UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA ANIMAL

CURSO DE DOUTORADO

EFEITOS DE UM ANÁLOGO À SUBSTÂNCIA APAZIGUADORA BOVINA SOBRE O CRESCIMENTO, COMPORTAMENTO, ESTRESSE E CARACTERÍSTICAS DE CARCAÇA DE BEZERROS DESMAMADOS

Douglas Gomes Vieira

Campo Grande, MS

2024

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Orientador: Profª Dr. Henrique Jorge Fernandes
Coorientador: Profº Dr. Marcelo Vedovatto

Tese apresentada à Universidade Federal de Mato Grosso do Sul, como requisito à obtenção do título de Doutor em Ciência Animal. Área de concentração: Produção Animal.

Campo Grande, MS

2024

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Certificado de aprovação

DOUGLAS GOMES VIEIRA

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EFFECTS OF A BOVINE APPEASING SUBSTANCE ANALOGOUS ON GROWTH, BEHAVIOR, STRESS, AND CARCASS CHARACTERISTICS OF WEANED CALVES

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Aprovado em: 06-05-2024

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DEDICATÓRIA

A Deus, aos meus pais José e Margarete fonte de todo amor e perseverança. Pois, sem estes não seria possível tamanha oportunidade e privilégio.

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Que me deu forças, sabedoria, saúde e discernimento nessa importante fase da minha vida. Além disso, agradeço também a Deus pelo dom da vida, sustentando meus passos para as minhas realizações.

À minha família, que é minha base onde me refúgio e busco força para permanecer e conquistar os objetivos que tracei para minha vida. Meu agradecimento especial a minha mãe, Margarete Tenório Gomes Vieira, ao meu pai Jose Gonzaga Vieira, minha irmã Keila Gomes Vieira Freitas, meu cunhado Enok Freitas e meu grande amigo que considero como um pai Max Antônio de Sousa, que em todos os momentos me motivaram.

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À Empresa Nutricorp, Araras, SP, 13601-000, Brasil, pela doação do produto e por nos incentivar a conduzir os experimentos.

Epígrafe

"Sometimes when you're in a dark place you think you've been buried, but you've actually been planted"

Christine Caine

Resumo

VIEIRA, D.G. Efeitos de um análogo à substância apaziguadora bovina sobre o crescimento, comportamento, estresse e características de carcaça de bezerros desmamados. 2024. 97f. Tese (Doutorado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, 2024.

Objetivos: Os objetivos foram: Cap.1) realizar uma revisão de literatura sobre a utilização de um análogo a substancia apaziguadora bovina (SAB) em bovinos de corte; avalizar os impactos da administração da ASAB. Cap.2) avaliar o efeito da aplicação da SAB no momento do desmame, sobre o crescimento, estresse, comportamento e resposta à vacinação de bezerros Nelore. Cap.3) aplicação ao desmame sobre ganho de peso, resposta à vacinação, estresse, comportamento diurno, características de carcaça e qualidade de carne de novilhas Brangus.

Material e métodos: Os animais de todos os experimentos foram divididos em 2 tratamentos: Salina, injeção de solução salina e SAB. Cap.2) um total de 90 bezerros *Bós taurus* indicus, [n = 90; peso corporal (PC) = 198 ± 30.8 kg e 8 ± 1 mês de idade]. Os bezerros foram criados do nascimento até o desmame em pastagem de capim-marandu [Urochloa brizantha (Hochst. ex A. Rich) R. D. Webster, cv. Marandu; onde tiveram livre acesso à água e uma mistura completa de minerais/vitaminas. Cap.3) foram utilizadas um total de 30 bezerras Brangus PC (199.8 ± 16 kg; 8 ± 1 mês de idade). As bezerras foram criadas do nascimento até o desmame em pastagem de Capim-Marandú (Urochloa brizantha cv Marandú) e no dia do desmame foram transportadas por 3 km em um único caminhão, para o confinamento experimental. Na chegada (d 0), as bezerras foram estratificadas pelo peso corporal e designadas aos tratamentos: SAB (n = 15; SecureCattle; Nutricorp, Araras, SP, Brazil) ou Saline (n = 15; solução salina, 0,9% NaCl).

Resultados: A aplicação da SAB: Cap.2) a SAB pode melhorar o sistema imunológico via maior produção de anticorpos a vacinas e maior adaptação dos animais ao novo ambiente e redução

da perda de peso causado por estresse: Cap.3) administração de SAB as bezerras na chegada ao confinamento, melhorou o GMD nos 45 dias iniciais, parecendo acelerar a adaptação ao novo esquema e ambiente. Além disso, aliviou (ou não; falta avaliar cortisol e haptoglobina) as reações fisiológicas induzidas pelo estresse.

Conclusões: A aplicação da SAB em bezerros desmamados pode aumentar a produção de enzimas antioxidantes, melhorar o sistema imunológico, melhorar a qualidade de carne e aumentar o ganho de peso aos primeiros 45 dias de desmame. Além disso, os autores concluíram que a diminuição observada na atividade enzimática é resultado do aumento da produção de espécies reativas de oxigênio e, portanto, da menor maciez da carne.

Palavras-chave: *Bos indicus*, desempenho, resposta imune, saúde, temperamento.

Abstract

VIEIRA, D.G. Effects of an analogue of the bovine appeasing substance on growth, behavior, stress, and carcass characteristics of weaned calves. 2024. 97f. Tese (Doutorado) - Faculdade de Medicina Veterinária e Zootecnia, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, 2024.

Objectives: The objectives were: Chapter 1) to carry out a literature review on the use of an analogue of bovine appeasing substance (SAB) in beef cattle; assess the impacts of SAB administration. Chapter 2) evaluates the effect of applying SAB at the time of weaning on the growth, stress, behavior, and response to vaccination of Nelore calves. Chapter 3) Application at weaning on weight gain, response to vaccination, stress, daytime behavior, carcass characteristics, and meat quality of Brangus heifers.

Material and methods: Animals from all experiments were divided into 2 treatments: Saline, saline injection, and SAB. Cap.2) a total of 90 Bós taurus indicus calves, [n = 90; body weight (BW) = 198 ± 30.8 kg and 8 ± 1 month of age]. The calves were raised from birth to weaning on Marandu grass pasture Urochloa brizantha (Hochst. ex A. Rich) R. D. Webster, cv. Marandu; where they had free access to water and a complete mineral/vitamin mix. Chap.3) a total of 30 Brangus PC calves (199.8 ± 16 kg; 8 ± 1 month of age) were used. The calves were raised from birth until weaning on Capim-Marandú pasture (Urochloa brizantha cv Marandú) and on the day of weaning, they were transported 3 km in a single truck, to the experimental confinement. Upon arrival (d 0), calves were stratified by body weight and assigned to treatments: SAB (n = 15; SecureCattle; Nutricorp, Araras, SP, Brazil) or Saline (n = 15; saline solution, 0.9% NaCl).

Results: The application of SAB: Chapter 2) SAB can improve the immune system via greater production of antibodies to vaccines and greater adaptation of animals to the new environment and reduction of weight loss caused by stress: Chapter 3) Administration of SAB the calves

upon arrival at the feedlot, ADG improved in the initial 45 days, appearing to accelerate adaptation to the new scheme and environment. Furthermore, it alleviated (or not; cortisol and haptoglobin remain to be evaluated) the physiological reactions induced by stress.

Key-words: *Bos indicus*, health, performance, immune response, temperament.

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CAPÍTULO 3. A administração de substâncias apaziguadoras na entrada no confinamento impactou o crescimento, o temperamento, o comportamento do curral, a imunocompetência e a qualidade da carne de novilhas de corte.

LISTA DE ABREVIATURAS

Abbreviation

ADF	Acid detergent fiber
ADG	Average daily gain
BAS	Bovine appeasing substance
BW	Body weight
CCW	Cold carcass weight
CL	Cooking loss
CP	Crude protein
DM	Dry matter
D	Day
DFD	Dark, firm, and dry
EE	Ether extract
EL	Exudation losses
IBR	*Infectious bovine rhinotracheitis*
MFI	Myofibrillar fragmentation index
MM	Minerls matter
NDF	Neutral detergent fiber
NaCl	Sodium chloride
NEFA	Non-esterified fatty acids
NEm	Net energy for maintenance
NEg	Net energy for gain
PI3	*Parainfluenza-3 virus*
REA	Rib eye area
SF	Shear force
TDN	Total digestible nutrients

SUMÁRIO

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1. INTRODUÇÃO

O desmame é um evento estressante na vida do bezerro, que leva a alterações no comportamento, nos mediadores hormonais do estresse, na função imune e, consequentemente, no crescimento pós desmame (Lynch et al., 2012). Um análogo à substância apaziguadora bovina (SAB; SecureCattle; Nutricorp, Araras, SP, Brazil), sintetizado através de uma mistura de ácidos graxos que replicam a composição de um feromônio produzido pelas vacas (Pageat, 2001; Capellozza et al., 2022), tem sido usado visando se modular o comportamento agonístico, estimulando a alimentação e o ganho de peso, melhorando o desempenho pós-desmame e reduzindo as concentrações médias de haptoglobinas nesta fase (Shafi et al., 2021).

Cooke et al. (2020) conduziram dois experimentos utilizando SAB. No experimento 1, 206 bezerros receberam uma aplicação tópica (5 mL/animal) de SAB ou água no momento da desmama. Os animais que receberam a SAB apresentaram maior peso vivo até 45 d após a aplicação e uma menor concentração de haptoglobina até 15 d após o desmame. No experimento 2 do mesmo estudo, 140 touros foram transportados até um confinamento e receberam os mesmos tratamentos descritos no experimento 1 anteriormente ao transporte. Os touros que receberam a SAB ganharam mais peso nos primeiros 15 dias de confinamento, porém menos peso dos d 15 até o d 45 deste. Esse estudo mostra a possibilidade de melhora no ganho de peso e na redução dos indicadores de fase aguda, como resultado da aplicação tópica de SAB em bovinos de diferentes categorias, submetidos a situações de estresse.

Esforços adicionais de pesquisa, no entanto, devem ser elencados para se elucidar os potenciais modos de ação da SAB, bem como as situações em que este produto possa melhorar a saúde, o desempenho e o bem-estar animal.

Nossa hipótese, neste estudo foi de que a aplicação tópica de SAB no momento da desmama iria reduzir o estresse, melhorar a adaptação ao novo ambiente, e, consequentemente, aumentar o ganho de peso de bezerros em diferentes sistemas de produção. Assim, o nosso

objetivo foi avaliar os efeitos do uso de um análogo à substância apaziguadora bovina (SAB) sobre o crescimento, comportamento e estresse de bezerros desmamados em pastagem ou confinados logo após o desmame.

2. REFERÊNCIAS

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SHAFI, T.A.; MD. SIDDIQUI, Q.; AND WANI, A.A. Chemical Signaling in Bovines: Understanding the Behavior and Way of Communication. **Bovine Science**, 2021.

CAPÍTULO 1. EFEITOS DA APLICAÇÃO DE UMA DE UM ANÁLOGO A SUBSTÂNCIA APAZIGUADORA BOVINA NO DESMAME DE BEZERROS DE CORTE: REVISÃO

O artigo a seguir foi redigido de acordo com as normas para publicação no periódico *Livestock Science*, exceto o idioma.

EFEITOS DA APLICAÇÃO DE UM ANÁLOGO A SUBSTÂNCIA APAZIGUADORA BOVINA NO DESMAME DE BEZERROS DE CORTE: REVISÃO

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Highlights

- A resposta dos bezerros ao estresse de desmame é complexa.
- As características envolvidas incluem físicas, fisiológicas e metabólicas.
- A administração de SAB pode melhorar a adaptação ao desmame através da expressão de padrões comportamentais e fisiológicos.
- A baixa qualidade da carne está diretamente associada ao estresse.
- A Substância Apaziguadora Bovina é uma alternativa promissora para melhorar o desempenho de animais de corte.

Resumo

O conhecimento sobre o comportamento bovino e os fatores que promovem o estresse animal tem sido foco de diversas publicações que buscam equacionar a relação entre ambiente, bem-estar e produção animal. Com implicações para a saúde, o bem-estar e a produtividade dos animais, a minimização do stress animal através de melhores procedimentos de gestão animal e/ou tecnologias, estão se tornando uma prioridade. Com o intuito de diminuir os impactos provocados pelo estresse neuroendócrino intrínseco da produção pecuária, alternativas vêm sendo desenvolvidas para melhorar o bem-estar e propiciar melhor adaptação dos animais a manejos rotineiros. Entre elas, pode ser citado a feromonioterapia, com a utilização de feromônios apaziguadores, como a Substância Apaziguadora Bovina (SAB), análogo ao feromônio produzido pelo complexo da glândula mamária no momento do parto com o intuito de proporcionar sensação de conforto e segurança para o recém-nascido e maior adaptabilidade a animais em pleno desenvolvimento. A presente revisão demonstra os principais atributos das respostas relacionadas ao estresse em ruminantes e descreve a utilização estratégica da SAB como alternativa para a redução do estresse em bezerros desmamados.

Palavras-chave: bovinos, comportamento, crescimento, estresse ao desmame, feromônio

Abstract

Knowledge about bovine behavior and the factors that promote animal stress has been the focus of several publications that seek to equate the relationship between environment, well-being and animal production. With implications for animal health, welfare and productivity, minimizing animal stress through improved animal management procedures and/or technologies is becoming a priority. In order to reduce the impacts caused by the intrinsic neuroendocrine stress of livestock production, alternatives have been developed to improve well-being and provide better adaptation of animals to routine management. Among them, pheromone therapy can be mentioned, with the use of calming pheromones, such as Bovine

Calming Substance (SAB), analogous to the pheromone produced by the mammary gland complex at the time of birth with the aim of providing a feeling of comfort and security for the baby. newborn and greater adaptability to animals in full development. The present review demonstrates the main attributes of stress-related responses in ruminants and describes the strategic use of SAB as an alternative for stress reduction.

***Keywords*:** behavior, growth, pheromone, stress, temperament cattle, weaning stress

1. Introdução

O bem-estar animal refere-se ao estado físico e mental de um animal ao lidar com o seu ambiente (Keeling et al., 2019). De forma geral, a capacidade de um animal responder a estímulos externos dá-lhe a capacidade de atingir um estado de saúde, prosperidade e bem-estar (Lynch, 2010).

A maioria dos eventos estressores têm algum impacto sobre os neurônios dopaminérgicos. O estresse ativa o sistema nervoso simpático e inibe o sistema nervoso parassimpático, que está associado a atividades como descansar e comer.

