UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

COMBINAÇÃO DE COMPOSTOS ANTIMICROBIANOS E MISTURA DE MICRO MINERAIS ORGÂNICOS E LEVEDURA NA DIETA SOBRE A QUALIDADE E ACEITABILIDADE DA CARNE BOVINA

Autora: Aylle Medeiros Matos
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MARINGÁ
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Tese apresentada, como parte das exigências para obtenção do título de DOUTORA EM ZOOTECNIA, no Programa de Pós-Graduação em Zootecnia da Universidade Estadual de Maringá – Área de concentração: Produção Animal.

MARINGÁ
Estado do Paraná
julho – 2021

Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá - PR, Brasil)

M433c

Matos, Aylle Medeiros
Combinação de compostos antimicrobianos e mistura de micro minerais orgânicos e levedura na dieta sobre a qualidade e aceitabilidade da carne bovina / Aylle Medeiros Matos. -- Maringá, PR, 2021.
92 f.: il., figs., tabs.

Orientador: Prof. Dr. Ivanor Nunes do Prado.
Tese (Doutorado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Departamento de Zootecnia, Programa de Pós-Graduação em Zootecnia, 2021.

1. Bovinos- Nutrição - Aditivos. 2. Consumidores. 3. Bovinos- Nutrição - Compostos Antimicrobianos. 4. Avaliação sensorial. 5. Bovinos- Nutrição - Confinamento . I. Prado, Ivanor Nunes do , orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Departamento de Zootecnia. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 23.ed. 636.2085

Marinalva Aparecida Spolon Almeida - 9/1094

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 08 de julho de 2021.

Profª Drª Ana Maria Bridi	Profª Drª Ana Guerrero Barrado
Drª Ana Carolina Pelaes Vital	Dr. Luiz Fernando Costa e Silva

Prof. Dr. Ivanor Nunes do Prado
Orientador

“Uma criança, um professor, um livro e um lápis podem mudar o mundo”.

(Malala Yousafzai)

“Quem não tem amigo mas tem um livro tem uma estrada”.

(Carolina Maria de Jesus)

Aos meus pais, João e Deusdet,
Aos meus irmãos, Andrelina e Caique,
A minha sobrinha Maria Luísa,
Ao meu noivo Jefferson
DEDICO

AGRADECIMENTOS

À Universidade Estadual de Maringá e ao Programa de Pós-Graduação em Zootecnia.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pela concessão de bolsa de estudo.

À empresa Alltech do Brasil, por ter possibilitado desenvolver este trabalho.

Ao meu orientador Prof. Dr. Ivanor Nunes do Prado, pela dedicação na orientação, por tantos ensinamentos, confiança, conselhos e sinceridade.

Ao Dr. Luiz Fernando Costa e Silva, pela disposição sempre simpática em auxiliar na elaboração e condução do experimento.

À Dr. Ana Carolina Pelaes Vital, que fez parte primordial de todo o meu doutorado me auxiliando nos experimentos, ensinando e aconselhando. Gratidão!

Aos amigos Vanessa Duarte e Murilo Augusto Tagiariolli, pelo desenvolvimento do trabalho, pelos dias divertidos na fazenda, pelas madrugadas no laboratório, pela ajuda de sempre.

À Solange Yung, por toda ajuda durante o doutorado.

A melhor estagiária Gabriela Keyla Monteiro Godoy, por toda a ajuda e amizade.

À Dra. Ana Guerrero, por auxiliar nas correções dos trabalhos.

Ao Prof. Dr. Rodolpho Martin do Prado, pela atenção e colaboração nos trabalhos científicos.

À Prof. Dra. Magali Soares dos Santos Pozza, pela participação na banca de qualificação e contribuições.

À Prof. Dra. Ana Maria Bridi, pela participação na banca de defesa e contribuições.

Aos funcionários da Fazenda Experimental de Iguatemi (FEI), em especial ao José Carlos da Silva, pelo apoio no experimento e pelos momentos de descontração.

