg Se, 1,720.00 mg/kg Zn, 135.00 mg/kg F, 64,000.00 UI/kg vitamin A, 8,000.00 UI/kg vitamin D3, 858.00 UI/kg vitamin E, 745.00 mg/kg sodium monensin, and 2,3 x 10^9 UFC saccharomyces cerevisae.

[3] Mineral mix FP = FOSBOVI® Confinamento CRINA®; DSM Tortuga; 14.0% Ca, 1.6% P, 3.6% S, 2.0% Mg, 3.4% K, 5.6% Na, 8.00 mg/kg Co, 540.00 mg/kg Cu, 6.70 mg/kg Cr, 27,50 mg/kg I, 1,070.00 mg/kg Mn, 6.70 mg/kg Se, 2,000.00 mg/kg Zn, 160.00 mg/kg F, 168,000.00 UI/kg vitamin A, 17,000.00 UI/kg vitamin D3, 1,740.00 UI/kg vitamin E, 90.00 mg/kg biotin, D-Limonene 1,140.00 mg/kg D-Limonene, and 2,70 x 10^9 UFC/kg Saccharomyces cerevisiae.

Table 3. The nucleotide sequences of the PCR primers were used to assay gene expression using real-time quantitative PCR.

NCBI	**Genes**	**Sequence primer (5' – 3')**	**Amplicon size (bp)**	**Final concentration in qPCR reaction**
XM_019984995.1	PPARγ	GGATCAGAAACAGACAAGTTGTTCA GCCAAAACGGCATCTCTGTG	149	300 nM
XM_019976328.1	mTOR	ATGTGCGAACACAGCAACAC CCTTTCACGTTCCTCTCCCC	146	300 nM
XM_019980560.1	Rps6k	GACCCGGAGAATGGTCAAGC CCAGCCTTGACATGGTGTGT	104	300 nM
XM_019960295.1	GAPDH	AGCCGTAACTTCTGTGCTGT GAAGGGGTCATTGATGGCGA	148	300 nM
NM_173979.3	ACTB	ATATTGCTGCGCTCGTGGTC GTACGAGTCCTTCTGGCCCAT	149	300 nM

PCR = polymerase chain reaction; NCBI = National Center for Biotechnology Information; PPARγ = peroxisome proliferator-activated receptor gamma; mTOR = mechanistic target of rapamycin; Rps6k = ribosomal protein S6 kinase; GAPDH = glyceraldehyde-3-phosphate dehydrogenase; ACTB beta-actin.

Table 4. Blood parameters on days 252, 462, and 561 of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item	**Maternal treatment**		**SEM**	***P - value***		
	LHA	**HHA**		**HA**	**Day**	**HA × Day**
Insulin, ulU/mL	10.98	11.07	1.50	0.953	0.068	0.900
Insulin-like growth factor-1[1], ng/mL	287.92	325.52	17.04	0.034	<0.001	0.236
Urea, mg/dL	23.86	23.34	0.86	0.546	<0.001	0.387
Albumin, g/dL	4.74	4.66	0.10	0.411	<0.001	0.749
Total protein, g/dL	11.25	11.08	0.20	0.413	<0.001	0.808
Amino aspartate-transferase, U/L	111.50	108.68	4.27	0.515	<0.001	0.375
Gamma-glutamyl transferase[1], U/L	28.02	27.81	1.30	0.872	<0.001	0.276
Glucose[1], mg/dL	113.52	108.44	2.43	0.043	<0.001	0.967
Creatinine[1], mg/dL	1.78	1.78	0.05	0.959	<0.001	0.164
Cholesterol, mg/dL	147.26	146.18	5.07	0.832	<0.001	0.323
Triglycerides[1], mg/dL	42.65	40.24	1.85	0.201	<0.001	0.921

LHA = low herbage allowance; HHA = high herbage allowance; SEM = standard error medium. Blood samples (10 mL) were collected from the jugular vein of all animals into commercial tubes (BD Vacutainer® SST II Advance) containing 158 USP units of sodium-heparin on days 252, 462, and 561. Samples were collected before morning supplementation, immediately placed on ice following collection, and then centrifuged at 3000 × g for 15 minutes at 4 °C. Plasma samples were stored frozen at −20 °C until laboratory analysis.