A retirada abrupta de um bezerro de sua mãe (desmame) entre os 3 e 6 meses de idade é uma prática padrão nos sistemas de produção de gado de corte (Enriquez et al., 2011). Bezerros desmamados podem sofrer um estresse nutricional, social e psicológico, que causa alterações comportamentais, metabólicas, fisiológicas e imunológicas (Lynch et al., 2010; Loughlin et al., 2014). Neste contexto, o desmame é uma das fases mais estressante da vida do animal, e aumenta significativamente as concentrações do cortisol plasmático (Cook et al., 2013), da noradrenalina (Schubach et al., 2020) e das proteínas de fase aguda, (Cornelis et al., 2005), bem como atua reduzindo a função imunológica e o comportamento lúdico dos animais (Hulbert et al., 2011).

Vários outros estudos também relataram as ligações entre o temperamento animal e a resposta ao estresse em bovinos de corte (Fell et al., 1999; King et al., 2006; Café et al., 2011). Estes estudos mostraram associações favoráveis entre o temperamento e as respostas de cortisol ao estresse induzidas pelo manejo, tanto em bovinos *Bos tauros* indicus, quanto em *Bos taurus* taurus.

Neste cenário, o objetivo com esta revisão é elucidar como o estresse ocorre e quais os possíveis efeitos da utilização da SAB sobre o estresse em bovinos.

2. Revisão de Literatura

O estresse é definido como a reação de um animal a fatores que potencialmente influenciam sua homeostase. Os animais que são incapazes de lidar com esses fatores são, então, classificados como estressados (Moberg, 2000).

O conhecimento dos tipos de estresse pode ajudar a se elaborar estratégias para reduzi-lo. Geralmente, no caso da pecuária, os estressores podem ser agrupados em três grandes categorias: (1) estresse fisiológico; (2) estresse físico; e (3) estresse psicológico.

1) Estresse Fisiológico: Envolve a ocorrências de alterações endócrinas e metabólicas devido a estressores psicológicos e físicos (Cappellozza e Marques, 2021)

2) Estresse Físico: Envolve a ocorrência de físicos, como calor, ruído, transporte e privação ou restrição alimentar, assim manejos como a castração ou a descorna (Chen et al., 2015).

3) Estresse Psicológico: Pode ser causado por uma ameaça ao bem-estar mental do animal, como o desmame, um ambiente novo ou a mistura de lotes de animais (Bains et al., 2015).

Sobre a perspectiva fisiológica, quando um animal recebe a ação de um agente estressor, a resposta imediata é a ativação do eixo hipotálamo-pituitária-adrenal (HPA) (Dodson & Smith, 2000), caracterizado pela síntese e liberação do hormônio liberador de corticotropina (CRH) e

vasopressina (VP) por seus respectivos neurônios, localizados no hipotálamo no núcleo paraventricular (Carroll and Forsberg, 2007). Em bovinos, o CRH tem ações estimulatórias mais influentes do que o VP (Carroll et al., 2007), de modo que, após sua ligação aos receptores de membrana na glândula pituitária, a proteína quinase-A é ativada e o 3′, 5 ′ AMP cíclico (cAMP) é produzido, levando a um influxo de cálcio que ativará a liberação do hormônio adrenocorticotrópico (ACTH) pela glândula pituitária (Link et al.,1993). Na glândula pituitária anterior, os corticotróficos são responsáveis pela produção de ACTH e sua principal função é promover a captação de colesterol, bem como a síntese e liberação de esteroides pela glândula adrenal (Carroll and Forsberg, 2007).

Na maioria das espécies de mamíferos, o cortisol é o glicocorticoide primário (Dodson & Smith, 2000) e geralmente classificado como “hormônio do estresse”. No metabolismo, o cortisol desencadeia várias respostas importantes, como a mobilização do glicogênio de músculos e de tecidos adiposos como um mecanismo para fornecer energia ao animal durante um desafio, a produção hepática de proteínas de fase aguda, uma maior síntese e liberação de catecolaminas e a supressão dos sistemas inflamatório e imunológico. Por outro lado, aumentos agudos nas concentrações de cortisol provocam uma resposta inflamatória transitória e temporária, enquanto em situações onde doenças ou estresse crônicos são observados, o cortisol permanece elevado por um grande período de tempo (Munck et al.,1984).

Em ruminantes, maiores concentrações de cortisol foram associadas a taxas de crescimento e desempenho reprodutivo reduzidos nas fêmeas (Colombo et al., 2020; Cappellozza et al., 2020).

A fim de estudar a resposta de bezerros ao desmame, Arthington et al. (2005) avaliaram os efeitos do desmame precoce e tradicional sobre marcadores de estresse e desempenho de crescimento de animais de corte. Esses autores evidenciaram que, no momento em que ocorreu o desmame tradicional (aproximadamente aos 300 dias de idade), os animais desmamados

precocemente (aproximadamente aos 89 dias de idade) eram cerca de 48 kg mais leves do que os animais desmamados tradicionalmente. Já o GMD (0 a 28 dias pós desmame = 0,87 vs. 0,40 kg / dia; 29 a 112 dias pós desmame = 1,38 vs. 1,18 kg / dia) e a eficiência alimentar (0 a 28 dias pós-desmame = 157 vs. 81 g / kg; 29 a 112 dias pós desmame = 159 vs. 136 g / kg) pós desmame foram maiores para os bezerros desmamados precocemente. Além disto, do desmame tradicional ao abate, os animais desmamados precocemente ganharam cerca de 30 kg a mais de PV e tiveram maior eficiência alimentar do que os bezerros desmamados tradicionalmente. Esses resultados foram explicados pelas concentrações relativamente estáveis de haptoglobina e ceruloplasmina em bezerros desmamados precocemente no período em que ocorreu o desmame tradicional e a uma resposta de fase aguda (RFA) diminuída, observada nestes animais, quando receberam um desafio patogênico.

Outro evento estressante é o transporte dos animais e a entrada destes em confinamentos, que podem ocorrer juntos ou em um curto período de tempo. Durante o transporte, os animais permanecem sem alimento e água por um período significativo de tempo. Em estudos para avaliar os efeitos metabólicos que as restrições alimentar e hídrica causam nos animais, Marques et al. (2012) relataram uma mobilização de tecidos (em especial, adiposos) e uma redução do desempenho no confinamento, também observada por Cooke et al. (2020).

Uma das alternativas no controle e prevenção do estresse, a feromônioterapia pode ser aplicada em bovinos, com efeitos sobre a ansiedade e a fobia, modulando o comportamento animal, com reflexos positivos no seu desempenho.

A substância apaziguadora bovina (SAB) é uma tecnologia que utiliza feromônios apaziguadores, constituídos por uma mistura de ácidos graxos com composição semelhante à substância originalmente produzida pelas vacas ao parto (Angeli et al 2020). A SAB tem sido utilizada para reduzir o comportamento agonístico em várias situações, ajudando na melhoria da produção de leite, na redução da contagem de células somáticas de vacas leiteiras, na

melhoria do desempenho e na redução da duração e dos custos com medicação em caso de bezerros leiteiros pré-desmame (Angeli et al 2020; Osella et al., 2018).

A administração da SAB em bezerros *Bos indicus* aumentou a resposta à vacinação, mostrando maior imunidade humoral contra IP3 e BVDV-1 (Vieira et al., 2023). Outro estudo com bezerros *Bos indicus* mostrou que a administração de SAB melhorou a imunidade humoral contra BRSV, BVDV e PI3 (Schubach et al., 2020). Esta melhora da imunidade humoral dos bezerros que recebem a SAB pode ser atribuída, pelo menos parcialmente, ao alívio do estresse provocado pelo desmame e pelo manejo de vacinação, e à consequente maior atividade do sistema imune.

Cooke et al. (2020) avaliando os impactos da administração de BAS em bovinos *Bos indicus* ao desmame e à entrada em confinamento, observaram que a administração do BAS aumentou o ganho de peso corporal e reduziu as concentrações plasmáticas de haptoglobina até 15 dias após sua aplicação.

Conclusões

A SAB se apresenta como uma alternativa para melhorar a adaptação dos animais ao ambiente, podendo melhorar o sistema imunológico e aumentar o desempenho.

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Conflitos de Interesse

Os autores declaram não haver conflitos de interesse

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CAPÍTULO 2. EFFECTS OF AN APPEASING SUBSTANCE APPLICATION AT WEANING ON GROWTH, STRESS, BEHAVIOR AND RESPONSE TO VACCINATION OF *BOS INDICUS* CALVES

O artigo a seguir foi redigido e publicado de acordo com as normas para publicação no periódico *Animals* (anexoI).

Effects of an appeasing substance application at weaning on growth, stress, behavior and response to vaccination of *Bos indicus* calves

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Simple Summary: Weaning is one of the most stressful experiences for beef calves during their life cycle; however, it is a crucial practice for the production system. Calming pheromones can al-leviate stress and reduce its negative impacts on behavior, growth, and the immune system. In this study, the administration of a bovine-appeasing substance enhanced body weight gain, re-duced temperament scores and serum cortisol concentration, and improved the behavior and re-sponse to vaccination. Therefore, the bovine-appeasing substance could be used in complement to the best weaning practices to reduce stress and its impact on performance, temperament, grazing behavior, and the immune system.

Abstract

An analog of a bovine-appeasing substance (BAS) was previously demonstrated to have calming effects, and it could be an alternative to alleviate the stress caused by weaning. Thus, the objective of this study was to evaluate the effects of BAS administration at weaning on growth, stress, be-havior, and response to vaccination of Nellore calves. Eighty-six Nellore calves (40 females and 46 males) were abruptly weaned and randomly assigned into 1 of 2 treatments: (1) saline solution (0.9% NaCl; n = 43) and (2) BAS (Secure Catte, IRSEA Group, Quartier Salignan, France; n = 43). The solutions were topically applied (5 mL/calf) to the nuchal skin area of each animal. On d 0, be-fore treatment application, calves were vaccinated against infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI3) virus, and bovine viral diarrhea virus types 1 and 2 (BVDV-1 and 2). Calves from each treatment were kept in different pastures for 15 d (time of BAS action) and then moved to a single pasture. Body weight (BW), blood samples, and temperament in the chute (entry score, chute score, and exit score) were collected on d 0, 3, 8, 15, 51, and 100, and behavior on pasture on d 1, 2, 4, 5, 6, 7, and 9. Calves assigned to BAS vs. Saline treatment tended to have greater BW on d 15 ($p = 0.10$), tended to have lower entry scores on d 8 and 51 ($p = 0.10$), and chute scores on d 8 ($p = 0.07$), and had

lower exit scores on d 8 ($p = 0.02$). Calves assigned to BAS vs. Saline treatment also had greater time grazing on d 7 and 9 ($p < 0.01$), eating concentrate on d 2, 5, and 6 ($p = 0.05$), walking on d 1, 2, 5, and 9 ($p < 0.01$), standing and ruminating on d 2, 7 and 9 ($p < 0.01$), and play-ing on d 2, 4, 6, 7, and 9 ($p < 0.01$). Furthermore, they had lower time lying on 1 and 2 ($p < 0.01$), standing on d 5 and 9 ($p < 0.01$), and vocalizing on d 1 and 2 ($p < 0.01$). Calves assigned to BAS vs. Saline treatment had greater serum titter concentrations of PI3 t on d 15 and 51 ($p = 0.05$) and BVDV-1 on d 51 ($p = 0.02$). However, they had lower serum concentrations of cortisol on d 3 ($p = 0.03$). BAS administration did not affect ($p \geq 0.12$) the serum titer concentration of IBR and BVDV-2 titers or the plasma concentration of haptoglobin and ceruloplasmin. The BAS administration im-proved BW, reduced temperament and serum cortisol concentration, and improved behavior and response to vaccination.

***Keywords*:** cortisol; immune system; pheromone; temperament

1. Introduction

Weaning is a highly stressful event for calves, exposing them to many physiological challenges that directly impact their performance, health, and welfare [1]. Upon weaning, calves experience multiple stressors, such as maternal separation and changes in the social and physical environment. Additionally, handling management at weaning is often used as a convenient time for vaccination, deworming, dehorning, castration, branding, and frequently commingling with a new group [2]. The encounter with such stressors can increase plasma cortisol and acute phase proteins and reduce feed and water intake, growth, and the immune system [3].

The behavior of calves is drastically affected for approximately one week after weaning [4]. The most characteristic behavior affected is an increased frequency of vocalization,

followed by increased time walking and standing and decreased time grazing, ruminating, playing, and resting [4]. Furthermore, increased aggressivity is frequently observed on pasture or in the corral after weaning [4].

One of the current strategies designed to reduce stress at weaning is using appeasing pheromones, as demonstrated in several species [5–7]. In cattle, an analog of a bo-vine-appeasing substance (BAS) was developed based on a mixture of fatty acids repre-senting the composition of the natural substance produced by the mammary gland [8,9]. The BAS is topically applied to the nuchal skin area of each animal, inducing the calves to inhale the product, which leads to calming effects for about 15 d, according to the manu-facturer (Secure Cattle, IRSEA Group, Quartier Salignan, France).

When the BAS was applied at weaning to grazing beef calves [9,10] or kept in a feed-lot [11,12], it increased the subsequent growth [9,10], reduced the plasma concentration of haptoglobin [10], concentration of cortisol on hair [12] and plasma [11], aggressivity [12], increased the plasma concentration of glucose and β-hydroxybutyrate [11], and improved the response to vaccination [12].

Although some studies have evaluated the application of BAS in calves [9–12], we are unaware of a study that evaluated the effects of the application of BAS at weaning on the temperament, behavior, and immune response of grazing *Bos indicus* beef calves. We hy-pothesized the BAS application at weaning would reduce the stress as observed in other studies [9–12], and as stress affects performance, behavior, and the immune system [3], we expected BAS application would improve growth, temperament in the corral, diurnal pasture behavior, and humoral immunity and reduce the acute phase response related to stress. Thus, the objective of this study was to evaluate the effects of BAS application at weaning on growth, stress, behavior, and response to vaccination of Nellore calves.

2. Materials and Methods

2.1 Animals, treatments, and sample collection

The experiment was conducted at the farm school at Universidade Federal do Mato Grosso do Sul in Terenos, MS, Brazil (20°26′50.8″ S 54°50′21.5″ W) during the dry season period, from May to August of 2021.

Eighty-six Nellore calves [40 females and 46 males; the average body weight (BW) was 198 ± 30.8 kg, and the average age was 8 ± 1 month] were abruptly weaned (d 0) and enrolled in the experiment. On d 0, calves were stratified by BW and sex and randomly as-signed into 1 of 2 treatments: (1) saline solution (0.9% NaCl; n = 43; 20 females and 23 males) and (2) BAS (Secure Cattle, IRSEA Group, Quartier Salignan, France; n = 43; 20 fe-males and 23 males). The BAS composition consists of a mixture of fatty acids, including palmitic, oleic, and linoleic acids (similar to the BAS produced by the mammary gland of the cow), added at 1% of the excipient and estimated to remain in treated animals for 15 d, according to the manufacturer [12]. The solutions were topically applied (5 mL/calf) to the nuchal skin area of each animal. Before the treatment applications, calves were segregated by treatment into two groups. Saline-treated calves were processed and immediately re-leased to a pasture before BAS administration in the other group. This treatment application method was chosen to avoid any cross-effects of BAS on Saline-treated calves.