Ao grupo de pesquisa, Venício Macedo de Carvalho, Vicente Alfonso Diaz Avila, Ednéia Bonin, Melina Aparecida Plastina Cardoso, Tatiane Rogelio Ramos, pelo auxílio durante a condução do experimento.

A todos que, direta ou indiretamente, contribuíram para a realização deste trabalho.

BIOGRAFIA

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No dia 08 de julho de 2021, submeteu-se à Defesa de Tese junto ao Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá.

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RESUMO

Este estudo foi realizado para avaliar o efeito da combinação de compostos antimicrobianos e uma mistura de microminerais orgânicos e levedura sobre a qualidade e aceitabilidade da carne de bovinos não castrados terminados em confinamento e alimentados com dieta de grãos. O total de 24 bovinos (*Bos taurus x* Nellore) aos 24 ± 3,2 meses de idade e com peso corporal (PC) de 385,5 ± 3,84 kg foram utilizados em um delineamento inteiramente ao acaso, distribuídos em quatro tratamentos de acordo com o peso inicial, sendo eles: CONT - sem aditivos, MONE - inclusão de 30 mg de monensina/kg de MS, MO+VM - inclusão de 30 mg de monensina/kg de MS + 30 mg de virginiamicina/kg de MS, MO+AD - inclusão de 30 mg de monensina/kg MS + 3,0 g de Advantage Confinamento®/100 kg/PC. O Advantage Confinamento® é composto por microminerais orgânicos e a levedura viva *Saccharomyces cerevisiae*. Após 84 dias de confinamento, os animais foram abatidos e foi coletado o músculo *Longissimus thoracis* (LT) para análises de qualidade de carne. A combinação de aditivos (MONE e MO+AD) reduziu o pH e aumentou os valores de L* ($P < 0,05$). A perda por gotejamento não foi alterada pelas dietas, mas a perda por cozimento foi reduzida ($P < 0,05$) aos 14 dias no tratamento com MO+AD. A carne de animais alimentados com as dietas MO+VM e MO+AD foi mais macia ($P < 0,05$). A inclusão de aditivos não alterou ($P > 0,05$) a oxidação lipídica, que foi afetada apenas pelo tempo de maturação ($P < 0,05$). Os aditivos não tiveram efeito ($P > 0,05$) sobre a atividade antioxidante da carne. Não foram observados resíduos de monensina e virginiamicina na carne. Não houve diferenças ($P < 0,05$) nos níveis de minerais da carne entre tratamentos. A carne maturada por sete dias foi melhor aceita pelos consumidores e a carne de animais alimentados com a dieta

MO+AD resultou em maior disposição de compra. A dieta não alterou ($P > 0,05$) o odor, o sabor e a aceitabilidade geral da carne. Nos primeiros sete dias de avaliação, a carne dos touros alimentados com a inclusão da dieta MONE ou dieta MO+AD apresentaram os maiores escores de avaliação visual pelo consumidor. A partir dos 7 dias a carne foi considerada indesejável para o consumo humano, para todas as dietas (score abaixo de 5). Em conclusão, a inclusão de composto antimicrobiano e Advantage Confinamento® na dieta de bovinos terminados em confinamento pode melhorar a cor e a textura da carne sem deixar resíduos na carne. Além disso, a suplementação de aditivos, principalmente monensina e Advantage Confinamento®, pode ser benéfica para a aceitação da carne e a intenção de compra pelo consumidor.