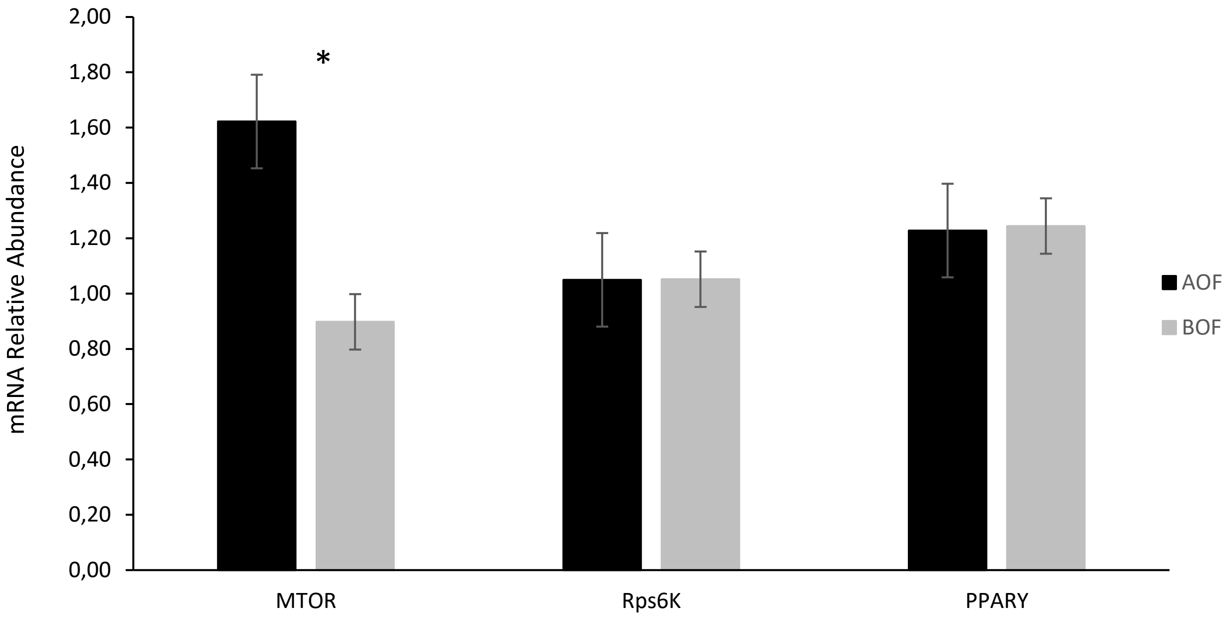

Figure 1. Gene expression profiles were detected using real-time PCR. Liver mRNA expression of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy. Actin beta (ACTB) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as reference genes for the normalization of gene expression in liver tissue samples. Values are mean ± SEM.

Table 5. Postweaning performance of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item	Maternal treatment		SEM	*P - value*
	LHA	**HHA**		
Growing phase - dry season, 84 days				
Supplement intake, kg	2.029	2.250	0.07	0.012
Supplement conversion, kg/kg of BW	2.869	3.001	0.11	0.273
Supplement efficiency, kg of BW/kg	0.351	0.335	0.01	0.253
Growing phase - wet season, 126 days				
Supplement intake, kg	2.981	3.244	0.10	0.052
Supplement conversion, kg/kg of BW	2.821	3.004	0.06	0.028
Supplement efficiency, kg of BW/kg	0.356	0.333	0.01	0.031
Finishing phase - feedlot, 101 days				
Dry matter intake, kg/day	8.488	9.036	0.28	0.105
Feed conversion, kg/kg of BW	7.162	6.080	0.13	0.045
Feed efficiency, kg of BW/kg	0.140	0.147	0.01	0.047
Body weight (BW), kg				
Day 252	179	196	5.93	0.005
Day 336	238	259	7.56	0.007
Day 462	371	394	9.19	0.018
Day 563	490	527	10.9	0.001
Average daily gain, kg/day				
Day 252 to 336	0.703	0.743	0.04	0.306
Day 336 to 462	1.064	1.073	0.03	0.777
Day 462 to 563	1.185	1.329	0.04	<0.001

LHA = low herbage allowance; HHA = high herbage allowance; SEM = standard error medium.

Table 6. Carcass traits of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item	**Maternal treatment**		**SEM**	***P* - *value***
	LHA	**HHA**		
Hot carcass weight, kg	286.9	305.3	6.32	0.005
Carcass transfer, g/kg	669.0	652.0	0.01	0.257
Carcass ADG, kg/day	0.787	0.852	0.03	0.039
Dressing percent, %	58.45	58.40	0.34	0.870
Backfat thickness, mm	4.15	4.24	0.29	0.747
pH	5.71	5.70	0.05	0.857
Cold carcass weight, kg	281.9	300.0	6.23	0.006
Cooling loss, kg	5.05	5.38	0.15	0.033
Cooling loss, %	1.76	1.76	0.05	0.937
Forequarter, kg	63.33	68.09	1.50	0.003
Thin flank, kg	17.09	17.75	0.55	0.234
Hindquarter, kg	60.48	63.87	1.37	0.017
Forequarter, %	44.90	45.48	0.38	0.131
Thin flank, %	12.09	11.89	0.23	0.392
Hindquarter, %	43.01	42.67	0.31	0.275

LHA = low herbage allowance; HHA = high herbage allowance; SEM = standard error medium; ADG = average daily gain.

CHAPTER 3 - *The following article is in accordance with Meat Science Journal's publication guidelines.*

IMPACT OF HERBAGE ALLOWANCE DURING MID AND LATE PREGNANCY OF *BOS INDICUS* NELLORE COWS ON THE MEAT PRODUCTION AND QUALITY OF MALE OFFSPRING RAISED WITHIN AN INTENSIVE PRODUCTION SYSTEM

Abstract

This study evaluated the influence of different herbage allowance (HA) levels from mid-gestation to calving on the meat production and quality of young Nellore bulls raised within an intensive production system. Two HA levels, low [LHA; 2.81 kg of forage dry matter (DM) per kg body weight (BW)] and high (HHA; 7.58 kg of forage DM per kg BW), were tested on 72 multiparous, lactating, Nellore beef cows pregnant with male calves on day -151. The cows were stratified by initial body weight (BW = 444 ± 42 kg) and body condition score (3.66 ± 0.28) and randomly allocated to 12 paddocks of *Urochloa brizantha* cv. Marandu. After calving, the cow-calf pairs were managed together until weaning. On day 252, the offspring were placed in 12 one-hectare paddocks and fed a supplement (1% of BW). After growing phase, paddocks were relocated to feedlot pens for a 101-day period and subsequently slaughtered on day 562 (LHA = 490 ± 7.8 kg and HHA = 527 ± 7.7 kg). The cold carcass weight was 19 kg heavier in HHA than LHA carcasses (289 vs. 308, $P = 0.020$). The *Longissimus* muscle area was 5.3% numerically greater for HHA compared to LHA (83.38 vs. 79.20, $P = 0.109$), while the ratio was higher for HHA compared to LHA (0.57 vs. 0.54, $P = 0.018$). The marbling (3.1 points), backfat thickness (4.2 mm), rump fat thickness (6.4 mm), and pH (5.7), were similar between maternal treatments ($P \geq 0.257$). Regarding meat quality, there was no interaction between maternal treatments × meat aging time (1, 7, or 14 days) for any assessed variable ($P \geq 0.102$). Thus, the low HA during the mid to late gestation of dams led to a decrease in meat production but did not impact the meat quality of young Nellore bulls raised within an intensive production system.