On d 0, before treatment application, calves were vaccinated against infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI3) virus, bovine viral diarrhea virus type 1 and 2 (BVDV-1 and 2), bovine respiratory syncytial virus, and *Mannheimia haemolytica* (2 mL s.c.; Bovi Shield Gold One Shot, Zoetis, São Paulo, SP, Brazil). From d 0 to d 15, calves were maintained in two similar 2-ha pastures (1 pasture/treatment) of marandu-grass [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu] complemented with hay [Urochloa *dictyoneura* (Fig. and De Not.) Veldkamp]. Calves from each treatment were kept in different

pastures for 15 d to avoid the cross-effects of BAS on Saline-treated calves. On d 15, calves were moved to a single 17-ha pasture (both treatments in the same pasture) of marandu grass, where they were kept until the end of the study (d 100). During the entire experiment, calves had free-choice access to water and protein concentrated supplement [guarantee levels of 300 g/kg of crude protein (CP), 246 g/kg of non-nitrogen protein, 75 g/kg of sodium, 45.2–52.8 g/kg of calcium, 7.30 g/kg of phosphorus, 7.54 g/kg of sulfur, 200 mg/kg of copper, 133 mg/kg of manganese, 600 mg/kg of zinc, 12 mg/kg of cobalt, 15 mg/kg of iodine, 3.6 mg/kg of selenium, and 160 mg/kg of monensin; target intake of 1 g/kg of BW; Criatec 30 Seca, Tec Agro Nutrição Animal, Campo Grande, MS, Brazil).

The BW of calves was collected individually at 08:00 a.m. on d 0, 8, 15, 51, and 100. Fasted BW was not obtained to avoid shrink-induced stress effects on blood parameters evaluated in the study [13]. Three trained technicians evaluated the temperament (same trained personnel during the entire experiment, who were blinded to treatments) in the corral on d 0, 3, 8, 15, 51, and 100. The entry and exit scores in the squeeze chute were evaluated according to Baszczak et al. [14], with scores 1 = animals that walked in or out of the chute; 2 = those that trotted to or from the chute; and 3 = those that ran or galloped in or out of the chute. The chute score was evaluated using an adaptation of Cooke et al. [15], where 1 = calm with no movement; 2 = restless movements; 3 = frequent movement; 4 = constant movement, vocalization, and shaking of the chute; and 5 = violent and continu-ous struggling.

After the handling in the squeeze chute, calves were sequentially allocated in a small pen (5 × 10 m) and in small groups (n = 5) to evaluate the pen score as described by Ham-mond et al. [16]. Briefly, one trained technician approached the group and evaluated the response of each calf, being scores 1 = nonaggressive (docile)—walks slowly, can ap-proach closely, not excited by technician or facilities; 2 = slightly aggressive—runs along fences, will stand in the corner if humans stay away, may pace fence; 3 = moderately aggressive—runs along fences,

head up and will run if humans move closer, stops before hitting gates and fences, avoids humans; 4 = aggressive—runs, stays in the back of group, head high and very aware of humans, may run into fences and gates even with some distance, will likely run into fences if alone in pen; and 5 = very aggressive—excited, runs into fences, runs over humans and anything else in path, "crazy". Temperament scores were averaged among technicians.

Blood samples were collected from a jugular vein (14 calves/treatment; 7 females and 7 males/treatment) on d 0, 3, 8, 15, 51, and 100 into two blood collection tubes (10 mL; Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) with and without sodium heparin for the collection of plasma and serum, respectively. After collection, blood samples were immediately stored on ice and then centrifuged at 1200× g for 30 min for plasma and se-rum harvest. Samples were stored at −20 °C for further analysis of serum concentrations of cortisol and antibody titters (against IBR, PI3, and BVDV-1 and 2) and plasma concentrations of haptoglobin and ceruloplasmin. Cortisol, ceruloplasmin, and haptoglobin were analyzed on d 0, 3, 8, 15, 51, and 100, and antibody titers were analyzed on d 0, 15, and 51. On d 0, calves were individually identified on both sides of the body, with large numbers, using hair dye to facilitate animal identification for behavior evaluation. The diurnal behavior was evaluated from 06:30 a.m. to 06:00 p.m. (678 min/d) on d 1, 2, 4, 5, 6, 7, and 9. An observation tower (elevated 5 m from the ground) was used to facilitate the visualization of the animals by the evaluators on pastures. The tower was built of metal, and the top had a 1.30-meter-high base that hid most of the evaluator's bodies from the visualization by the calves. A pair of evaluators (one using binoculars and the other recording the behavior in the spreadsheet; each pair was replaced after four hours of evaluation) scanned the calf's behavior at each 10-minute interval, and the specific behavior detected during each scan was counted for 10 min for the statistics analysis. The variables evaluated in each scan were adapted from Enríquez et al. [4], being grazing, eating concentrate, drinking water, walking, lying, lying ruminating, standing, standing

ruminating, playing (jumping, running, with no sign of stress), and vocalizing. The time spent on each activity was calculated as a percent of the total time evaluated per day (678 min) for the subsequent statistical analysis.

The herbage mass was evaluated on d 0, 15, 51, and 100 using the comparative yield method [17], and the samples collected were dried at 60 °C for 5 d and weighed. Herbage allowance was calculated as the average herbage mass divided by the average total BW of calves in each pasture [18]. Hand-picked forage samples were also collected on d 0, 15, 51, and 100. Afterward, samples were dried at 60 °C for 5 d and ground at 1 mm for later chemical composition analysis.

2.2 Laboratory analysis

Antibody titers against IBR, PI3, and BVDV-1 and 2 viruses were assessed using procedures outlined by Rosenbaum et al. [19]. Individual serum samples were evaluated for the greatest dilution of antibody titers that achieved total protection of cells against those viruses and are reported as log2. Calves with antibody titers ≥ 4 for each virus were considered seropositive and assigned a value of 1, whereas calves with antibody titers < 4 were considered seronegative and assigned a value of 0. These scores were utilized to determine the percentage of calves that had positive seroconversion for antibody protection against those viruses, as previously described by Richeson et al. [20].

Plasma concentrations of haptoglobin were analyzed as described by Cooke and Arthington [21] and ceruloplasmin as described by Demetriou et al. [22]. The inter and intra-assay CV was 3.9% and 6.4% for haptoglobin and 2.0% and 4.3% for ceruloplasmin, respectively. The serum concentration of cortisol was analyzed (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) as previously described by Cooke et

al. [23] due to 100% cross-reactivity between bovine and human cortisol and accomplished within a single assay with an intra-assay with a CV of 8.52%.

Forage samples were analyzed according to AOAC [24]: CP, method 976.05; ether extract (EE), method 920.39; and ash, method 942.05. The concentrations of lignin, neutral detergent fiber (NDF), and acid (ADF) were analyzed as described by Van Soest et al. [25]. The total digestible nutrients (TDN) concentrations were calculated as described by Weiss et al. [26], and the net energy for maintenance (NEm) and gain (NEg) was determined by the equations proposed by the NASEM [27]. The chemical compositions of forage and hay are described in Table 1.

Table 1. Chemical composition of marundu-grass [Urochloa brizantha (Hochst. ex A. Rich) R. D. Webster, cv. Marandu] and dictyoneura-hay [Urochloa dictyoneura (Fig. and De Not.) Veldkamp] consumed by calves, herbage mass, and herbage allowance.

Items [1]	**Saline Pasture**			**BAS Pasture**			**Saline and BAS in the Same Pasture**	
	Forage d0	**Forage d15**	**Hay d0 to d15**	**Forage d0**	**Forage d15**	**Hay d0 to d15**	**Forage d51**	**Forage d100**
g/kg of DM								
Crude protein	81.0	77.0	101	89.0	80.0	103	91.0	83.0
NDF	622	680	759	620	676	759	645	663
ADF	339	402	403	336	387	404	354	297
Lignin	21.0	45.0	58.0	18.0	45.0	57.0	39.0	31.0
Ether extract	24.0	28.0	19.0	27.0	24.0	19.0	24.0	34.0
Ash	109	118	39.0	110	119	44.0	105	98.0
TDN	649	585	610	659	580	627	612	643
Mcal/kg of DM								
NEm	1.47	1.26	1.34	1.50	1.24	1.40	1.35	1.45
NEg	0.88	0.69	0.77	0.91	0.67	0.82	0.77	0.86
Herbage mass, kg DM/ha	4956	1840		4893	1930		1752	2554
Herbage allowance, kg DM/kg BW	0.56	0.21		0.54	0.22		0.10	0.17

[1] DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; TDN, total digestible nutrients; NEm, net energy for maintenance; NEg, net energy for gain.

2.3 Statistical analyses

The calf was considered the experimental unit for all analyses. All continuous variables were analyzed using the MIXED procedure, and binomial variables (only the percentual of seroconversion against IBR, PI3, and BVDV-1 and 2) were analyzed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version SAS University), with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. All variables were analyzed as repeated measures, and the statistical mod-el used was:

$$Yijk = \mu + Ti + Dj + Ak + Sl + (TD)ij + eijkl$$

where Yijkl = observation of the effect of treatment i per days of collection j in animal k and sex l; μ = overall mean; Ti (fixed) = effect of treatment [i = 1 (Saline) and 2 (BAS)]; Dj (fixed) = effect of days (j = 0, ..., 100); Ak (random) = animal effect (k = 1, ..., 86); Sl (random) = sex effect [l = 1 (male) and 2 (female)]; TDij (fixed) = interaction between treatment i and day j; and eijkl = random error associated with each observation.

The results of day 0 for BW, temperament, and plasma and serum variables were included as covariates in each respective analysis but were removed from the model when $p > 0.10$. The Toeplitz covariance structure was selected for BW, and the first-order auto-regressive covariance structure was selected for all other variables. The covariance structures were selected according to the lowest Akaike information criterion. Means were separated using pairwise differences (PDIFF), and all results were reported as the least squares mean (LSMEANS), followed by the standard error of the mean (SEM). Significance was defined as $p \leq 0.05$, and tendency was defined as $p > 0.05$ and ≤ 0.10. The Pearson correlations were analyzed using the CORR procedure of SAS (to evaluate correlations be-tween BW, temperament, and blood variables).

554 **3. Results**

555 No treatment × day interaction or main treatment effects were detected ($P \geq 0.29$) for
556 BW. However, BAS-treated heifers had greater ADG (treatment effects; $P = 0.05$) from d 6 to
557 45 than Saline-treated heifers (Table 2).

558

559 **Table 2.** Growth performance of Nellore calves receiving saline solution (Saline; n = 43) or bo-
560 vine-appeasing substance (BAS; n = 43) at weaning (d 0).

Items	Treatments[1]		SEM	*P*-value	
	Saline	BAS		Treatment	Treatment × day
Body weight, kg				0.60	0.10
d 0	198	198	0.96		
d 8	196	196	0.96		
d 15	194[b]	197[a]	0.96		
d 51	203	203	0.97		
d 100	195	196	0.98		
Average daily gain, kg/d				0.09	
d 0 to 8	-1.179	-0.268	0.07		
d 8 to 15	-0.278[b]	0.158[a]	0.07		
d 15 to 51	0.226	0.164	0.07		
d 51 to 100	-0.152	-0.134	0.07		

561 [a,b] Within a row, without a common superscript, differ ($p \leq 0.05$) or tend to differ ($p \leq 0.10$).
562

563 Upon treatment administration, an effect of treatment ($p \leq 0.05$) and a tendency for
564 treatment × day interaction were detected ($p = 0.10$) for the entry score, in which BAS-treated
565 calves had lower scores on d 8 and 51 compared to Saline-treated calves (Ta-ble 3).
566 Furthermore, a tendency for treatment × day effect ($p = 0.07$) was detected for chute score ($p =$
567 0.02) and exit score, being lower for BAS-treated calves only on d 8, compared to Saline-treated
568 calves (Table 3). No effects of treatment or treatment × day interaction ($p \geq 0.11$) were detected
569 for the pen score (Table 3).

570

571 **Table 3.** Temperament of Nellore calves receiving saline solution (Saline; n = 43) or bovine
572 appeasing substance (BAS; n = 43) at weaning.