Palavras-chave: Aditivos, compostos antimicrobianos, consumidores, *Saccharomyces cerevisiae*, sensorial, visual

ABSTRACT

This study was carried out to evaluate the effect of the combination of antimicrobial compounds and a blend of organic microminerals and yeast on meat quality and acceptability from bulls finished in feedlot and fed a high-grain diet. A total of 24 bulls (Bos taurus x Nellore) at 24 ± 3.2 months of age and with body weight (BW) of 385.5 ± 3.84 kg were used in a completely randomized design distributed in four treatments according to the initial weight, being them: CONT without additives, MONE inclusion of 30 mg of monensin/kg of DM, MO+VM inclusion of 30 mg of monensin/kg of DM + 30 mg of virginiamycin/kg of DM, MO+AD inclusion of 30 mg monensin/kg DM + 3.0 g Advantage Confinamento®/100 kg/BW. Advantage Confinamento® is composed of organic micro minerals and live yeast *Saccharomyces cerevisiae*. After 84 days in feed-lot, the bulls were slaughtered and *Longissimus thoracis* (LT) was collected for meat quality analyses. The combination of additives (MONE and MO+AD) reduced pH and increased L* values ($P < 0.05$). Drip loss was not altered ($P > 0.05$) by diets, but cooking loss was reduced ($P < 0.05$) at 14 days of ageing in meat of bulls from MO+AD diet. Meat from bulls fed with MO+VM and MO+AD diets was softer ($P < 0.05$). The inclusion of additives did not alter ($P > 0.05$) lipid oxidation, which was affected ($P < 0.05$) only by ageing time. The additives had no effect ($P> 0.05$) on meat antioxidant activity. Monensin and virginiamycin residues were not found in meat. There were no differences ($P > 0.05$) in meat mineral levels among diets. Meat aged for 7 days was better accepted by consumers and meat from bulls fed with MO+AD diet resulted in greater willingness to buy. The diet did not alter ($P > 0.05$) the odor, flavor and general acceptability of the meat. In the first seven days of evaluation, meat from bulls fed with the inclusion of MONE diet or MO+AD diet presented the highest visual evaluation scores by the consumer. From 7 days onwards, meat was considered undesirable for human consumption, for all diets (score below 5). Furthermore, the inclusion of additives, mainly antimicrobial compounds and Advantage Confinamento®, can be beneficial for meat acceptance and consumer's purchase intention.

Keyword: Additives, antimicrobial compounds, consumers, *Saccharomyces cerevisiae,* sensory, visual

I- INTRODUÇÃO

A produção agropecuária é uma das principais atividades econômicas do Brasil se destacando como o maior exportador de carne bovina do mundo. Em maio de 2021 o Brasil exportou cerca de 150 mil toneladas de carne e no acumulado do ano, foram exportadas 710 mil toneladas, destas, cerca de 85% era carne *in natura* (ABIEC, 2021). Esses altos números produtivos são reflexo de um processo de desenvolvimento responsável por elevar a produtividade, aumentando a sua competitividade no mercado internacional.

Os consumidores de carne, tanto do mercado interno como externo, estão se tornando cada vez mais exigentes na busca por produtos de maior qualidade como, por exemplo, cor, maciez, sabor, quantidade de gordura e segurança, como também, sobre todo o processo produtivo animal. O bem-estar animal, sustentabilidade da produção, respeito ao meio ambiente, alimento ofertado aos animais, resíduos na carne e o uso de tecnologias, como a rastreabilidade são informações buscadas pelos consumidores (Salnikova & Grunert, 2020; Acebrón & Dopico, 2000). Nesse sentido, o produtor vem buscando por tecnologias de manejo que permita a produção de carne de melhor qualidade e de forma eficiente, podendo ser obtido com um sistema de produção intensivo, como o sistema de terminação em confinamento (Silva et al., 2010).

As dietas de ruminantes terminados em confinamento têm maior quantidade de grãos (>70%), ricos em amido (Pinto & Millen, 2018; Rivaroli et al., 2016; Souza et al., 2019). O amido é fermentado rapidamente no rúmen aumentando a ocorrência de distúrbios metabólicos (González et al., 2012). Alternativas são usadas para minimizar os efeitos da rápida fermentação do amido, incluindo aditivos como propólis (Valero et al., 2014), óleos essenciais (Fugita et al., 2018; Souza et al., 2019), ionóforos (Zawadzki et al., 2011), antibióticos (Souza et al., 2018) e leveduras (Geng et al., 2015).