Keywords: Aged steak, Fetal programing, Herbage allowance, Meat quality, Nellore.

1. Introduction

The global demand for meat is steadily increasing, and Brazil plays a crucial role as a global beef producer and exporter, potentially helping to meet the growing demand for meat (FAO, 2009; Malafaia et al., 2021). According to FAO (2009), a significant portion of this demand will be met by tropical and subtropical regions, where *Bos indicus* and *Bos indicus*-influenced cattle predominate (Cooke et al., 2020). However, the beef from these cattle is considered to have lower quality, mainly due to reduced tenderness and intramuscular fat (Ramos et al., 2023). It's important to note that meat quality is influenced by numerous factors, including fetal programming. Several studies have indicated that maternal nutrition during gestation can impact the development of muscular, adipose, and connective tissues, thereby influencing meat production and quality (Du et al., 2010; Du et al., 2013; Du et al., 2017).

Nonetheless, much of the research that gave rise to these concepts was conducted with mice and subsequently validated with *Bos taurus* beef cows (Moriel et al., 2021; Waldon et al., 2023). Responses related to maternal insults during gestation differ between *B. taurus* and *B. indicus* breeds (Cooke et al., 2020). Therefore, the limited knowledge regarding fetal programming in *B. indicus* genetics presents an excellent opportunity for new research (Moriel et al., 2021). Recently, our research team investigated the effects of supplementing Nellore (*B. indicus*) beef cows during the second and third trimesters of gestation, with a focus on improving meat quality

(Ramirez-Zamudio et al., 2022). Nonetheless, meat quality was not influenced by maternal supplementation. These outcomes may be related to the ample forage supply during gestation, which did not impose a significant restriction on the cows, as well as lenient rearing performances (Ramirez-Zamudio et al., 2022; Rodrigues et al., 2021).

Herbage allowance (HA) can significantly influence forage intake and productive performance (Chilibroste et al., 2012), potentially leading to either nutrient surplus or restriction during pregnancy, subsequently impacting meat quality. Additionally, grazing spring-calving beef cows often face nutrient restrictions during the second and third trimesters of gestation (Millen et al., 2011). Therefore, we hypothesized that a low HA during the mid to late gestation period in *B. indicus* Nellore cows would result in poorer meat production and quality in male offspring compared to cohorts born from cows subjected to a high HA. To investigate this hypothesis, our experiment aimed to assess the influence of different HA levels from mid-gestation to calving on the carcass traits and meat quality of young Nellore bulls raised within an intensive production system.

2. Materials and Methods

The current study was conducted at the Experimental Farm of Agência Paulista de Tecnologia dos Agronegócios, Regional Center of Colina (APTA-Colina), Colina, SP, Brazil (20°43’5’’S and 48°32’38’’W) from June 2019 to June 2021. Procedures used in this study were approved by the Ethics Committee on Animal Use of Development Decentralization Department (CEUA-DDD, protocol no 0004/2020). This manuscript describes the meat quality responses of the male offspring reared within an intensive production system for slaughter. Two other companion manuscripts (Souza, 2021, *manuscript in progress*; Cidrini et al., 2024, *manuscript in* *progress*) describe the pre- and postpartum responses of cows, as well as offspring responses from birth to slaughter. In them, a full description of the treatments applied to beef cows and the management scheme of the offspring after birth can be found.

2.1. Experimental management

Pregnant cows and treatments

Shortly, at day 140 ± 15 of gestation (day -151 of the study), 72 multiparous Nellore beef cows, aged 4.5 ± 1.5 years, and lactating, pregnant with male calves via artificial insemination (utilizing 3 bulls), underwent stratification based on their initial body weight (BW = 444 ± 42 kg) and body condition score (BCS = 3.66 ± 0.28). Following stratification, these cows were randomly distributed across one of 12 paddocks formed of *Urochloa brizantha* cv. Marandu (two paddocks per block). Each paddock accommodated 6 cows and 7.5 hectares area. Previous studies from our research group were based to define the treatments (Rodrigues et al., 2021), establishing both a maximum and minimum HA levels. A lower HA was defined as a maximum of 3.50 kilograms of dry matter (DM) per kilogram of BW, while a higher HA was set as a minimum of 6.50 kilograms of DM per kilogram of BW, resulting in two treatments based on HA: low (LHA; 2.81 kg of forage DM per kg BW) and high (HHA; 7.58 kg of forage DM per kg BW; Sollenberger et al., 2005). These treatments were randomly assigned to paddocks within each block, resulting in 6 paddocks for each maternal treatment. Treatments were applied from day -151 until day 0 (calving and the end of treatment application). All cows received daily protein supplementation at 0.10% of BW from day -151 to 0 (Table 1). Due to eight unexpected sexing errors, four calf losses or refusals, one calf death, and three spontaneous abortions, only 56 cow-calf pairs remained in the study.

Cow-calf phase

After calving (from day 0 to 221), all cow-calf pairs were managed as a single group in the same pasture area (7332 kg of DM per ha and 5.9% CP) with free-choice access to mineral salt. The milk production of cows subjected to low and high HA during gestation was similar ($P \geq 0.490$), ensuring that any effect on the offspring would come from fetal programming effects. On day 222, calves were transferred to be weaned in a single pen (25 × 25 m) for a 30-day preconditioning period with *ad libitum* access to Tifton 85 hay, water, and supplement. The composition of the mineral salt and supplement (dry season supplement). used can be found in Table 1.