Items	Treatments[1]		SEM	*P*-value	
	Saline	BAS		Treatment	Treatment × day
Entry score, (1–3)				0.05	0.10
d 0	1.59	1.63	0.07		
d 3	1.65	1.50	0.07		
d 8	1.96 [a]	1.71 [b]	0.07		
d 15	1.59	1.56	0.07		
d 51	1.87 [a]	1.69 [b]	0.07		
d 100	1.48	1.37	0.07		
Chute score, (1–5)				0.32	0.07
d 0	2.40	2.52	0.10		
d 3	2.38	2.37	0.10		
d 8	2.52 [a]	2.21 [b]	0.10		
d 15	2.04	2.07	0.10		
d 51	2.26	2.06	0.10		
d 100	2.13	1.93	0.10		
Exit score, (1– 3)				0.10	0.02
d 0	1.87	1.92	0.07		
d 3	1.81	1.77	0.07		
d 8	2.09 a	1.72 b	0.07		
d 15	1.82	1.67	0.07		
d 51	1.94	1.94	0.07		
d 100	1.77	1.65	0.07		
Pen score (1–5)	2.31	2.40	0.05	0.22	0.11

573 a,b Within a row, without a common superscript, differ ($p \leq 0.05$) or tend to differ ($p \leq 0.10$).
574

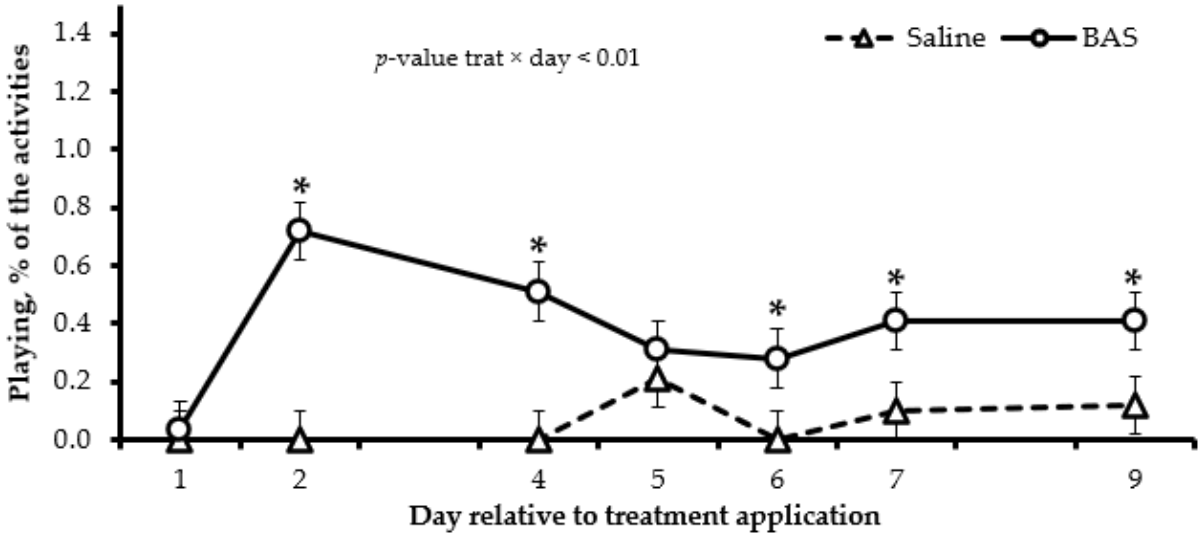
575 The diurnal behavior was affected by treatments, and a treatment × day interaction was
576 detected in several variables. The BAS-treated calves spent more time grazing ($p < 0.01$; on d
577 7 and 9), eating concentrate ($p = 0.05$; on d 2, 5 and 6), walking ($p < 0.01$; on d 1, 2, 5 and 9),
578 and standing ruminating ($p < 0.01$; on d 2, 7 and 9) and less time lying ($p < 0.01$; on d 1 and 2)
579 and standing ($p < 0.01$; on d 5 and 9) compared to Saline-treated calves (Table 4). No effects
580 of treatment or its interaction with days ($p \geq 0.18$) were detected for drinking water and lying
581 and ruminating (Table 4). Furthermore, BAS-treated calves spent more time playing ($p < 0.01$;
582 on d 2, 4, 6, 7, and 9) and less time vocalizing ($p < 0.01$; on d 1, 2, and 3) compared to Saline-
583 treated calves (Figure 1 and 2).

584 **Table 4.** Diurnal pasture behavior of Nellore calves receiving saline solution (Saline; n = 14)
585 or bovine appeasing substance (BAS; n = 14) at weaning.

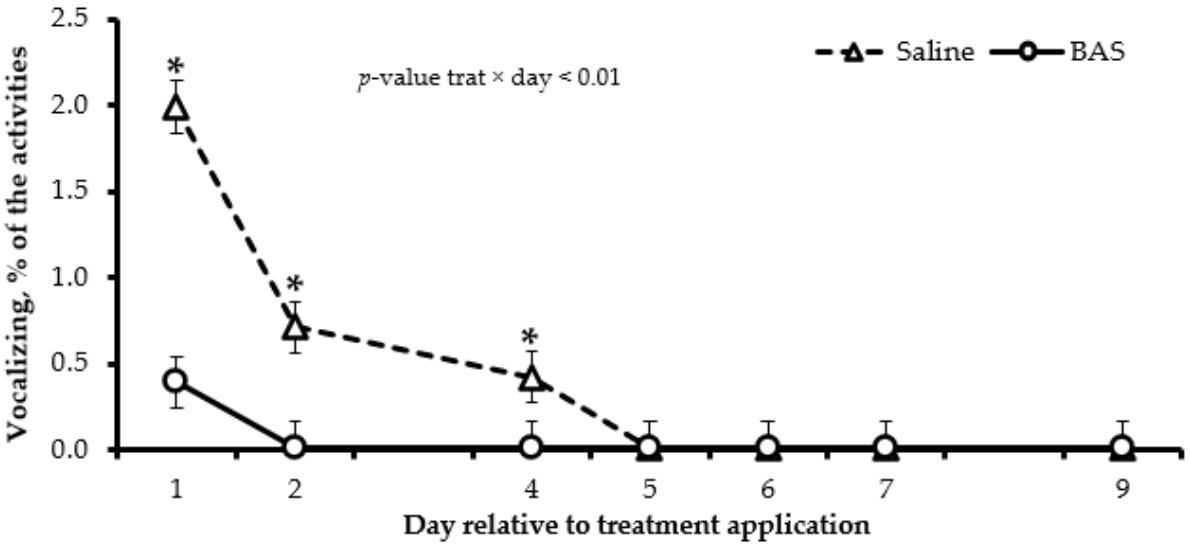
Items, % of the Activities	Treatments		SEM	*p*-Value	
	Saline	BAS		Treatment	Treatment × Day
Grazing				0.56	<0.01
d 1	27.9	27.4	1.84		
d 2	28.3	27.8	1.84		
d 4	36.5	35.7	1.84		
d 5	42.1	42.4	1.84		
d 6	43.2	42.1	1.84		
d 7	45.1 [b]	50.2 [a]	1.84		
d 9	36.3 [b]	49.2 [a]	1.84		
Eating concentrate				<0.01	0.05
d 1	0.30	0.65	0.58		
d 2	1.95 [b]	3.39 [a]	0.58		
d 4	1.54	2.14	0.58		
d 5	2.69 [b]	4.84 [a]	0.58		
d 6	1.45 [b]	4.86 [a]	0.58		
d 7	1.84	2.45	0.58		
d 9	3.16	3.37	0.58		
Drinking water	1.04	0.85	0.18	0.46	0.53
Walking				<0.01	<0.01
d 1	12.6 [b]	20.9 [a]	1.07		
d 2	5.34 [b]	14.8 [a]	1.07		
d 4	6.95	6.74	1.07		
d 5	6.42 [b]	9.70 [a]	1.07		
d 6	6.53	4.96	1.07		
d 7	7.36	5.61	1.07		
d 9	8.27 [b]	12.9 [a]	1.07		
Lying				<0.01	<0.01
d 1	21.7 [a]	6.22 [b]	1.53		
d 2	30.2 [a]	17.3 [b]	1.53		
d 4	30.6	32.8	1.53		
d 5	26.3	22.8	1.53		
d 6	21.2	18.1	1.53		
d 7	16.7	19.7	1.53		
d 9	21.9	19.2	1.53		
Lying ruminating	2.97	3.76	0.41	0.18	0.42
Standing				0.62	<0.01
d 1	29.9	33.9	1.75		
d 2	31.9	28.8	1.75		
d 4	20.1	17.4	1.75		
d 5	24.3 [a]	12.5 [b]	1.75		
d 6	21.1	19.9	1.75		
d 7	15.8	18.3	1.75		
d 9	10.4 [b]	17.5 [a]	1.75		
Standing ruminating				<0.01	0.01

d 1	1.19	2.06	0.74
d 2	1.03 [b]	4.32 [a]	0.74
d 4	2.05	1.33	0.74
d 5	2.80	3.14	0.74
d 6	3.94	2.89	0.74
d 7	1.33 [b]	3.37 [a]	0.74
d 9	1.53 [b]	4.60 [a]	0.74

586 [a,b] Within a row, without a common superscript, differ ($p \leq 0.05$) or tend to differ ($p \leq 0.10$).
587 Total time evaluated = 678 min/day.
588



589 **Figure 1**. Playing behaviors of Nellore calves receiving saline solution (Saline; n = 14) or bo-
590 vine-appeasing substance (BAS; n = 14) at weaning. Total time evaluated = 678 min/day. *
591 Repre-sents differences ($p \leq 0.05$) between treatments on each day.
592



593 **Figure 2**. Vocalizing behaviors of Nellore calves receiving saline solution (Saline; n = 14) or
594 bo-vine-appeasing substance (BAS; n = 14) at weaning. Total time evaluated = 678 min/day. *
595 Repre-sents differences ($p \leq 0.05$) between treatments on each day.

596

597 The response to vaccination was affected by treatments, and a treatment × day inter-
598 action was detected (p = 0.05) for PI3 titters, being greater for BAS-treated calves on d 15 and
599 51 compared to Saline-treated calves (Table 5). A treatment × day interaction also tended to be
600 detected (p = 0.10) for PI3 seroconversion, being greater for BAS-treated calves on d 15,
601 compared to Saline-treated calves. Furthermore, a treatment × day interaction was detected (p
602 = 0.02) for BVDV-1 titters, being greater for BAS-treated calves on d 51 com-pared to Saline-
603 treated calves. No effects of treatment × day (p = 0.69) were detected, but treatment effects
604 tended to be detected (p = 0.09) for BVDV-1 seroconversion, being greater for BAS-treated
605 calves than Saline-treated calves. No effects of treatment or its interaction with days ($p \geq 0.12$)
606 were detected for titers and seroconversion of IBR and BVDV-2 (Table 5).

607

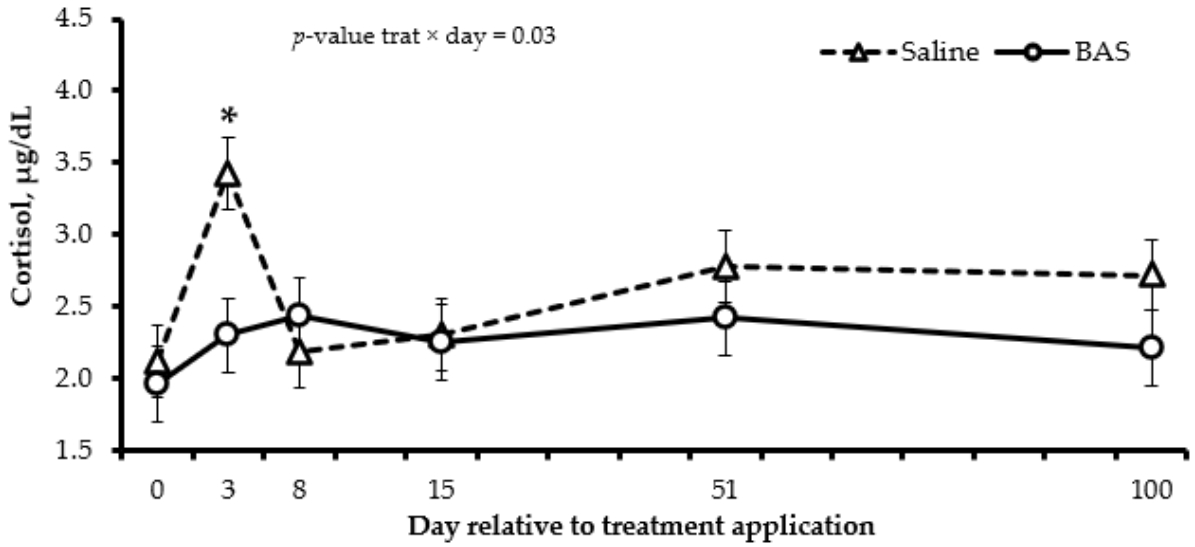
608 **Table 5.** Response to vaccination and acute phase response of Nellore calves receiving saline
609 solution (Saline; n = 14) or bovine-appeasing substance (BAS; n = 14) at weaning.

Items [1]	Treatments		SEM	*p*-Value	
	Saline	BAS		Treatment	Treatment × Day
Response to vaccination					
IBR					
Titers, log_2	2.94	3.06	0.53	0.85	0.66
Seroconversion, % total	91.7	100	5.87	0.33	0.33
PI_3					
Titers, log_2				0.03	0.05
d 0	0.00	0.00	0.53		
d 15	3.21 [b]	5.49 [a]	0.53		
d 51	4.82 [b]	6.15 [a]	0.53		
Seroconversion, % total				0.12	0.10
d 0	0.00	0.00	0.09		
d 15	66.7 [b]	100 [a]	0.09		
d 51	100	100	0.09		
BVDV-1					
Titers, log_2				0.10	0.02
d 0	0.26	0.40	0.61		
d 15	0.26	0.57	0.61		
d 51	4.26 [b]	6.40 [a]	0.61		
Seroconversion, % total	66.7	100	12.0	0.09	0.69
BVDV-2					
Titers, log_2	1.06	1.28	0.42	0.72	0.87
Seroconversion, % total	66.7	66.7	12.2	1.00	1.00
Acute phase response					
Haptoglobin, mg/mL	0.41	0.43	0.02	0.46	0.40
Ceruloplasmin, mg/mL	15.7	14.8	0.64	0.36	0.41

610 [1] On d 0, calves were vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea
611 vi-rus type 1 and 2 (BVDV-1 and 2), parainfluenza-3 (PI3) virus, bovine respiratory syncytial
612 virus, and Mannheimia haemolytica (2 mL s.c.; Bovi Shield Gold One Shot, Zoetis). [a,b] Within
613 a row, without a common superscript, differ ($p \leq 0.05$) or tend to differ ($p \leq 0.10$).
614

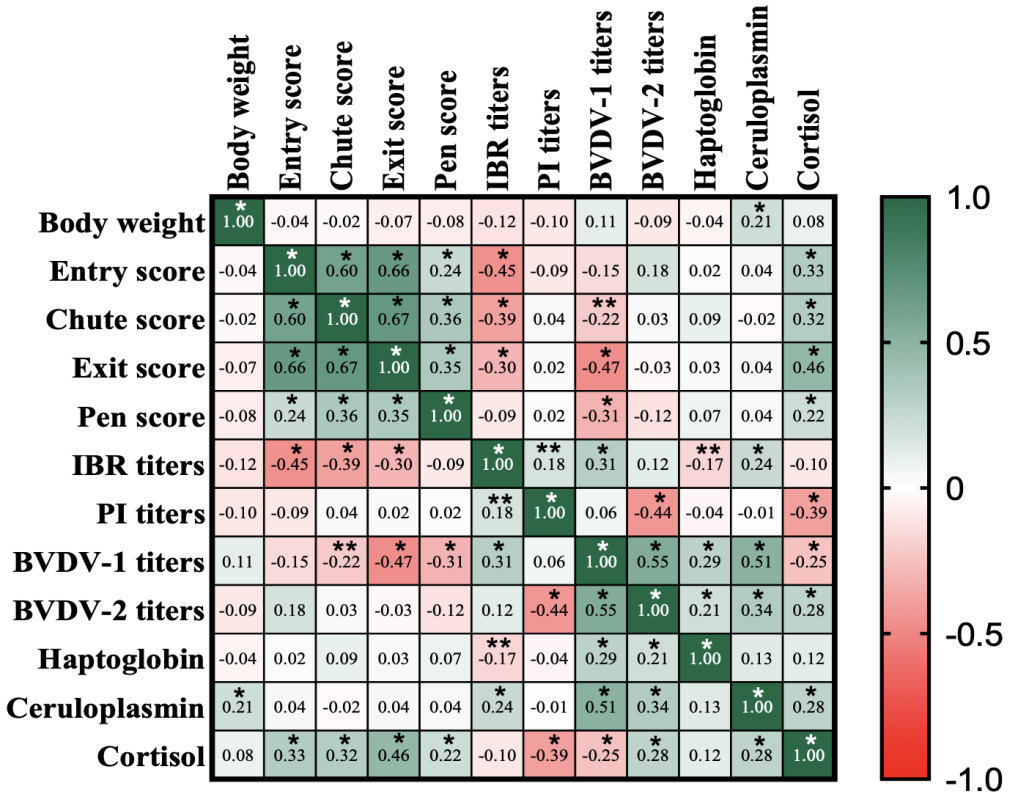
615 The plasma concentration of haptoglobin and ceruloplasmin was not affected by
616 treatment × day or treatment ($p \geq 0.36$; Table 5). However, a treatment × day interaction was
617 detected ($p = 0.03$) for serum cortisol concentration, which was lower for BAS-treated calves
618 on d 3, compared to Saline-treated calves (Figure 3).