Antibióticos e ionóforos são os aditivos alimentares mais utilizados com o intuíto de aumentar a eficiência da utilização dos alimentos ingeridos pela modulação da fermentação ruminal em bovinos (Russell & Strobel, 1989; McAllister et al., 2020). No entanto, proibições e restrições do uso rotineiro de antibióticos e ionóforos na alimentação animal vêm sendo impostas por países importadores e consumidores por possíveis danos à saúde humana (Cameron & McAllister, 2016).

As leveduras, principalmente a *Saccharomyces cerevisiae*, estão sendo utilizadas como aditivos microbianos na alimentação animal. Quando suplementados na dieta, alguns autores relataram melhora no desempenho (Ovinge et al., 2018) e promoção da saúde (Shen et al., 2019) dos animais. Os microminerais também vêm sendo utilizados na sua forma orgânica, por apresentar maior biodisponibilidade em relação às formas inorgânicas, acarretando em melhor adsorção dos nutrientes e, portanto, menor excressão ao ambiente (Pino & Heinrichs, 2016). Alguns microminerais quando associados à levedura apresentam alta capacidade antioxidante (Ye et al., 2020) e pode ser benéfico para o tempo de prateleira da carne, mantendo um produto de qualidade por mais tempo. Portanto, a combinação de leveduras e microminerais pode ser uma alternativa aos ionóforos e antibióticos, uma vez que estes possuem restrições em alguns países.

II- REVISÃO DE LITERATURA

Aditivos na alimentação de bovinos confinados

A Instrução Normativa n°13/2004 define aditivos como “substância, microrganismo ou produto formulado, adicionado intencionalmente aos produtos, que não é utilizado normalmente como ingrediente, tenha ou não valor nutritivo e que melhore as características dos produtos destinados à alimentação animal dos produtos animais, melhore o desempenho dos animais sadios ou atenda às necessidades nutricionais”.

Dentre os aditivos melhoradores de desempenho animal, destacam-se os antibióticos e ionóforos desde a década de 1970/1980. Essas substâncias têm sido utilizadas na produção de ruminantes há décadas como moduladores da fermentação ruminal, promovendo redução da ocorrência de distúrbios metabólicos, reduzindo perdas de amônia pela excreção urinária e de metano (Novilla et al., 2017; Russell, 1987; Schelling, 1984) e consequentemente, melhorando o desempenho animal e eficiência alimentar (Bergen & Bates, 1984).

Mecanismo de ação da monensina sódica

Os ionóforos, especificamente a monensina sódica, são moléculas produzidas a partir de cepas do gênero *Streptomyces* (Kirchhelle, 2018; Russel and Strobel, 1989; Wallace et al., 2002). A monensina sódica atua de forma análoga aos antibióticos, por sua capacidade inibidora sobre bactérias gram-positivas (Teather & Forster, 1998), modulando a dinâmica ruminal e aumentando a eficiência energética, melhorando o metabolismo de nitrogênio e reduzindo o risco de acidose (Nagaraja & Titgemeyer, 2007; Schelling, 1984). A monensina é uma molécula lipofílica e se liga a prótons, aderindo à membrana celular externa das bactérias, que são ricas em lipídios, catalisando a entrada ou saída de determinados íons (Russell, 1989). Inicialmente, ocorre elevada saída de K^+ e entrada de H^+ para dentro da célula, causando redução do pH. A célula reage para estabilizar o pH e o balanço iônico celular, exportando H^+ para o meio por intermédio das bombas de Na^+/K^+ e de próton ATPase (Schelling, 1984). Por meio do mecanismo da bomba iônica, na tentativa de manter sua osmolaridade, a célula bacteriana utiliza sua energia, de forma excessiva, até reduzir suas reservas, afetando o crescimento das bactérias Gram-positivas e favorecendo as bactérias Gram-negativas.

A monensina possui baixa ou quase nenhuma atividade contra bactérias Gram-negativas devido a sua parede celular composta por peptideoglicanos e membrana externa constituída por lipopolissacarídeos, proteínas e lipoproteínas (Novilla, 2018).