Growing phase

From day 252 to 462, the offspring were allocated to twelve 1-hectare paddocks of *Urochloa brizantha* cv. Marandu and equipped with water troughs and feeders, ensuring a minimum of 50 cm per animal. This phase was divided into 84 days of the dry season (4467 kg of DM/ha and 6.4% CP) and 126 days of the wet season (3762 kg of DM/ha and 10.6% CP). During this time, the animals received a supplement equivalent to 1% of their BW, with their weights measured every 42 days for supplement supply adjustments. Detailed information of supplements compositions for both dry and wet seasons can be found in Table 1.

Finishing phase

On day 462, the offspring were relocated from the paddocks to 12 open-air feedlot pens, each with an area of 60 m^2. Each pen was equipped with a covered feed bunk measuring 4 meters in length, and water fountain with a capacity of 100 l, featuring high-flow valves. Over a 101-day period in the feedlot, the animals were provided with three different diets, detailed in Table 1. During the initial 14 days, they received the acclimation diet, followed by a 7-day period during which a mixture of the acclimation and growth diets (in a 50:50 ratio) was provided. From day 21 until day 42, the animals were fed the growth diet. Subsequently, for 7 days, they received a mixture of the growth and finishing diets (in a 50:50 ratio), and after day 49, only the finishing diet was provided. The diets were formulated to meet the nutrient requirements of Nellore bulls to achieve a daily weight gain of 1.4 kg, in accordance with the recommendations of the National Academies of Sciences, Engineering, and Medicine (2016). The diets were offered daily *ad libitum*, and orts were weighed to measure dry matter intake (DMI). The quantity of feed provided was adjusted to maintain 3% to 5% orts.

Slaughter procedure

On experimental day 562, all young bulls were transported to a commercial packing plant (Minerva Foods; Barretos, SP, Brazil), located approximately 20 km from the research facility. The offspring were slaughtered with average BW of 490 ± 7.8 for the LHA and 527 ± 7.7 for the HFA. At the slaughterhouse, the animals were placed in resting pens for 18 hours with free access to water and then subjected to humane slaughter under Brazilian Federal Inspection. Following the slaughter, all carcasses were individually identified for subsequent analyses. All procedures adhered to the guidelines outlined in the 'Regulamento de Inspeção Sanitária e Industrial para Produtos de Origem Animal' (Brasil, 2000).

2.2. Sample collection

Carcass ultrasound was performed on offspring on day 561 using a veterinary ultrasound machine, Piemedical—Scanner 200, with a linear probe (ASP-18) and a frequency of 3.5 MHz.

Longissimus muscle area (LMA; cm²), backfat thickness (BFT; mm), and marbling (points 1 to 10) were measured through ultrasound images. Images were taken between the 12th and 13th ribs, transverse to the *Longissimus thoracis* muscle. Thus, fat thickness was measured in the distal middle third of the rib-eye area. Vegetable oil was used as an acoustic coupling agent in all evaluations.

At the slaughterhouse, approximately 24 hours post-slaughter, after cold-chamber storage, all carcasses were weighed to determine cold carcass and four 2.54 cm thick steaks were obtained from the *Longissimus thoracis* of three animals per experimental unit (randomly selected). The steaks were taken from the left side of the carcass between the 9th and 13th ribs, individually identified, and vacuum-packed (99% vacuum) using a Selovac Sealer machine (Selovac, São Paulo, SP, Brazil). All steaks were then stored at -20°C until laboratory analysis. Steaks from day 1 of aging time were used for myofibril fragmentation index, sarcomere length, and malondialdehyde concentration analyses. Additionally, three steaks each animal were stored (aged) for 7 and 14 days in a chilling chamber (4 ± 1°C) to simulate typical Brazilian market conditions. Shear force, cooking loss, water holding capacity, and instrumental meat color were analyzed at 1, 7, and 14 days of aging time.

2.3. Laboratorial Analysis

Myofibril fragmentation index

Before the meat quality analyses started, the samples were thawed at 4 °C for 24 h. Myofibril fragmentation indices (MFI) were determined on muscle according to Culler, Smith, and Cross (1978). Four grams of minced muscle was homogenized for 30 s in 10 vol (v/w) of a 2 °C isolating medium consisting of 100 mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM MgCl, and 1 mM sodium azide. The protein concentration of the myofibril suspension was determined by the biuret method as described by Gornall, Bardawill, and David (1949). An aliquot of the myofibril suspension was diluted with an isolating medium to reach a protein concentration of 0.5 ± 0.05 mg/ml. Protein concentration was determined by the biuret method. The diluted myofibril suspension was stirred and poured into a cuvette; absorbance of this suspension was measured immediately at 540 nm. Absorbance was multiplied by 200 to give an MFI for each sample.

Sarcomere length

The sarcomere length was measured as described by Cross, West, and Dutson (1981). The sample (the remaining 1-day aging time sample from Warner-Bratzler shear force) was incubated in 0.1 M $NaHPO_4$ + 0.2 M Sucrose buffer overnight at 4º C. After that, the fibers were removed and subjected to Laser diffusion (Thorlabs; model HNL020R, USA). For the calculation the sarcomere length has used the equation below:

$$\mu = \frac{0.6328 \times \mathrm{D} \times \sqrt{\left(\frac{T^2}{D}\right) + 1}}{\mathrm{T}}$$

where:

D = Distance (mm) of the sample to the sheet where the image will be drawn (preferably 100 mm); T = Space (mm) between the diffraction bands; laser wavelength (632.8×10^{-3}).