619



620 **Figure 3**. Serum cortisol concentration of Nellore calves receiving saline solution (Saline; n =
621 14) or bo-vine-appeasing substance (BAS; n = 14) at weaning. *Represents differences ($p \leq$
622 0.05) between treatments on each day.
623

624 Significative Pearson correlations were detected between several experiment variables
625 ($p \leq 0.05$; Figure 4). The BW had positive correlations only with plasma ceruloplasmin
626 concentration. Temperament variables (entry, chute, exit, and pen scores) were all positively
627 correlated, as expected, and temperament variables had negative correlations with IBR and
628 BVDV-1 titter concentrations but had positive correlations with serum cortisol concentration.
629 However, BVDV-2 titter concentration was positively correlated with serum cortisol
630 concentration. No correlations between temperament variables and plasma haptoglobin and
631 ceruloplasmin were detected ($p > 0.05$).
632



633

634 **Figure 4**. Heat map of Pearson correlations between variables in the experiment. Green colors
635 represent positive correlations, and red colors represent negative correlations. The number in
636 each cell rep-resents the Pearson correlation coefficients. * Represents differences ($p \leq 0.05$),
637 and ** represents tendencies ($p \leq 0.10$).
638

639 ## 4. Discussion

640 Weaning is a highly stressful event for calves, exposing them to physiological, physical,
641 and psychological challenges that directly impact their performance, health, and welfare [1]. In
642 the current experiment, BAS application was able to improve BW, reduce stress, and improve
643 behavior and immune response immediately after weaning, corroborating the immediate
644 window of efficacy of BAS (15 d).

645 In the current experiment, the BAS-treated calves had greater BW only on d 15, com-
646 pared to the Saline-treated calves. Other studies also observed improved ADG in calves when
647 BAS was applied at weaning [9–11]. The absence of BAS effects after d 15 is probably related
648 to the time of action of the product, which is about 15 d, according to the manufacturer. The
649 improved ADG in BAS-treated calves was probably a consequence of the reduced stress

response (i.e., lower temperament scores, lower serum concentration of cortisol, and lower vocalization) and its effects on behavior (mainly by increasing time spent grazing and consuming supplements).

In the current study, the animals did not gain BW from the beginning to the end of the experiment (d 0 to 100). This is explained by the low quality of the forage/hay and the low herbage allowance during the study, which is a common scenario in tropical regions. Alt-hough a protein supplement was offered to calves post-weaning to attenuate the low crude protein concentrations in forage and hay, it was not enough to promote a continuous gain post-weaning. This scenario increased the stress of the experimental calves; however, all animals from both treatments were subjected to the same environment and stress conditions.

The temperament scores and the serum concentration of cortisol were reduced by BAS application, indicating less stress on those calves, mainly during the first two weeks after weaning. In a study conducted in a feedlot with crossbreed (90% British × 10% Nellore) weaned calves, BAS administration reduced the exit velocity by 14 d after weaning, and it also reduced the hair concentration of cortisol on d 14 [12]. In another study with weaned Angus-influenced calves raised in a feedlot, the BAS administration at weaning reduced the plasma concentration of cortisol and increased the plasma concentration of glucose 7 d after weaning [11]. In addition to those results from previous studies with *Bos taurus*-influenced calves raised in a feedlot, we are unaware of a study that evaluated the BAS application on temperament and serum concentration of cortisol in *Bos indicus* calves raised in pasture. Our study demonstrates that BAS administration can reduce the adrenocortical response elicited by weaning, and this reduction can affect calf temperament and BW.

The mechanism through which BAS administration leads to reduced cortisol production is still unclear. However, it is known that BAS target organs involved in pheromone perception include the main olfactory epithelium (MOE) and vomeronasal organ (VNO; [28,29]). The

675 MOE is responsible for the recognition of traditional odor molecules and chemical and
676 environmental signals without specificity or meaning, whereas the VNO is related to
677 pheromone recognition, carrying specific chemosensory signals through the receptors [30],
678 leading to the occurrence of a neuroendocrine cascade [29]. The VNO neurons can encode
679 stimulus strength, activating an entire neural subpopulation and conducting an electrochemical
680 signal to the calf brain [28], stimulating the hypothalamus to exhibit an appropriate
681 neuroendocrine response unique to the specific subpopulation of neurons stimulated in the
682 VNO and causing calming effects in the animal [29].

683 An increase in vocalization and a decrease in time playing are traditional character-istics
684 of behavior presented by calves after weaning due to the high psychological stress caused by
685 the separation from the dam [4]. In the current study, the BAS administration assuaged these
686 effects, which corroborates with the results of temperament and cortisol. In another study, the
687 BAS application reduced the number of escape attempts and mounts on subsequent days after
688 weaning, showing the fast-calming effect of the product on the animal after administration [12].

689 In the current study, the BAS application increased the time spent grazing, eating
690 concentrate, walking, and standing ruminating and reduced the time spent lying. The effects of
691 BAS on physical activity were also evaluated by Schubach et al. [12], but using a pedometer.
692 They found that BAS-treated calves tended to engage in more allogrooming bouts and presented
693 more steps after weaning. All these results show that BAS-treated calves were more active after
694 weaning, and the increased walking in our experiment was more related to exploratory activity
695 than a greater stress response. Increased time spent grazing, eating concentrate, and ruminating
696 in BAS-treated calves shows faster adaptation to the new environment due to the lower stress.
697 These changes in behavior could also be responsible for the improved BW in BAS-treated
698 calves on d 15 of the study. The lower se-rum cortisol concentration detected on d 3 in BAS vs.

699 Saline-treated calves was probably responsible for causing these behavior changes between
700 treatments.

701 Weaning stress-related responses are well known to have a prominent impact on the
702 immune system, leading to immunosuppression in calves [29]. In the current experiment, the
703 BAS application increased the response to vaccination, showing greater humoral immunity
704 against PI3 and BVDV-1. Another study with *Bos taurus*-influenced calves also observed better
705 responses to vaccination after BAS application [12]. Cortisol affects the immune system in
706 several ways, including reducing immune cell proliferation and differentiation, effector cell
707 function, and increasing cytokine expression [31]. In our correlation analysis, serum cortisol
708 concentration was negatively correlated with the serum concentration of PI3 and BVDV-1
709 titers, showing the negative effects of cortisol on the immune system. However, surprisingly,
710 cortisol was positively correlated with BVDV-2 titer con-centration. The reason for that is
711 unknown and deserves further investigation. However, it could explain the lack of BAS effects
712 on the BVDV-2 titer concentration.

713 Acute-phase proteins also affect the immune system and are normally increased by
714 stress [29], but in the current study, haptoglobin and ceruloplasmin were not affected by BAS
715 application. The effects of BAS on acute-phase proteins are inconsistent. Some studies have
716 shown decreased concentrations of haptoglobin [9,12], while others have found no differences
717 [11,32]. In our study, the serum cortisol concentration was reduced by BAS only on d 3, and
718 cortisol has been reported to trigger an acute, transient, and temporary inflammatory cascade
719 that traditionally happens approximately 48 to 72 h after the cortisol peak [29]. Based on that
720 rationale, if the cortisol peak was on d 3, the inflammatory response should have happened after
721 d 3, and in this case, the inflammatory reaction could have happened between d 3 and 8. If our
722 rationale is correct, we could have lost the timing of blood collection for ceruloplasmin and

haptoglobin analysis (blood collection was made on d 0, 3, 8, 15, 51, and 51), and further studies with less spaced-out blood collection de-serve to be conducted.

5. Conclusion

The administration of a bovine-appeasing substance at weaning resulted in lower serum cortisol concentrations, concomitant with lower temperament scores, while at the chute, calves consequently engaged in desirable behaviors immediately post-weaning, such as grazing, eating, and rumination, which ultimately led to enhanced body weight gain and response to vaccination. The bovine-appeasing substance could be used in complement to the best weaning practices to reduce stress and its impact on performance, temperament, pasture behavior, and the immune system.

Conflict of interest statement

The authors O.A.d.S. works at Nutricorp and B.I.C. worked at Nutricorp during the experiment execution, so they were not involved in experiment execution, laboratory and statistical analysis, or draft writing. The other authors declare that they have no conflicts of interest.

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CAPÍTULO 3. APPEASING SUBSTANCE ADMINISTRATION AT FEEDLOT ENTRY IMPACTED GROWTH, TEMPERAMENT, PEN BEHAVIOR, IMMUNOCOMPETENCE, AND MEAT QUALITY OF BEEF HEIFERS

O artigo a seguir foi redigido e submetido de acordo com as normas para publicação no periódico *Livestock Science* (anexoII).

852 **Appeasing substance administration at feedlot entry impacted growth, temperament,**
853 **pen behavior, immunocompetence, and meat quality of beef heifers**

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857

858 **Highlights**

859 • The bovine appeasing substance (BAS) administration increased growth for 45 d.

860 • BAS reduced plasma ceruloplasmin and serum cortisol concentrations on d 15 and 45.

861 • BAS improved immune system and temperament.

862 • BAS increased the time walking and eating and reduced the time standing and seeking.

863 BAS tended to improve meat quality.

864

Abstract

This experiment evaluated the effects of bovine appeasing substance (BAS) administration at feedlot entry on growth, temperament, inflammation, response to vaccination, pen behavior, carcass characteristics, and meat quality of beef heifers. Thirty heifers [½ Nellore × ½ Angus; 198 ± 16.1 kg; 7 ± 1 mo] were weaned and assigned to 1 of 2 treatments (d 0): 1) BAS (n = 15; SecureCattle; IRSEA Group, Quartier Salignan, France) or 2) Saline (n = 15; 0.9% NaCl). Treatments were topically applied (5 mL/calf) at the nuchal skin area of each animal. On d 0, heifers were also vaccinated against respiratory diseases. Heifers from each treatment were kept in different pens for 15 d post-treatment administration and then moved to a single pen. Body weight, blood samples, and temperament traits were collected on d 0, 2, 6, 15, 45, and 150. Group-pen behavior was evaluated from d 0 to d 9. On d 150, heifers were slaughtered, and carcass characteristics and quality were analyzed. Administering BAS at feedlot entry increased (P = 0.05) average daily gain from d 6 to 45, but not (P = 0.31) after d 45. Moreover, BAS reduced ($P \leq 0.03$) plasma ceruloplasmin and serum cortisol concentrations on d 15 and 45 but did not ($P \geq 0.77$) affect plasma haptoglobin. Furthermore, BAS administration improved the response to vaccination, increasing (P = 0.03) infectious bovine rhinotracheitis titers on d 15 and 45 and tending (P = 0.09) to increase parainfluenza-3 virus titers on d 45. The BAS administration reduced ($P < 0.01$) the entry scores on d 6, 15, and 45, tended to reduce (P = 0.08) chute scores from d 2 to 150, and reduced (P = 0.05) exit scores on d 2, 6, and 15. The BAS administration increased ($P \leq 0.04$) walking, drinking, and eating time while also tending ($P \leq 0.10$) to increase lying and ruminating time. Moreover, time standing and seeking were reduced ($P < 0.01$) and tended to reduce (P = 0.10) the time vocalizing by BAS administration. Lastly, BAS tended ($P \leq 0.10$) to increase the myofibrillar fragmentation index and to reduce the thiobarbituric acid reactive substances concentration in meat. In summary, BAS administration at feedlot entry increased growth, reduced stress, and inflammation, and

890 improved humoral immune responses, behavior, and meat quality of heifers, besides no effects
891 on carcass characteristics.
892
893 ***Keywords*****:** bovine appeasing substance, ceruloplasmin, cortisol, pheromone, stress,
894 temperament
895

896 **1. Introduction**

897 Weaning and feedlot entry are two of the most critical events in beef production
898 systems, exposing animals to various stressors and health challenges (Duff and Galyean, 2007).
899 These events stimulate adrenocortical and acute-phase protein responses that stimulate
900 immediate and long-term impacts on calf growth, immunity, temperament, and meat quality
901 (Carroll and Forsberg, 2007; Cappellozza et al., 2020). In some scenarios, feedlot entry is
902 conducted immediately after weaning and thus, strategies for mitigating different stressors in a
903 short period are essential.

904 An alternative approach that can be used to alleviate specific stress-related responses is
905 the use of appeasing pheromones. These have been studied and isolated in several species and
906 act through chemoreceptors located in the vomeronasal organ and the olfactory epithelium,
907 generating signals to the central nervous system to induce a behavioral and physiological
908 response in a conspecific (Liberles, 2014). In cattle, a synthetic analogue of bovine appeasing
909 substance (BAS) has been created and is based on a mixture of fatty acids, reproducing the
910 composition of the natural substance produced by the sebaceous gland on the skin of the
911 mammary gland of the cow (Liberles, 2014; Cappellozza et al., 2022).

912 Recent research has described that BAS administration at weaning increased body
913 weight (BW), reduced reactivity and serum cortisol concentration, and improved behavior and
914 response to vaccination of grazing beef cattle (Vieira et al., 2023). In an experiment conducted

by Cappellozza et al. (2020), BAS administration immediately before transporting the steers to the slaughterhouse reduced the meat pH and the risks of having dark, firm, and dry (DFD) cuts on those animals, which is often observed in high stressed animals, leading to reduced carcass and meat quality (Carrasco-García et al., 2020).

We are unaware of one experiment that evaluated the long-term effects of BAS administration at feedlot entry on the growth, stress, temperament, carcass, and meat quality of beef heifers. Thus, we hypothesized that BAS administration at feedlot entry would reduce the stress and the stress-related effects on growth, physiological responses, temperament, pen behavior, and meat quality of beef heifers. Therefore, the objective of this experiment was to evaluate the effects of BAS administration at feedlot entry on growth, inflammation, response to vaccination, temperament, pen behavior, carcass characteristics, and meat quality of beef heifers.