No rúmen, a monensina sódica altera as razões dos ácidos graxos voláteis, aumentado o ácido graxo propiônico e reduzindo os ácidos graxos acéticos e butíricos (Nagaraja et al., 2012). Goodrich et al. (1984) revisaram 228 ensaios dos USA, onde o melhoramento na eficiência, na média geral, foi de 7,5% para bovinos suplementados com monensina sódica (246 mg/dia) e o aumento no ganho médio diário de 13,5%. Da mesma forma, na Europa, Wilkinson et al. (1980) reportaram aumento de 14,0% no ganho médio diário de bovinos terminados em pastagens e suplementados com 200 mg/dia de monensina sódica.

Mecanismo de ação da virginiamicina

Derivada de *Streptomyces virginiae,* a virginiamicina inibe o crescimento de bactérias Gram-positivas interrompendo a síntese de proteínas (Cocito, 1983; Coe et al., 1999), porém, possui efeito quase nulo na maioria das bactérias Gram-negativas devido à impermeabilidade da parede celular (Cocito, 1979). A virginiamicina é constituída por dois componentes, o fator M (grupo das estreptograminas A) e o fator S (grupo das estreptograminas B), que individualmente são bacteriostáticos, mas, combinados são bactericidas (Cocito, 1983). A atividade inibidora sinérgica dos fatores A e B é atribuída às alterações conformacionais na subunidade ribossômica de bactérias Gram-positivas induzida pela ligação de compostos do tipo A, inibindo a síntese de proteína no interior da célula bacteriana. A virginiamicina altera a fermentação ruminal aumentando proporções de propionato e diminuindo a produção de ácido lático, metano e degradação de proteínas (Fiems et al., 1990; Nagaraja et al., 1987; Nagaraja & Taylor, 1987), melhorando o desempenho animal e a eficiência alimentar de bovinos terminados em confinamento, recebendo milho na dieta e suplementados com até 26 mg/kg da matéria seca de virginiamicina (Castagnino et al., 2018; Montano et al., 2015).

Restrição ao uso de antibióticos e ionóforos

O uso de antibióticos e ionóforos na dieta animal vem sendo questionado e menos aceito pelos consumidores pelos possíveis resíduos na carne e seus derivados e, consequentemente, seleção à resistência cruzada aos antibióticos em bactérias patogênicas do trato gastrointestinal de animais de produção (Russell & Houlihan, 2003, Fernqvist & Ekelund, 2014; Kirchhelle, 2018). Assim, a Autoridade Europeia da Segurança do Alimento (OJEU, 2003) baniu na União Europeia o uso dessas substâncias em 2006. No Brasil, a ANVISA considerou que as pesquisas relativas à resistência e resíduos na carne ou leite não são relevantes do ponto de vista da decisão sobre a continuidade ou não do uso dessas moléculas como aditivo.

No entanto, o uso racional de antimicrobianos desempenha papel vital na eficiência da produção animal. Uma estimativa de que a restrição total ao uso dos antibióticos e ionóforos, poderia reduzir a eficiência alimentar em até 10% ou mais, assim, seria necessário aumentar a produção de ingredientes para os animais, como milho e soja, e consequentemente aumentar a área plantada e o desmatamento (Hao et al., 2014).

Uma alternativa seria a substituição parcial dos antibióticos e ionóforos a longo prazo para reduzir o impacto sobre a produção e economia na pecuária. Neste sentido, para atender as exigências do mercado consumidor que busca por alimentos seguros, pesquisas estão sendo realizadas com o propósito de desenvolver novos compostos e substituir os antibióticos e ionóforos, parcial ou totalmente, por substâncias que não representem riscos à saúde humana, mas permitindo manter a competitividade e eficiência do sistema.

Leveduras e micro minerais orgânicos

Leveduras como S. *cerevisiae* é uma alternativa viável que vem sendo utilizada em dietas alto grão de bovinos em confinamento e alguns benefícios podem ser observados como a redução de ocorrência de acidose ruminal no rebanho e melhora o desempenho animal e eficiência alimentar (Stephens et al., 2010; Mcallister et al., 2011).