Malonaldehyde concentration

The method used to measure lipid oxidation was Thiobarbituric Acid Reactive Substances (TBARS) described by Pikul, Leszczynski, and Kummerow (1989). Five g of meat sample was weighed and 20 mL of trichloroacetic acid (7.5%) added and homogenized for 2 minutes. Samples were filtered and 5 ml of the filtrate was added in test tubes with 5 mL of TBA (0.02M thiobarbituric acid) solution. The malonaldehyde concentration was determined by a spectrophotometer (Thermo ScientifictTM MultiskanTM GO) with an absorbance of 540 nm.

pH and Warner – Bratzler shear force

The pH was measured using a potentiometer with digital identification, a temperature compensation sensor, and a glass electrode suitable for deep pH analysis (model 1001-001, Sentron; Amsterdam, The Netherlands). The Warner–Bratzler shear force analysis was determined according to procedures of AMSA (1995). A steak sample (2.54 cm) was cooked on a pre-heated (180 °C) electric oven (Feri90, Venâncio, Venâcio Aires, RS, Brazil) with its internal temperature monitored by thermocouples to prevent the temperature variation from cooking. The samples were turned when it reached an internal temperature of 40 °C and take out from the oven with the sample reached an internal temperature of 72 °C, the sample was removed from the oven and kept at room temperature. From each steak, eight homogeneous cylinders, 1.27 cm in diameter, parallel to the orientation of the muscle fibres, avoiding connective tissue and fat, were obtained using a stainless-steel sampler. The cylindrical samples were sheared perpendicularly to the orientation of the muscle fibres, using shear force equipment (model CT3 25K – texture analyser- Brookfield) with a Warner–Bratzler blade. The device was equipped with a stainless-steel blade of 1.18 mm od thickness and 126.77 mm of height containing a veeshaped (60 angle) cutting edge, with a capacity of 25 kg at a velocity of 20 cm/min. The results were presented in Newton (N).

Cooking loss and water holding capacity

Cooking losses calculated from drip and evaporation losses of beef steaks, whereby cooking losses was calculated by the difference in weight before and after cooking the steak and the values were expressed in percentage. The water holding capacity (WHC) was calculated by the difference of weight of a meat sample (approximately 2 grams) before and after subjected to the pressure of 10 kg for five minutes.

Display - Instrumental meat color

The beef samples were thawed 24 hours in refrigerated room before the analysis. The meat samples were packed in polystyrene trays over-wrapped with a retractile film (oxygen permeable) without touching the muscle and displayed in a refrigerated expositor (Metalfrio, model: VB40R, Metalfrio Solutions Ltda - Brazil) at 4 ± 1 °C and fluorescent light (1,080 lx, 12 h), simulating typical Brazilian markets real conditions. The colour was measured at 30 min of oxygen exposed, 1, 7 and 14 days. Three measurements were made of colour for each steak using a portable spectrophotometer CR-400 (Konica Minolta sensing, Ins., Tokyo, Japan) using D65 illuminant and 10° standard observer, which was calibrated before use. The colour was evaluated for lightness (L*), redness (a*) and yellowness (b*) based on the CIELab systm (Commission Internationale de I'Eclairage), and chroma (C*). The hue-angle (an indicator of the angle at which a vector radiates into the red-yellow quadrant; h*) (Cañeque et al., 2004; Pflanzer & de Felício, 2011) and ΔE values were calculated as:

$$h^* = tan^{-1\frac{b^*}{a^*}}$$

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$$

where ΔL^*, Δa^* and Δb^* are the derivatives of corresponding parameters.

2.4. Statistical analyses

Initially, the mathematical assumptions of data normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett test) were tested. The data were analyzed as a complete block design using the MIXED procedure of SAS (version 9.4) with paddock as the experimental unit, while calf (paddock) was included as random effects in all statistical analyses. pH, shear force, cooking loss, water holding capacity, and display were analyzed as repeated measures and tested for fixed effects of maternal treatments, day of the study, and all resulting interactions, using calf (paddock) as the subjects. The covariance structure was selected based on the lowest Bayesian information criterion. Malonaldehyde concentration, sarcomere length, and myofibril fragmentation index were tested for fixed effects of maternal treatment using calf (paddock) as a random effect. For all variables, the initial BW of the cow was included as a covariate ($P \leq 0.05$). All results are reported as least-square means. Means were separated by PDIFF when a significant F-test was detected. A probability of $P \leq 0.05$ was considered significant, while tendencies were considered when $0.05 < P \leq 0.10$ for all tests.

3. Results

The CCW of offspring born to cows subjected to LHA during mid to late gestation was 19 kg lighter compared to their HHA cohorts (289 vs. 308, $P = 0.020$). The *Longissimus* muscle area was 5.3% greater for HHA compared to LHA (83.38 vs. 79.20, $P = 0.109$), while the ratio was 5.6% higher for HHA (0.57 vs. 0.54, $P = 0.018$). The remaining carcass traits showed no significant differences between maternal treatments ($P \geq 0.257$, Table 2), with mean values of 3.1 points, 4.2 mm, 6.4 mm, and 5.7 for marbling, backfat thickness, rump fat thickness, and pH, respectively.

Regarding the meat quality parameters assessed only on day 1, no effects of maternal treatments were observed (Table 3; P ≥ 0.131). The variables sarcomere length, myofibrillar fragmentation index, and malonaldehyde presented respective means of 1.79 µm, 79.9, and 0.189 mg/kg of meat.

There was no interaction between maternal treatments × aging time for any variable evaluated (P ≥ 0.102; Table 3). Furthermore, there were no significant effects of HA on the measured variables (P ≥ 0.313), except for a trend indicating higher water holding capacity in HHA steaks compared to LHA (32.96 vs. 31.76, P = 0.074). When considering the impact of aging time, cooking loss showed no significant differences among steaks stored for 1, 7, and 14 days (P = 0.235), while yellowness tended towards higher values on day 1 (P = 0.085). However, the other parameters displayed significant variations with aging time (P ≤ 0.012). Shear force and redness decreased over time, while lightness was lower on day 1. Thawing loss and chroma increased over the storage period. Water holding capacity was higher on day 14 of aging. Hue values increased with aging time, and pH initially decreased from 5.81 on day 1 to 5.80, followed by a subsequent increase to 5.88 on day 14.