2. Materials and Methods

The experiment was conducted in an experimental feedlot located at Fazenda Agropecuária Cedron, Anastácio, MS, Brazil (20°30'03.5"S 55°53'34.1"W). All practices utilized were reviewed and approved by the Institutional Animal Care and Use Committee of Universidade Estadual de Mato Grosso do Sul under protocol nº 2.135/2020.

2.1 Animals, treatments, and sample collection

Thirty crossbreed weaned heifers [½ Nellore × ½ Angus; 198 ± 16.1 kg of BW; 7 ± 1 mo of age] were selected for the experiment. Those were previously raised on Marandu-grass pasture [*Urochloa brizantha* (Hochst. ex A. Rich) R. D. Webster, cv. Marandu] and on the day of weaning, were abruptly separated from their dams and transported 3 km in a single truck to the experimental feedlot. Afterward (d 0), heifers were stratified by BW and assigned to 1 of 2

940 treatments: 1) BAS (n = 15; SecureCattle; IRSEA Group, Quartier Salignan, France) or 2)
941 Saline (n = 15; saline solution, 0.9% NaCl). The solutions were topically applied (5 mL/calf)
942 to the nuchal skin area of each animal. Before the treatment administration, heifers were
943 segregated into two groups (1 group/treatment). Saline-treated heifers were processed and
944 immediately released to a feedlot pen before the BAS administration to the other group. This
945 treatment administration method was chosen to avoid any cross-effects of BAS on Saline-
946 treated heifers. Additionally, on d 0, before treatment administration, heifers were vaccinated
947 against infectious bovine rhinotracheitis (IBR), parainfluenza-3 virus (PI_3), bovine viral
948 diarrhea virus type 1 (BVDV-1) and 2 (BVDV-2; 2 mL s.c.; Biopoligen HS; Biogenesis Bago
949 SA, Buenos Aires, Argentina).

950 From d 0 to d 15, heifers were maintained in two similar pens (1 pen/treatment),
951 separated from each other for about 200 m of distance to avoid cross-effects of BAS on Saline-
952 treated heifers. On d 15, heifers were moved to a single pen (both treatments in the same pen),
953 where they were kept until the end of the experiment (d 150).

954 Heifers were offered a total mixed ration (TMR) offered with three initial adaptation
955 formulas to prevent metabolic disorders (1 wk each; d 0 to 21) and one final ration (d 22 to 150;
956 Table 1). The TMR was provided *ad libitum*, twice daily (0700h and 1600h), and the quantity
957 offered was adjusted daily to ensure 5% residuals. The offered TMR samples were collected
958 daily, frozen at -20°C, and pooled by wk for subsequent nutritional profile analysis.

959 Full BW and blood samples were collected on d 0, 2, 6, 15, 45, and 150. Fasted BW was
960 not obtained to avoid shrink-induced stress effects on traits evaluated in this experiment
961 (Marques et al., 2012). Blood samples were collected from a jugular vein into two blood
962 collection tubes (10 mL; Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) with and
963 without sodium heparin for collection of plasma and serum, respectively. After collection,
964 blood samples were immediately stored on ice and then centrifuged at 1200 × g for 30 min for

965 plasma and serum harvest. Samples were stored at -20ºC until further analysis of plasma
966 concentrations of haptoglobin and ceruloplasmin and serum concentration of cortisol and
967 antibody titters (i.e., against IBR, $PI_{3,}$ and BVDV-1 and 2). Haptoglobin, ceruloplasmin, and
968 cortisol were analyzed from samples collected on d 0, 2, 6, 15, 45, and 150, while antibody
969 titers were analyzed in samples from d 0, 15, and 45.

970 Three trained technicians evaluated the temperament (i.e., the same trained personnel
971 who were blinded to treatments during the entire experiment) in the corral on d 0, 2, 6, 15, 45,
972 and 150. The entry and exit scores in the squeeze chute were evaluated according to Baszczak
973 et al. (2006), with scores 1 = animals that walked in or out of the chute; 2 = those that trotted to
974 or from the chute; and 3 = those that ran or galloped in or out of the chute. The chute score was
975 evaluated using an adaptation of Cooke et al. (2011) criterion, where 1 = calm with no
976 movement; 2 = restless movements; 3 = frequent movement; 4 = constant movement,
977 vocalization, shaking of the chute; and 5 = violent and continuous struggling. All heifers were
978 handled calmly and smoothly without an electric shock or physical contact until they reached
979 or left the chute.

980 On d 0, the heifers were individually identified on both sides of the body, with large
981 numbers using hair dye to facilitate animal identification for behavioral evaluation. Behavior
982 was evaluated for nine consecutive days (d 0 to d 9) during twelve consecutive hours [from
983 0600h to 1800h; except for the days of handling in the corral (d 0, 2, and 6), when heifers were
984 evaluated only after the handling (i.e., from 1000h to 1800h)] with an interval of 5 minutes
985 between each scan. The variables evaluated were adapted from Enríquez et al. (2010): walking,
986 drinking water, eating, lying, lying ruminating, standing idle, standing ruminating, playing
987 (jumping, running, no sign of stress), seeking (walking beside the fence, with head held high,
988 looking for the dam), and vocalizing. The variables of total lying (lying + lying ruminating),

total standing (standing idle + standing ruminating), and total ruminating (lying ruminating + standing ruminating) were later calculated.

On d 150, all heifers were loaded into a single truck, and transported for 115 km to a commercial slaughterhouse. Heifers were slaughtered using the technique of brain concussion and section of the jugular vein. After slaughter, the carcass of each heifer was divided into two halves, weighed, and refrigerated (2 to 4°C) for 24 h. After this period, pH and temperature were collected in the *semimembranosus* muscle using a pH meter (Mettler M1120x; Mettler Toledo, Columbus, OH, USA) and thermometer (Clink Termômetro Digital; Clink Comércio de Importação e Exportação LTDA; Joinvile, SC, Brazil) devices. Then, a transverse cut was performed in the *longissimus dorsi* muscle between the 12^{th} and 13^{th} ribs to assess the subcutaneous fat thickness and ribeye area (REA). The subcutaneous fat thickness was measured using a digital caliper (Stainless Steel Caliper; Dexter, China). The REA was determined by taking its format on a tracing paper with the area evaluated in an area meter equipment (LI-3100 Area Meter, LI-COR Environmental, Lincoln, NE, USA).

Ribeye samples (2.5 cm thick) were collected from the *longissimus dorsi* muscle (between the 12^{th} and 13^{th} ribs), vacuum-packaged, identified, and frozen at -20°C for subsequent determination of the Warner–Bratzler shear force (WBSF), marbling score, fat color, meat color, exudate loss, cooking loss, myofibrillar fragmentation index (MFI) and thiobarbituric acid reactive substances (TBARS) concentration.

2.2 Laboratory analysis

The TMR samples were dried for 72 h at 55°C and ground through a 1-mm sieve. Then, these were analyzed according to AOAC (2000) for crude protein (method 976.05), ash (method 942.05), and ether extract (method 920.39). The concentrations of neutral and acid detergent fiber were analyzed as described by Van Soest et al. (1991). The total digestible nutrient

concentrations were calculated as described by Weiss et al. (1992), and net energy for maintenance and gain by the equations proposed by the NASEM (2016). The nutritional profile of TMR diets is described in Table 1.

Plasma concentrations of haptoglobin were analyzed as described by Cooke and Arthington (2013) and ceruloplasmin as described by Demetriou et al. (1974). The inter- and intra-assay CV were 3.6% and 5.4% for haptoglobin and 2.2% and 4.7% for ceruloplasmin, respectively. The serum concentration of cortisol was analyzed (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) as previously described by Cooke et al. (2017) due to 100% cross-reactivity between bovine and human cortisol and accomplished within a single assay with an intra-assay with a CV of 7.6%.

Antibody titers against IBR, PI_3, and BVDV-1 and 2 viruses were assessed using procedures outlined by Rosenbaum et al. (1970). Individual serum samples were evaluated for the greatest dilution of antibody titers that achieved total protection of cells against those viruses and are reported as $\log_2$. Heifers with antibody titers ≥ 4 for each virus were considered seropositive and assigned a value of 1, whereas those with antibody titers < 4 were considered seronegative and assigned a value of 0. These scores were utilized to determine the percentage of heifers that had positive seroconversion for antibody protection against the aforementioned viruses, as previously described by Richeson et al. (2008).

Meat samples were evaluated for tenderness through the WBSF method (AMSA, 2016) using six replicates (1.27-cm diameter), with the fiber direction parallel to the longest dimension of the strip and perpendicular to the direction of the blade, using WBSF equipment (G-R Manufacturing Co., Manhattan, KS, USA) equipped with a Warner–Bratzler blade. Marbling was scored according to the USDA Quality Grade in six classes, where: slight = 400, small = 500, modest = 600, moderate = 700, slightly abundant = 800 and moderately abundant = 900. Fat and meat color were evaluated using a colorimeter (MiniScan XE Plus; HunterLab,

Reston, VA, USA) with a D65 light source, with 10° of observation angle and 30 mm opening of the measuring cell. The luminosity (L*), redness (a*), and yellowness (b*) scales of the CIE Lab system were used.

Meat samples were evaluated for exudate and cooking losses following the methodologies reported in AMSA (2016). Briefly, each steak was weighed on aluminum trays and thawed for 24 h at 4°C to obtain the exudate losses. The steaks were then cooked in an oven with upper and lower heaters (Forno Elétrico Crystal Plus Advanced, Layr Eletrodomésticos, São Paulo, SP, Brazil) at 170°C until reaching 71°C in the center. The temperature was determined with individual thermocouples thermometers (Taylor 1478-21, Taylor Precision Products, Oak Brook, IL, USA) inserted into the geometric center of each pile. The samples were removed from the oven, dried, and weighed to obtain the cooking losses. The MFI was evaluated using the procedures described by Ramos and Gomide (2007) and TBARS concentration according to Lemon (1975).

2.3 Statistical analyses

One Saline-treated heifer had to be removed from the experiment because of its temperament, which challenged her handling in the working chute. The heifers were considered the experimental units for all analyses, and data were analyzed using MIXED (for quantitative variables) and GLIMMIX (for binomial variables) procedures of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4). The Satterthwaite approximation was selected to determine the denominator of degrees of freedom for the test of fixed effects. Average daily gain (ADG), carcass traits, and meat quality traits were tested for fixed effect of treatment and using calf(treatment) as a random effect. All other variables were analyzed as repeated measures and tested for fixed effects of treatment, day, and treatment × day, using calf(treatment) as a random variable and subject. The results of d 0 were included as covariates in each respective analysis

but were removed from the model when $P > 0.10$. The Toeplitz covariance structure was selected for BW, and the first-order autoregressive covariance structure was selected for all other variables. The covariance structures were selected according to the lowest Akaike information criterion. Means were separated using PDIFF, and all results were reported as LSMEANS followed by SEM. Significance was defined as $P \leq 0.05$, and tendency when $P > 0.05$ and ≤ 0.10.

3. Results

No treatment × day interaction or main treatment effects were detected ($P \geq 0.29$) for BW. However, BAS-treated heifers had greater ADG (treatment effects; $P = 0.05$) from d 6 to 45 than Saline-treated heifers (Table 2).

No treatment × day interaction or main treatment effects were detected ($P \geq 0.77$) for plasma concentration of haptoglobin (Figure 1). However, BAS-treated heifers had lower plasma concentrations of ceruloplasmin and serum concentrations of cortisol (treatment × day effects; $P \leq 0.03$) on d 15 and 45, compared to Saline-treated heifers (Figure 1). Furthermore, BAS-treated heifers had greater IBR titer concentration (treatment × day effects; $P = 0.03$) on d 15 and 45 and tended to have greater IBR seroconversion (treatment × day effects; $P = 0.09$) on d 15 vs. Saline-treated heifers (Table 3). In addition, BAS-treated heifers tended to have greater PI_3 titer concentration (treatment × day effects; $P = 0.09$) on d 45, whereas no effects were noted ($P \geq 0.21$) for PI_3 seroconversion (Table 3). Surprisingly, despite being present in the vaccine, titer against BVDV-1 and BVDV-2 was not produced by any heifer, regardless of the treatment.

The BAS-treated heifers had lower entry scores on d 6, 15, and 45 (treatment × day effects; $P < 0.01$), tended to have lower chute scores from d 2 to 150 (treatment × day effects;

P = 0.08), and had lower exit scores on d 2, 6, and 15 (treatment × day effects; P = 0.05) compared to Saline-treated heifers (Table 4).

The diurnal behavior was affected by treatments, and when evaluated in min/d, BAS-treated heifer spent more time walking, drinking, and eating (treatment effects; $P \leq 0.04$), tended to have greater total time lying and ruminating (treatment effects; $P \leq 0.10$), while spending less time standing idle, total time standing and seeking (treatment effects; $P < 0.01$). Finally, BAS-treated heifers tended to spend less time vocalizing (treatment effects; P = 0.10; Table 5). When the behavior was evaluated in percentual of the activities, BAS-treated heifers had greater percentual walking, drinking, and eating (treatment effects; $P \leq 0.03$) and lower percentual standing idle and seeking (treatment effects; $P < 0.01$; Table 5). Treatments did not affect other behavior variables in min/d or percentual of activities ($P \geq 0.11$; Table 5).

Treatments did not affect carcass characteristics ($P \geq 0.11$), neither WBSF, marbling score, fat color, meat color, exudate, or cooking losses (Table 6). However, BAS-treated heifers tended ($P \leq 0.10$) to have greater MFI and lower TBARS concentration vs. Saline-treated heifers (Table 6).

4. Discussion

Weaning and feedlot entry are two of the most critical events in beef production systems, exposing animals to various stressors and health challenges (Duff e Galyean, 2007). In this experiment, BAS administration at feedlot entry reduced the stress and the stress-related effects on growth, inflammation, immune system, temperament, pen behavior, and meat quality of heifers.

The BAS administration improved the ADG from d 6 to 45 but did not improve the BW at slaughter (d 150). Several experiments reported an improvement in ADG in the weeks post-BAS administration (~4 to 6 wk; Cappellozza et al., 2020; Colombo et al., 2020; Cooke et al.,

2020; Vieira et al., 2023), but not for a longer time (100 d; Vieira et al., 2023). In the current experiment, the absence of BAS effects after d 45 is probably related to the time of action of the product, which is about 15 d, according to the manufacturer. The improved ADG in BAS-treated heifers was probably a consequence of the reduced stress response (i.e., lower temperament scores, lower serum cortisol concentration, and lower plasma ceruloplasmin concentration) and its effects on behavior (mainly by increasing time spent eating and ruminating and decreasing the time seeking and vocalizing) indicating faster adaptation to the new environment condition.