No ambiente ruminal, as leveduras parecem atuar removendo oxigênio do rúmen ao realizarem a oxidação da glicose via respiração aeróbica, consumindo o oxigênio decorrente dos processos metabólicos ruminais, favorecendo o crescimento de bactérias estritamente anaeróbicas (Newbold et al., 1996). Outro mecanismo utilizado pelas leveduras no rúmem é a liberação de nutrientes, uma vez que elas possuem carboidratos, lipídeos, vitaminas do complexo B e aminoácidos (Yousri, 1982) além dos ácidos málico e dicarboxílico, estimulando o crescimento de algumas bactérias ruminais, como é o caso da *Selenomonas ruminantium*, bactérias consumidoras de ácido lático, produto da ação das bactérias amilolíticas, transformando-o em ácidos graxos que serão utilizados como energia pelo animal (Nisbet & Martin, 1990). Assim, há equilíbrio do pH ruminal, reduzindo o risco de distúrbios metabólicos relacionados com a limitação do crescimento de microrganismos celulolíticos e fibrolíticos, aumentando a ingestão de alimentos e a digestibilidade da fibra (Shurson, 2018; Desnoyers et al., 2009).

Em estudo avaliando a inclusão de 1 ou 2,5 g/100 kg de peso corporal de *S. Cerevisiae* em bovinos de corte, alimentados com dietas com 80% de concentrado Monnerat et al. (2013) observaram menor pH na dieta com 1 g de levedura e uma menor produção de ácido butírico ocorreu com 2,5 g de levedura. Ao passo que, Ovinge et al. (2018) avaliaram o efeito da *S. cerevisiae* em dietas à base de milho floculado para bovinos de corte e observaram que a inclusão de levedura melhorou a digestibilidade da fibra da dieta, em contrapartida, não provocou diferenças no crescimento e eficiência alimentar e caracteríticas de carcaça dos animais.

Os minerais são nutrientes indispensáveis para o desenvolvimento animal, pois fazem parte dos processos metabólicos e bioquímicos atuando como cofatores enzimáticos. Eles são dividos em duas classes, macrominerais (cálcio, magnésio, fósforo, potássio, sódio, cloro e enxofre e microsminerais (cromo, cobalto, cobre, iodo manganês, molibdênio, níquel, selênio e zinco).

Os minerais estão disponíveis para alimentação animal na forma inorgânica, como óxidos, sulfatos, cloretos, carbonatos e fosfatos, sendo mais utilizada na suplementação mineral de bovinos devido ao menor custo em relação aos minerais orgânicos. Em contrapartida, os minerais orgânicos são combinações de um ou mais minerais com substâncias orgânicas, como aminoácidos, carboidratos ou até mesmo proteína. Esses minerais se tornam mais biodisponíveis aumentando a retenção no organismo e, aumentando sua a absorção no intestino (Zanetti, 2014), estimulando o desempenho animal e a eficiência alimentar (Genther-Schroeder et al., 2016; Budde et al., 2019).

Leveduras e microminerais sobre a qualidade da carne

Embora a literatura tenha abordado bem o efeito da utilização de leveduras e microminerais sobre o desempenho animal e eficiência alimentar em bovinos, e que estes resultados sejam positivos e promissores, existe grande necessidade de melhor compreender os efeitos destes compostos sobre a qualidade de carne e, sobretudo, sobre a aceitabilidade da carne pelo consumidor.

Gomes et al. (2009) observaram que a suplementação com levedura e com monensina, em associação ou separadamente, não teve efeito importante sobre o pH da carcaça, a cor, a força de cisalhamento e as perdas por exsudação da carne. Da mesma forma, Geng et al. (2015) avaliaram a qualidade da carne de 45 bovinos não castrados recebendo dietas com dose de 0,8 g/animal/dia e 50 g/animal/dia e dieta controle basal com monensina (34,5 mg/kg de MS) e observaram que a inclusão da *S. cerevisiae* não alteraram coloração da carne e apresentaram carne mais macia.