4. Discussion

In the present study, a 6.6% increase in cold carcass weight was observed, associated with a larger *Longissimus* muscle area and an improved ratio in the HHA offspring carcasses. Together, these parameters confirm the hypothesis that the offspring of Nellore cows subjected to better nutritional conditions during gestation exhibit higher meat production. This connection arises from the fact that fetal muscle fiber formation occurs during the mid-third of gestation (hyperplasia), with virtually all muscle fibers formed at birth (Kuang et al., 2007). Subsequent growth ensues through an increase in the diameter and length of muscle cells (hypertrophy).

Previous studies evaluating the same animals from the current trial demonstrate that cows from HHA maternal treatment cows gave birth to calves that were 4.2 kg heavier with a higher density of muscle fibers compared to LHA (Souza, 2021, *manuscript in progress*), in addition to exhibiting a higher body weight throughout the production cycle (Cidrini et al., 2024, *manuscript in progress*). This justifies the greater cold carcass weight and meat production of offspring from cows subjected to this maternal treatment. These findings align with other studies that found heavier carcasses when taurine cows were subjected to better nutritional conditions during gestation (Stalker et al., 2007; Underwood et al., 2010).

However, these results diverge of Ramirez-Zamudio et al. (2022) and Fernandes et al. (2023), who did not observe effects of maternal nutrition on carcass characteristics. These authors attribute the similarity in characteristics to the low level of nutritional restriction during the gestational phase, which can be compensated through physiological and metabolic adaptations in the dam (Rotta et al., 2015) to maintain nutrient supply to the fetus. In our study, we chose to provide an intensive level of supplementation to the offspring, ensuring conditions to express their productive potential and preventing possible epigenetic alterations that could silence the expression of genes differentially expressed by nutrition during the gestational phase and compromise our hypothesis.

Regarding the degree of carcass finishing (fatness), no effects were observed on marbling, backfat thickness, and rump fat thickness. In contrast, Underwood et al. (2010) found carcasses with greater backfat thickness when offering better forage conditions during the mid-third of gestation. This occurs due to fetal programming on adipose tissue, especially on subcutaneous and visceral fat, which predominantly develops in beef cattle during the mid-gestation to neonatal stage, occurring just before the development of intramuscular adipocytes (Du et al., 2013). In contrast, marbling fat has a more extended window, with the potential formation of intracellular fat adipocytes up to 250 days post-partum in cattle (Du et al., 2017).

Nonetheless, *B. indicus* breeds are less prone to accumulate fat in the carcass compared to *B. taurus* breeds. This is attributed to the smaller volume of adipocytes, a factor that may be related to an inherent mechanism facilitating heat dissipation and ensuring adaptation to tropical and subtropical regions (Cooke et al., 2020). This reduced genetic predisposition may have minimized the effects of fetal programming on fat deposition in the carcasses of the HHA maternal treatment in the present study. These conclusions are supported by the findings of Ramirez-Zamudio et al. (2022) and Fernandes et al. (2023), who also did not observe effects of better nutritional conditions during gestation on carcass finishing. Furthermore, in our study, the animals were not castrated, and this condition makes them even more prone to deposit lean tissue (Silva et al., 2019).

There was no difference in sarcomere length among the offspring from different maternal treatments. This aligns with the findings related to fatness, as subcutaneous fat contributes to carcass protection (thermal insulation) during cold room storage. It also reduces the impact of rapid cooling by preventing fiber shortening, which, in turn, helps maintain sarcomere length and preserves beef tenderness (Tait et al., 2005).

The absence of effect on the analyzed variables in steaks aged for 1, 7, or 14 days indicates that, contrary to our hypothesis, fetal programming, in general, was unable to alter the meat quality of Nellore offspring raised within intensive systems. Characteristics highly relevant to consumer perception of meat quality, such as tenderness (Shear Force), color, and juiciness, were only affected by aging time. It is worth noting that, following the methodology described by Destefanis et al. (2008), steaks from the offspring of both treatments were considered tough at the 1-day aging time with 62.75 N, and intermediate on days 7 and 14 with 49.32 N and 43.67 N, respectively (tenderness classes: tender <42.87 N; intermediate = 42.87 N ≤ WBSF ≤52.68 N; and tough >52.68 N). This reinforces the perception that steaks from *B. indicus* animals do not exhibit tender meat, and processes like aging contribute to meat tenderness, especially in non-castrated animals.

5. Conclusion

In summary, under the conditions analyzed in our study, the results indicate that a low HA during the mid to late gestation period of the dams reduces meat production. However, it did not result in poorer meat quality in young Nellore bulls raised within an intensive production system compared to cohorts born from cows subjected to a high HA. Furthermore, these responses related to meat quality may be associated with the genetic predisposition and sexual condition of the offspring.

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7. Conflict of Interest Statement

The authors declare no conflict of interest.