Administering BAS reduced the plasma concentrations of ceruloplasmin and serum cortisol concentrations on d 15 and 45. In other experiments, BAS administration also reduced cortisol serum, plasma, or hair concentrations (Colombo et al., 2020; Schubach et al., 2020; Vieira et al., 2023), but not on plasma ceruloplasmin (Vieira et al., 2023). Our experiment demonstrated that weaning and feedlot entry elicited an adrenocortical response (i.e., increasing the serum concentration of cortisol) and were responsible for eliciting an inflammatory response (i.e., increasing the plasma concentration of ceruloplasmin and haptoglobin), suggesting that BAS was effective in attenuating this cascade of events. According to Cappellozza et al. (2022), acute increases in cortisol have been reported to trigger an acute, transient, and temporary inflammatory cascade, and it has been associated with reduced growth rates.

The mechanism through which BAS administration leads to reduced cortisol production is still unclear. However, it is known that BAS targets organs involved in pheromone perception, including the main olfactory epithelium (MOE) and vomeronasal organ (VNO; Cappellozza et al., 2022; Kekan et al., 2017). The MOE is responsible for the recognition of traditional odor molecules and chemical and environmental signals without specificity or meaning, whereas the VNO is related to pheromone recognition, carrying specific chemosensory signals through the receptors (Grus et al., 2005), leading to the occurrence of a

neuroendocrine cascade (Cappellozza et al., 2022). The VNO neurons can encode stimulus strength, activating an entire neural subpopulation and conducting an electrochemical signal to the calf brain (Kekan et al., 2017), stimulating the hypothalamus to exhibit an appropriate neuroendocrine response unique to the specific subpopulation of neurons stimulated in the VNO and causing calming effects in the animal (Cappellozza et al., 2022).

The increase on IBR and PI_3 titer concentrations following BAS administration suggests an improvement in the immune system by the product. In another experiment where the stress amount likely was alleviated (i.e., animals were kept in the same location and grazing the same pasture before and after weaning), BAS administration at weaning concurrently with vaccination against respiratory diseases increased the serum titer concentration against PI_3 and BVDV-1 (Vieira et al., 2023). The improvement of the immune system in BAS-treated heifers is related to less adrenocortical stimulation (i.e., less production of cortisol). Cortisol affects the immune system in several ways, including reducing immune cell proliferation and differentiation, affecting cell function, and increasing cytokine expression (Carrol et al., 2007).

Heifers that received BAS presented an alleviated post-weaning stress response and lower temperament scores (mainly chute scores) for 150 d. However, the most intense effects were observed for the first 45 d of the experiment. Over 100 days, Vieira et al. (2023) observed a reduced temperament score for only 51 d in calves that received a BAS administration at weaning. It is likely that the current experiment had more stressful weaning practices than the ones reported by Vieira et al. (2023; i.e., including transportation and change from grazing to a feedlot environment), and this greater stress could have caused emotional trauma, and, thus, becoming more reactive for a longer period. This could explain the lower production of cortisol and ceruloplasmin that led to a decrease in temperament score observed in BAS-treated heifers.

The BAS administration affected the pen behavior, mainly increasing the time spent walking, drinking, eating, lying, and ruminating and decreasing the time spent standing,

seeking, and vocalizing. The BAS effects on the behavior were also evaluated by Schubach et al. (2020), and BAS-treated calves at weaning and kept on a feedlot tended to engage in more allogrooming bouts, and presented more steps after weaning. In the experiment of Vieira et al. (2023), BAS-treated calves at weaning and kept in a grazing system increased the time spent grazing, eating concentrate, walking, and standing ruminating and reduced the time spent lying, agreeing with results detected in the current experiment. All these results demonstrate that BAS-treated heifers were more active after weaning and the increased walking in our experiment was more related to exploratory activity than a greater stress response. Increased time spent drinking, eating, and ruminating in BAS-treated heifers also suggests a faster adaptation to a novel environment due to the lower stress.

The BAS-treated heifers presented less time seeking and vocalizing. An increase in time seeking for the dam and vocalizing are traditional characteristics of behavior presented by post-weaning calves due to the high psychological stress caused by the separation from their dams (Enriquez et al., 2010). In other experiments, BAS administration at weaning reduced the number of escape attempts and mounts on subsequent days after weaning (Schubach et al. (2020), and increased the time playing, and decreased the time vocalizing (Vieira et al., 2023). All these effects on behavior observed herein could be explained by the reduced temperament, serum cortisol and plasma ceruloplasmin concentrations in BAS-treated heifers.

Stressful situations are often observed as animals are shipped to slaughter, considering that this management involves human handling procedures, transport to the slaughter facility, loading and unloading, arrival in a novel environment (i.e., slaughter facility), as well as feed and water deprivation (Cappellozza et al., 2020). Furthermore, more stressed animals before slaughter had reduced carcass and meat quality (Carrasco-García et al. 2020). However, in the current experiment, BAS administration did not affect carcass characteristics, WBSF, marbling score, fat color, meat color, exudate loss, or cooking loss. In an experiment conducted by

Cappellozza et al. (2020), BAS administration immediately before transporting steers to the slaughterhouse reduced the meat pH and the risks of having DFD meat but did not affect the meat color. In our experiment, the lack of effects on several variables of carcass and meat quality could be related to a very long period of BAS administration before the slaughter (150 d), reducing the chance to detect effects, compared to Cappellozza et al. (2020) that applied this technology immediately before transportation to the slaughterhouse.

In the current experiment, BAS administration tended to increase MFI in meat. The MFI is one of the most important indicators for measuring increased meat tenderness and proteolysis, indicating both I-band breaks and loss of myofibril integrity (Taylor et al., 1995), and stress normally reduces the MFI by affecting the pH and glycogen concentration in the muscle (Hamoen et al., 2013). In the current experiment, BAS administration did not reduce the serum concentration of cortisol on the day before the slaughter (d 150) but tended to decrease chute scores during the entire experiment, including d 150. As BAS-treated heifers were calmer during the entire experiment, we hypothesize that they were also calmer in the slaughterhouse, thus storing less glycogen in the muscle and improving the MFI by this mechanism.

The BAS administration tended to reduce TBARS concentration in meat. The TBARS concentration in meat measures lipid oxidation, and it is a primary cause of quality defects in meat products, including changes in flavor, color, texture, and nutritive value (Deters and Hansen, 2020). According to the same authors, transit and handling in the slaughter facilities increase oxidative stress through psychological stress, feed and water deprivation, and physical exertion. As BAS-treated heifers were calmer during the entire experiment, we hypothesize they were also calmer during transportation and slaughter facilities and this stress reduction was responsible for reducing the TBARS concentration in meat. We did not evaluate temperament or collected blood samples (i.e., to evaluate cortisol concentration and oxidant/antioxidant

system markers) during transportation and in the slaughterhouse to test this rationale, and it deserves further investigation.

5. Conclusion

In summary, BAS administration reduced the impact of weaning and feedlot entry stress on growth, immune system, serum cortisol concentration, plasma ceruloplasmin concentration, improved the behavior and adaptation to a new environment (i.e., feedlot pen), and besides no effect on carcass, improved meat quality (i.e., increased MFI and decreased TBARS concentration on meat). Research is still warranted to examine the benefits of further repeated BAS administration (i.e., at weaning and before transportation to the slaughter facilities on temperament, physiological stress, oxidative stress, and meat quality of beef cattle.

Conflict of interest statement

The authors Osvaldo A. de Sousa works at Nutricorp, and Bruno I. Cappellozza worked at Nutricorp during the experiment execution, so they were involved only on experiment planning and reviewing the manuscript draft. The other authors declare that they have no conflict of interest.

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Table 1. Composition and nutritional profile of the total mixed ration offered *ad libitum* to the heifers.

Items	Periods			
	d 0 to 7	d 8 to 14	d 15 to 21	d 22 to 150
Composition, dry matter (DM) basis				
Grass silage[1], %	80.7	66.3	43.1	12.0
Sugar cane bagasse, %	0.00	0.00	0.00	7.16
Ground corn, %	10.0	24.9	37.7	59.7
Soybean hull, %	6.00	4.14	13.5	14.3
Soybean meal, %	1.60	2.48	2.70	2.60
Commercial mix[2], %	1.70	2.24	3.00	4.29
Nutritional profile[3], (DM) basis				
CP, %	9.61	10.8	12.5	14.0
Ash, %	7.89	6.93	5.81	4.10
EE, %	2.27	3.02	3.74	4.86
NDF %	63.4	53.5	45.9	32.5
ADF, %	39.9	33.0	28.1	19.3
TDN[4], %	53.9	59.0	66.1	73.9
NEm[5], Mcal/kg	1.37	1.45	1.54	1.63
NEg[5], Mcal/kg	0.79	0.86	0.96	1.04

[1]*Panicum maximum* cv. Tanzânia.

[2]Nutriforte Concentrado Confinamento (Nutriforte, Dourados, MS. Brazil). Composition: crude protein (CP) = 90%; total digestible nutrients (TDN) = 67%; ether extract (EE) = 0.7; calcium = 0.25%; phosporus = 0.14%; sodium = 0.25%, potassium = 8500 mg/kg; cobalt = 1.0 mg/kg; selenium = 2.0 mg/kg; zinc = 450 mg/kg; and sodium monensin = 600 mg/kg.

[3]Neutral detergent fiber (NDF); acid detergent fiber (ADF), net energy for maintenance (NEm) and gain (NEg).

[4]Calculated as proposed by Weiss et al. (1992).

[5]Calculated using the equations proposed by the NASEM (2016).

Table 2. Growth performance of beef heifers receiving saline solution (Saline; n = 14) or bovine appeasing substance (BAS; n = 15) at feedlot entry (d 0).

Items	Treatments[1]		SEM	*P*-value	
	Saline	BAS		Treatment	Treatment × day
Body weight, kg				0.88	0.29
d 0	198	198	3.67		
d 6	214	211	3.67		
d 15	231	232	3.67		
d 45	275	280	3.72		
d 150	420	418	3.67		
Average daily gain, kg/d					
d 0 to 6	2.62	2.19	0.27	0.27	
d 0 to 15	2.18	2.29	0.14	0.58	
d 0 to 45	1.70	1.84	0.09	0.27	
d 0 to 150	1.48	1.47	0.04	0.82	
d 6 to 15	1.88	2.35	0.16	0.05	
d 6 to 45	1.56	1.79	0.08	0.05	
d 6 to 150	1.43	1.44	0.04	0.93	
d 15 to 45	1.46	1.63	0.10	0.26	
d 15 to 150	1.40	1.37	0.04	0.61	
d 45 to 150	1.39	1.32	0.04	0.31	

[1]Saline = saline solution (0.9% NaCl) and BAS (Secure Cattle, IRSEA Group, Quartier Salignan, France). Solutions (5 ml) were topically applied to the nuchal skin area of each animal.

Table 3. Response to vaccination of beef heifers receiving saline solution (Saline; n = 14) or bovine appeasing substance (BAS; n = 15) at feedlot entry (d 0).

Items[1]	Treatments[2]		SEM	*P*-value	
	Saline	BAS		Treatment	Treatment × day
IBR					
Titers, log_2				< 0.01	0.03
d 0	0.00	0.00	0.35		
d 15	1.50[a]	3.17[b]	0.35		
d 45	2.17[a]	3.84[b]	0.35		
Seroconversion, % total				0.12	0.09
d 0	0.00	0.00	8.60		
d 15	66.7[x]	100[y]	8.60		
d 45	100	100	8.60		
PI_3					
Titers, log_2				0.10	0.09
d 0	0.32	0.57	0.41		
d 15	3.59	4.50	0.41		
d 45	2.87[x]	4.67[y]	0.41		
Seroconversion, % total	75.0	100	13.0	0.21	0.34

[1] On d 0, before treatment administration, heifers were vaccinated against infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI_3) virus, bovine viral diarrhea virus type 1 and 2 (BVDV-1 and 2; 2 mL s.c.; Biopoligen HS; Biogenesis Bago SA, Buenos Aires, Argentina).

[2] Saline = saline solution (0.9% NaCl) and BAS (Secure Cattle, IRSEA Group, Quartier Salignan, France). Solutions (5 ml) were topically applied to the nuchal skin area of each animal.

[a-b]Within a row, without a common superscript differ ($P \leq 0.05$).

[x-y]Within a row, without a common superscript tends to differ ($P \leq 0.10$).

Table 4. Temperament scores in the chute of beef heifers receiving saline solution (Saline; n = 14) or bovine appeasing substance (BAS; n = 15) at feedlot entry (d 0).

Items	Treatments[1]		SEM	*P*-value	
	Saline	BAS		Treatment	Treatment × day
Entry score, 1 - 3				0.02	<0.01
d 0	1.34	1.40	0.07		
d 2	1.16	1.08	0.07		
d 6	1.46[a]	1.17[b]	0.07		
d 15	1.50[a]	1.17[b]	0.07		
d 45	1.63[a]	1.31[b]	0.07		
d 150	1.30	1.45	0.07		
Chute score, 1 - 5				0.01	0.08
d 0	1.71	1.69	0.16		
d 2	2.10[x]	1.69[y]	0.16		
d 6	2.12[x]	1.47[y]	0.16		
d 15	1.97[x]	1.46[y]	0.16		
d 45	1.85[x]	1.45[y]	0.16		
d 150	1.86[x]	1.47[y]	0.16		
Exit score, 1 - 3				0.01	0.05
d 0	1.69	1.60	0.11		
d 2	1.63[a]	1.30[b]	0.11		
d 6	1.79[a]	1.40[b]	0.11		
d 15	1.76[a]	1.25[b]	0.11		
d 45	1.77	1.61	0.11		
d 150	1.62	1.55	0.11		

[1] Saline = saline solution (0.9% NaCl) and BAS (Secure Cattle, IRSEA Group, Quartier Salignan, France). Solutions (5 ml) were topically applied to the nuchal skin area of each animal.

[a-b]Within a row, without a common superscript differ ($P \leq 0.05$).

[x-y]Within a row, without a common superscript tends to differ ($P \leq 0.10$).

Table 5. Pen behavior of beef heifers receiving saline solution (Saline; n = 14) or bovine appeasing substance (BAS; n = 15) at feedlot entry (d 0).