De acordo com Patel et al. (2019), as fontes orgânicas de microminerais têm forte impacto no metabolismo dos animais, e pode levar a diferenciação na qualidade da carcaça e da carne, e provavelmente está relacionado com a alta biodisponibilidade de cofatores essenciais aos processos bioquímicos e metabólicos, em comparação aos animais suplementados apenas com minerais inorgânicos.

Dentro deste contexto, a utlização de moléculas na dieta que podem melhorar a qualidade da carne se torna um objetivo de interesse uma vez que a carne é uma das commodities mais perecíveis devido ao seu pH moderado e alto teor de nutrientes e umidade. Os principais fatores que contribuem para a deterioração da carne e consequentemente perda de qualidade incluem a ação bacteriana pelo pH elevado ou pelas reações bioquímicas e⁄ou metabólicas; mudanças na coloração, o que atinge diretamente a percepção do consumidor no momento da compra (Pellissery et al., 2020). Ao mesmo tempo em que ocorrem essas alterações na sua coloração a oxidação lipídica também vai acontecendo e contribuindo para a deterioração da qualidade final da carne (Gutierrez et al., 2018) resultando no desenvolvimento de odores e sabores desagradáveis, tornando-a inaceitável para consumo humano.

Nesse sentido, compostos antioxidantes quando adicionados à dieta animal podem promover também melhor capacidade antioxidante na carne (Ornaghi et al., 2020), resultando em marior tempo de vida de prateleira. A ação antioxidante está ligada à capacidade de se ligar aos radicais livres e inibir processos de estresse oxidativo que potencializam a oxidação dos lipídeos provocando a oxidação da carne. As enzimas antioxidantes glutationa peroxidases contendo selênio catalisam a redução de lipídios e peróxidos de hidrogênio para produtos menos prejudiciais, proporcionando defesa contra o estresse oxidativo (Hardy e Hardy, 2004).

Cozzi et al., (2011), avaliando a carne de oitenta e quatro bovinos jovens não castrados da raça Charolês, suplementados com levedura enriquecida com selênio (0,3 mg de Se/kg MS) na dieta, observaram que essa suplementação levou a menores perdas por gotejamento na carne embalada a vácuo maturada por 11 dias. Essa menor perda por gotejamento muscular, pode ser explicada pela redução da oxidação da membrana celular (Dunshea et al., 2005). Além disso, o tratamento com levedura enriquecida com selênio aumentou o conteúdo de selênio no tecido muscular e reduziu a força de cisalhamento. Em contrapartida, nenhuma melhoria na qualidade da carne de bezerros suplementados com selênio enriquecido com levedura na (0,5 mg/kg) foi observada por Skřivanova et al. (2007).

Outro importante micromineral na dieta de bovinos é o zinco, que desempenha papel importante no metabolismo de proteínas, que são necessários para o crescimento e desenvolvimento dos tecidos musculares. O cromo, por sua vez, tem sido usado para manipular a qualidade da carne devido ao seu papel no metabolismo muscular (Hong et al., 2002) e provoca um maior acúmulo de energia no processo de deposição de músculos consequentemente, reduzindo a deposição de gordura, se tornando uma estratégia útil para melhorar a qualidade da carne (Najafpanah et al., 2014). Vellini et al. (2020) observaram tendência de produção de carne mais macia suplementando bovinos da raça Nelore com zinco associado à metionina de cromo.

Aceitabilidade da carne pelo consumidor

Considerando a importância da carne bovina e o valor econômico que ela representa para o agronegócio brasileiro, torna-se necessário conhecer o que pode influenciar a aceitabilidade da carne pelo mercado consumidor.