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Table 1. Feed ingredients and chemical composition of the supplements and diets throughout the cow/calf, growing, and finishing phase of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item, g/kg of DM	**Cow/calf phase**		**Growing phase**		**Finishing phase**		
	Pre-calving	**Post-calving**	**Dry season**	**Wet season**	**Diet A**	**Diet B**	**Diet C**
Ground corn	145	-	638	734	140	247	508
Soybean meal	390	-	314	221	140	120	85.0
Citrus pulp	-	-	-	-	140	247	140
Nutri gordura[1]	-	-	-	-	12.0	18.0	42.0
Corn silage	-	-	-	-	530	330	187
Urea	115	-	-	-	10.0	10.0	10.0
Mineral mix[2]	350	-	-	-	-	-	-
Mineral mix[3]	-	100	-	-	-	-	-
Mineral mix[4]	-	-	48.0	45.0	-	-	-
Mineral mix[5]	-	-	-	-	28.0	28.0	28.0
Crude protein	500	-	250	200	157	151	130
TDN	450	-	700	750	810	840	860
Type	SUP	MS	SUP	SUP	TMR	TMR	TMR
Intake, % of BW	0.1	*Ad libitum*	1.0	1.0	*Ad libitum*	*Ad libitum*	*Ad libitum*
NEm, Mcal/kg	-	-	-	-	1.65	1.70	1.74

NEg, Mcal/kg	-	-	-	-	0.95	1.05	1.18

TDN = total digestible nutrient; MS = Mineral salt; SUP = supplement; TMR = total mixed ration; BW = body weight. Supplement offered 84 days during the dry season and 126 days during the wet season. For the first 14 days, the acclimation diet (diet A) was provided, followed by a mixture of the acclimation and growth diets (diet B; 50:50) for the subsequent 7 days. From day 21 to day 42, the animals received the growth diet, followed by a mixture of the growth and finishing diets (diet C; 50:50) for 7 days, and after day 49, the animals received the finishing diet exclusively.

[1] Nutri Gordura, calcium salts of FA from soybean oil (Nutricorp, Araras, SP, Brazil).

[2] Mineral mix = 3,5% Ca, 2,0% P, 8,0% Na, 1,5% S, 5000mg/kg Mg, 45mg/kg Co, 400mg/kg Cu, 25mg/kg I, 260mg/kg Mn, 9mg/kg Se, 1700mg/kg Z, 200mg/kg F.

[3] Mineral mix = 16,0% Ca, 8,0% P, 10,7% Na, 1,2% S, 5000mg/kg Mg, 107mg/kg Co, 1300mg/kg Cu, 70mg/kg I, 1000 mg/kg Mn, 18mg/kg Se, 4000mg/kg Zn, and 800 mg/kg F.

[4] Mineral mix = FOSBOVI® Confinamento N; DSM Tortuga; 84.3% non-protein nitrogen – protein, 12.2% Ca, 1.35% P, 2.15% S, 1.45% Mg, 2.15% K, 4.65% Na, 6.50 mg/kg Co, 465.00 mg/kg Cu, 5.80 mg/kg Cr, 23.50 mg/kg I, 915.00 mg/kg Mn, 5.80 mg/kg Se, 1,720.00 mg/kg Zn, 135.00 mg/kg F, 64,000.00 UI/kg vitamin A, 8,000.00 UI/kg vitamin D3, 858.00 UI/kg vitamin E, 745.00 mg/kg sodium monensin, and 2,3 x 10^9 UFC Saccharomyces cerevisae.

[5] Mineral mix FP = FOSBOVI® Confinamento CRINA®; DSM Tortuga; 14.0% Ca, 1.6% P, 3.6% S, 2.0% Mg, 3.4% K, 5.6% Na, 8.00 mg/kg Co, 540.00 mg/kg Cu, 6.70 mg/kg Cr, 27,50 mg/kg I, 1,070.00 mg/kg Mn, 6.70 mg/kg Se, 2,000.00 mg/kg Zn, 160.00 mg/kg F, 168,000.00 UI/kg vitamin A, 17,000.00 UI/kg vitamin D3, 1,740.00 UI/kg vitamin E, 90.00 mg/kg biotin, D-Limonene 1,140.00 mg/kg D-Limonene, and 2,70 x 10^9 UFC/kg Saccharomyces cerevisiae.

Table 2. Carcass traits and meat quality parameters of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item	**Maternal treatment**		**SEM**	***P* - *value***
	LHA	**HHA**		
	Carcass traits			
Cold carcass weight, kg	288.5	308.1	8.02	0.020
Longissimus muscle area, cm²	79.20	83.38	2.51	0.109
Ratio	0.54	0.57	0.01	0.018
Marbling, points	3.20	3.03	0.15	0.257
Backfat thickness, mm	4.15	4.24	0.29	0.747
Rump fat thickness, mm	6.37	6.41	0.47	0.927
	Meat quality parameters			
Sarcomere length, µm	1.79	1.78	0.03	0.794
Myofibrillar fragmentation index	79.95	78.85	4.36	0.802
Malonaldehyde, mg/kg of meat	0.179	0.198	0.01	0.131

LHA = low herbage allowance; HHA = high herbage allowance; SEM = standard error medium; ADG = average daily gain.

Table 3. Meat quality parameters of offspring born to cows submitted to two different herbage allowances (HA) during mid and late pregnancy.

Item	**Maternal treatment**		**SEM**	**Aging time, days**			**SEM**	***P - value***		
	LHA	**HHA**		**1**	**7**	**14**		**HA**	**Day**	**HA × Day**
Shear force, N	51.57	52.25	3.29	62.75c	49.32b	43.67a	2.70	0.838	<0.001	0.145
pH	5.84	5.82	0.09	5.81b	5.80ab	5.88c	0.08	0.866	0.012	0.484
Lightness	36.47	36.41	0.93	34.58a	37.05b	37.69b	0.81	0.954	<0.001	0.880
Redness	15.74	16.28	0.52	18.47c	15.25b	14.32a	0.46	0.313	<0.001	0.604
Yellowness	7.66	8.07	0.57	8.36b	7.63a	7.61a	0.56	0.475	0.085	0.158
Chroma	17.65	18.25	0.61	20.28b	17.16a	16.41a	0.50	0.334	0.001	0.516
Hue	26.12	26.19	1.71	23.69ab	26.51b	28.26c	1.19	0.968	0.002	0.381
Water holding capacity, %	31.76	32.96	0.65	33.95b	32.75b	30.39a	0.97	0.074	0.006	0.152
Thawing loss, %	3.31	3.52	0.23	3.84b	3.20a	3.20a	0.18	0.386	<0.001	0.102
Cooking loss, %	22.19	22.26	0.76	23.03c	21.73a	21.91b	0.65	0.929	0.235	0.508

LHA = low herbage allowance; HHA = high herbage allowance; SEM = standard error medium.