Items	Treatments[1]		SEM	*P*-value
	Saline	BAS		
min/day				
Walking	37.8	53.1	2.34	<0.01
Drinking water	9.52	11.9	0.81	0.04
Eating	126	145	4.09	<0.01
Lying	115	119	4.34	0.50
Lying ruminating	57.1	67.9	4.73	0.11
Total lying	172	187	6.45	0.10
Standing idle	207	170	5.74	<0.01
Standing ruminating	15.6	14.9	2.22	0.82
Total standing	223	185	7.25	<0.01
Total ruminating	72.8	82.8	4.11	0.09
Playing	5.91	4.81	0.92	0.40
Seeking	4.64	1.30	0.32	<0.01
Vocalizing	9.56	5.96	1.54	0.10
% of the activities				
Walking	6.61	11.1	0.45	<0.01
Drinking water	1.55	1.95	0.13	0.03
Eating	20.5	23.3	0.69	<0.01
Lying	19.4	19.8	0.81	0.69
Lying ruminating	9.13	10.5	0.74	0.20
Total lying	28.6	30.3	1.13	0.26
Standing idle	36.5	28.7	1.17	<0.01
Standing ruminating	2.43	2.28	0.35	0.76
Total standing	38.9	31.0	1.39	<0.01
Total ruminating	11.6	12.8	0.65	0.20
Playing	0.94	0.75	0.14	0.36
Seeking	0.93	0.23	0.06	<0.01
Vocalizing	2.05	1.32	0.45	0.26

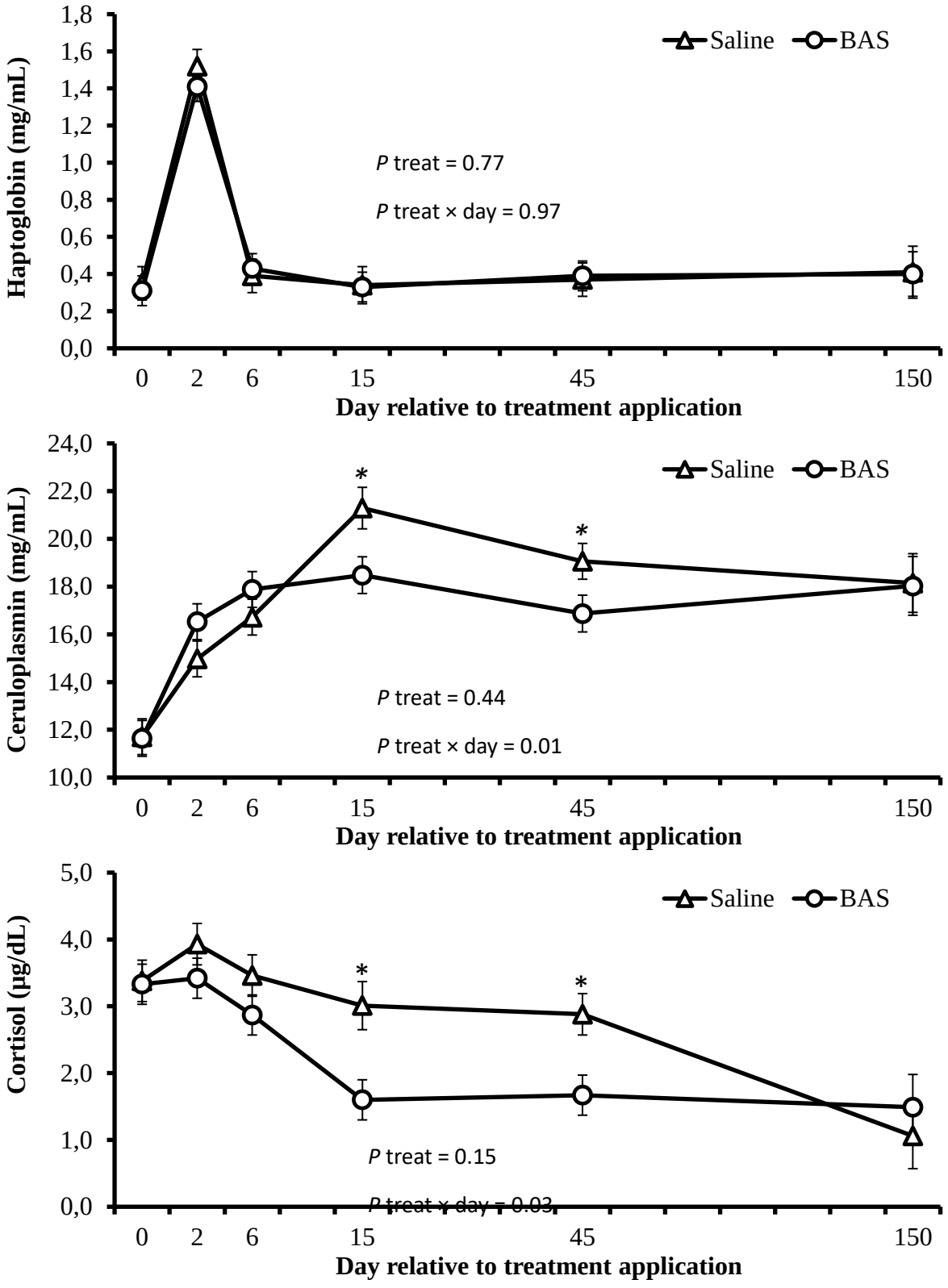
[1] Saline = saline solution (0.9% NaCl) and BAS (Secure Cattle, IRSEA Group, Quartier Salignan, France). Solutions (5 ml) were topically applied to the nuchal skin area of each animal.

Table 6. Carcass characteristics and meat quality of beef heifers receiving saline solution (Saline; n = 14) or bovine appeasing substance (BAS; n = 15) at feedlot entry (d 0).

Items	Treatments[1]		SEM	*P*-value
	Saline	BAS		
Carcass traits				
Hot carcass weight, kg	220.54	221.33	3.45	0.87
Carcass yield, %	51.75	51.92	0.27	0.67
pH (24h)	5.77	5.76	0.06	0.94
Temperature (24h), ºC	7.60	8.02	0.19	0.11
Fat thickness, mm	6.99	7.99	0.45	0.13
Ribeye area, cm^2	71.9	72.8	1.91	0.76
Meat quality traits[2]				
WBSF, kgF/cm^2	7.20	6.91	0.47	0.68
Marbling score, points	430.0	440	15.8	0.66
Fat color				
L*	65.0	65.4	0.51	0.57
a*	6.57	6.95	0.27	0.34
b*	11.4	11.5	0.33	0.84
Meat color				
L*	39.9	39.3	0.84	0.60
a*	18.4	18.1	0.66	0.77
b*	8.95	8.33	0.52	0.41
Exudate loss,%	4.90	4.83	0.51	0.93
Cooking loss,%	21.7	25.6	1.67	0.12
MFI	89.2	97.9	3.54	0.10
TBARS, mg/g	9.19	9.09	0.03	0.06

[1] Saline = saline solution (0.9% NaCl) and BAS (Secure Cattle, IRSEA Group, Quartier Salignan, France). Solutions (5 ml) were topically applied to the nuchal skin area of each animal.

[2]Warner–Bratzler shear force (WBSF), luminosity (L*), green to red (a*), and blue to yellow (b*), thiobarbituric acid reactive substances (TBARS); myofibrillar fragmentation index (MFI).



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1396 **Figure 1**. Plasma concentrations of haptoglobin and ceruloplasmin and serum concentration of
1397 cortisol of beef heifers receiving saline solution (Saline; 0.9% NaCl; n = 14) or bovine
1398 appeasing substance (BAS; Secure Cattle, IRSEA Group, Quartier Salignan, France; n = 15) at

feedlot entry (d 0). Solutions (5 ml) were topically applied to the nuchal skin area of each animal. *$P \leq 0.05$.

IMPLICAÇÕES E CONSIDERAÇÕES FINAIS

A substância apaziguadora bovina (SAB) se apresenta como uma alternativa para melhorar a adaptação dos animais ao ambiente, podendo melhorar o sistema imunológico e aumentar o desempenho.

Com animais em pastejo, a administração da SAB ao desmame resultou em menores concentrações séricas de cortisol, concomitantemente a menores pontuações de temperamento no tronco. Os bezerros desenvolveram ainda comportamentos desejáveis imediatamente após o desmame, tais como pastar, comer e ruminar. Tudo isto os levou a um maior ganho de peso corporal e a uma maior resposta à vacinação. A substância apaziguadora bovina pode ser usada como complemento às melhores práticas de desmame para reduzir o stress e o seu impacto no desempenho, temperamento, comportamento no pasto e sistema imunológico.

Em animais desmamados e encaminhados imediatamente ao confinamento, a administração de BAS reduziu o impacto do stress do desmame e da entrada no confinamento sobre o crescimento, o sistema imunológico, a concentração sérica de cortisol e a concentração plasmática de ceruloplasmina, além de melhorar o comportamento e a adaptação dos animais a um novo ambiente. Apesar de não ter efeito sobre a carcaça, ela foi capaz de melhorar a qualidade da carne produzida.

Apesar da administração da SAB ter-se mostrado eficiente em todas as situações de desmama, pode-se sugerir a continuidade de estudos que examine os benefícios de uma administração repetida de BAS, ao desmame e antes do transporte para o abate, sobre o temperamento, o stress fisiológico, o stress oxidativo e a qualidade da carne de bovinos de carne de bovino.

ANEXOS

animals

MDPI

Article

Effects of an Appeasing Substance Application at Weaning on Growth, Stress, Behavior, and Response to Vaccination of *Bos indicus* Calves

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Simple Summary: Weaning is one of the most stressful experiences for beef calves during their life cycle; however, it is a crucial practice for the production system. Calming pheromones can alleviate stress and reduce its negative impacts on behavior, growth, and the immune system. In this study, the administration of a bovine-appeasing substance enhanced body weight gain, reduced temperament scores and serum cortisol concentration, and improved the behavior and response to vaccination. Therefore, the bovine-appeasing substance could be used in complement to the best weaning practices to reduce stress and its impact on performance, temperament, grazing behavior, and the immune system.

Abstract: An analog of a bovine-appeasing substance (BAS) was previously demonstrated to have calming effects, and it could be an alternative to alleviate the stress caused by weaning. Thus, the objective of this study was to evaluate the effects of BAS administration at weaning on growth, stress, behavior, and response to vaccination of Nellore calves. Eighty-six Nellore calves (40 females and 46 males) were abruptly weaned and randomly assigned into 1 of 2 treatments: (1) saline solution (0.9% NaCl; $n = 43$) and (2) BAS (Secure Catte, IRSEA Group, Quartier Salignan, France; $n = 43$). The solutions were topically applied (5 mL/calf) to the nuchal skin area of each animal. On d 0, before treatment application, calves were vaccinated against infectious bovine rhinotracheitis (IBR), parainfluenza-3 (PI_3) virus, and bovine viral diarrhea virus types 1 and 2 (BVDV-1 and 2). Calves from each treatment were kept in different pastures for 15 d (time of BAS action) and then moved to a single pasture. Body weight (BW), blood samples, and temperament in the chute (entry score, chute score, and exit score) were collected on d 0, 3, 8, 15, 51, and 100, and behavior on pasture on d 1, 2, 4, 5, 6, 7, and 9. Calves assigned to BAS vs. Saline treatment tended to have greater BW on d 15 ($p = 0.10$), tended to have lower entry scores on d 8 and 51 ($p = 0.10$), and chute scores on d 8 ($p = 0.07$), and had lower exit scores on d 8 ($p = 0.02$). Calves assigned to BAS vs. Saline treatment also had greater time grazing on d 7 and 9 ($p < 0.01$), eating concentrate on d 2, 5, and 6 ($p = 0.05$), walking on d 1, 2, 5, and 9 ($p < 0.01$), standing and ruminating on d 2, 7 and 9 ($p < 0.01$), and playing on d 2, 4, 6, 7, and 9 ($p < 0.01$). Furthermore, they had lower time lying on 1 and 2 ($p < 0.01$), standing on d 5 and 9 ($p < 0.01$), and vocalizing on d 1 and 2 ($p < 0.01$). Calves assigned to BAS vs. Saline treatment had greater serum titer concentrations of PI_3 t on d 15 and 51 ($p = 0.05$) and BVDV-1 on d 51 ($p = 0.02$). However, they had lower serum concentrations of cortisol on d 3 ($p = 0.03$). BAS administration did not affect ($p \geq 0.12$) the serum titer concentration of IBR and BVDV-2 titers or

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Livestock Science

Appeasing substance administration at feedlot entry impacted growth, behavior, immunocompetence, and meat quality of beef heifers

--Manuscript Draft--

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Abstract:	This experiment evaluated the effects of bovine appeasing substance (BAS) administration at feedlot entry on growth, temperament, inflammation, response to vaccination, pen behavior, carcass characteristics, and meat quality of beef heifers. Thirty heifers [½ Nellore × ½ Angus; 198 ± 16.1 kg; 7 ± 1 mo] were weaned and assigned to 1 of 2 treatments (d 0): 1) BAS (n = 15; SecureCattle; IRSEA Group, Quartier Salignan, France) or 2) Saline (n = 15; 0.9% NaCl). Treatments were topically applied (5 mL/calf) at the nuchal skin area of each animal. On d 0, heifers were also vaccinated against respiratory diseases. Heifers from each treatment were kept in different pens for 15 d post-treatment administration and then moved to a single pen. Body weight, blood samples, and temperament traits were collected on d 0, 2, 6, 15, 45, and 150. Group-pen behavior was evaluated from d 0 to d 9. On d 150, heifers were slaughtered, and carcass characteristics and quality were analyzed. Administering BAS at feedlot entry increased (P = 0.05) average daily gain from d 6 to 45, but not (P = 0.31) after d 45. Moreover, BAS reduced (P ≤ 0.03) plasma ceruloplasmin and serum cortisol concentrations on d 15 and 45 but did not (P ≥ 0.77) affect plasma haptoglobin. Furthermore, BAS administration improved the response to vaccination, increasing (P = 0.03) infectious bovine rhinotracheitis titers on d 15 and 45 and tending (P = 0.09) to increase parainfluenza-3 virus titers on d 45. The BAS administration reduced (P < 0.01) the entry scores on d 6, 15, and 45, tended to reduce (P = 0.08) chute scores from d 2 to 150, and reduced (P = 0.05) exit scores on d 2, 6, and 15. The BAS administration increased (P ≤ 0.04) walking, drinking, and eating time while also tending (P ≤ 0.10) to increase lying and ruminating time. Moreover, time standing and seeking were reduced (P < 0.01) and tended to reduce (P = 0.10) the time vocalizing by BAS administration. Lastly, BAS tended (P ≤ 0.10) to increase the myofibrillar fragmentation index and to reduce the thiobarbituric acid reactive substances concentration in meat. In summary, BAS administration at feedlot entry increased growth, reduced stress, and inflammation, and improved humoral immune responses, behavior, and meat quality of heifers, besides no effects on

Anexo II – Artigo submetido (Capítulo 3) ao periódico cientifico *Livestock Science*