CONSIDERAÇÕES GERAIS

A prole nascida de vacas com alta oferta de forragem (AOF) durante o terço médio e final da gestação apresentou níveis mais baixos de glicose no sangue, concentrações mais altas de IGF-1 e exibiram maior expressão de mTOR, resultando em um crescimento superior ao longo de todo o ciclo de produção, quando comparada com animais nascidos de vacas com baixa oferta de forragem (BOF).

Durante a fase de terminação, a prole de AOF demonstrou um ganho de peso diário de carcaça 0,065 kg/dia maior, acompanhado de um aumento no consumo de matéria seca (DMI) e uma melhor eficiência alimentar. Isso resultou em um peso corporal 37 kg superior e um peso da carcaça quente 18 kg maior no abate, em comparação com animais nascidos de vacas com BOF. No entanto, essa diferença não se traduziu em uma qualidade de carne inferior em tourinhos Nelore criados dentro de um sistema de produção intensivo, nascidos de vacas submetidas a uma BOF.

Esses resultados sugerem que uma alta oferta de forragem durante o terço médio e final da gestação em vacas Nelore *B. indicus* melhora o crescimento pós-desmame da prole, com efeitos que se estendem por todo o ciclo de produção. Essas informações têm um impacto significativo na cadeia produtiva, uma vez que evidenciam que ajustes de manejo que não requerem investimentos adicionais, como o manejo correto do pasto, podem gerar impactos positivos ao longo da vida dos animais e aumentar o lucro dos pecuaristas envolvidos na atividade de cria, recria e engorda.

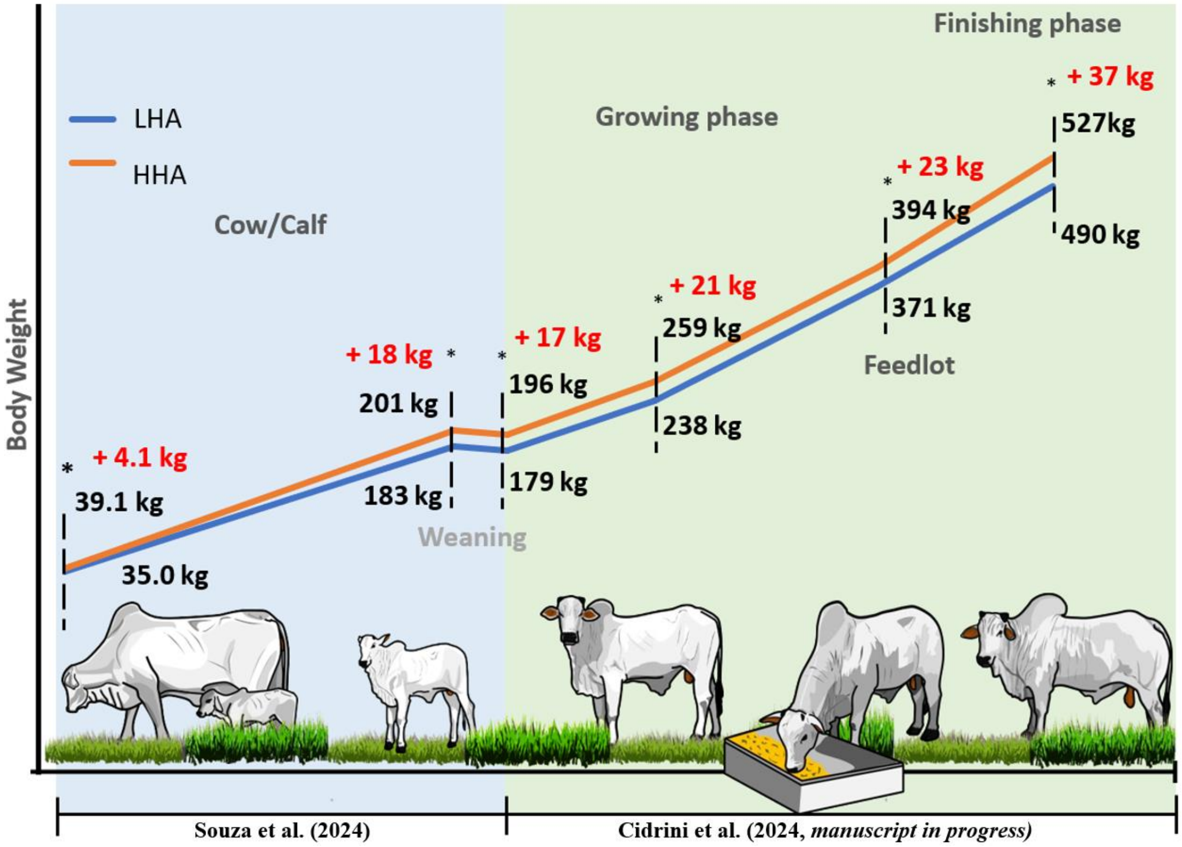
ILUSTRAÇÕES E FOTOGRAFIAS

Cow management and treatments

Growing phase

Finishing phase

Results


Finishing phase
LHA
HHA
Growing phase
Cow/Calf
Body Weight
* + 37 kg
527kg
490 kg
* + 23 kg
394 kg
371 kg
Feedlot
* + 21 kg
259 kg
238 kg
+ 18 kg *
201 kg
183 kg
* + 17 kg
196 kg
179 kg
Weaning
* + 4.1 kg
39.1 kg
35.0 kg
Souza et al. (2024)
Cidrini et al. (2024, *manuscript in progress)*