A qualidade é um conceito composto e muito difícil de definir e medir de modo simples e único, incluindo aspectos objetivos, tais como parâmetros tecnológicos: pH, capacidade de retenção de água, oxidação lipídica e proteica; parâmetros nutricionais: porcentagem de lipídeos, teor de ômega 3 e 6; parâmetros sanitários: quais medicamentos foram fornecidos aos animais ao longo do processo produtivo; sustentabilidade e bem-estar animal. E também aspectos subjetivos, como: maciez e suculência. Todas estas características são importantes, pois estão relacionadas à aceitabilidade, palatabilidade e perdas que ocorrem durante o processamento e armazenamento da carne (Mancini & Hunt, 2005, Priolo et al., 2001).

Diversos fatores podem influenciar a qualidade sensorial e aceitabilidade da carne. A cor da carne é importantíssima na avaliação visual e/ou sensorial, pois é o primeiro atributo que o consumidor avalia nas gôndolas dos supermercados, que muitas vezes tem preferência por uma cor vermelha cereja/vivo (Killinger et al., 2004). Carnes com coloração pálida ou vermelha escura são rejeitadas pelo consumidor.

Em estudo realizado por Milewski e Zaleska (2011), a aceitabilidade da carne de suínos suplementados com *S. Cerevisiae* não foram afetados pela suplementação. Da mesma forma, Dávila-Ramirez et al. (2020) observaram que a maioria das qualidades sensoriais (cor, aparência, odor, sabor, maciez e suculência) não foram afetados pela suplementação com *S. Cerevisiae*, exceto para a cor visual, em que os provadores observaram maior intensidade no controle em comparação com a dieta suplementada com 0,3% de YC.

Após seu cozimento, o parâmetro que influencia diretamente a aceitabilidade do consumidor é a maciez, força mecânica realizada durante o processo de mastigação (Etaio et al., 2013). Muitos fatores podem afetar esse parâmetro, como dieta fornecida, idade do animal, condição sexual, manejo pré-abate, tipo e tamanho das fibras entre outros (Font-i-Furnols & Guerrero, 2014, Guerrero et al., 2013). Uma forma de tornar a carne mais macia é o processo de maturação, onde o seu amaciamento ocorre ao longo dos dias pela ação principalmente da calpaína e calpastatina (Rivaroli et al., 2020; Ornaghi et al., 2020). No entanto, esse período de maturação não pode ser muito prolongado devido à ação das bactérias que começam a deteriorar a carne, tornando-a menos atrativa ao consumidor, uma vez que, a carne neste estado apresenta uma coloração escura, e pode provocar ranço durante o consumo devido a oxidação lipídica que ocorre durante a vida de prateleira.

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III. OBJETIVOS GERAIS

Avaliar a qualidade e aceitabilidade sensorial e visual da carne de bovinos não castrados jovens alimentados com dieta de alto grão em confinamento com adição de uma combinação de compostos antimicrobianos e mistura de microminerais orgânicos e levedura.

CAPÍTULO IV

(Meat Science)

Antimicrobial compounds and a blend of organic minerals traces and yeast on meat quality of crossbred (European *vs.* Nellore) bulls finished in feedlot and fed high-grain diet

Abstract

This study was realized to study the meat quality (by pH, colour, dripping loss, texture, lipid oxidation and antioxidant activity) of meat from 24 crossbred bulls (European vs. Nellore, 24 ± 3.2 months; 385.5 ± 3.84 kg) finished in feedlot for 84 days and fed with diets supplemented with antimicrobials compounds and their combinations. The bulls were fed 4 diets: CONT – without additives; MONE – inclusion of 30 mg of monensin/kg of dry matter intake (DMI); MO+VM – inclusion of 30 mg of monensin + 30 mg of virginiamycin/kg DMI; MO+AD – inclusion of 30 mg of monensin/kg DMI + 3.0 g of Advantage Confinamento®/100 kg BW. The Advantage Confinamento® is composed by organic minerals trace and live yeast *Saccharomyces cerevisae*. The pH, colour, drip loss, cooking loss, texture, lipid oxidation and antioxidant activity were evaluated at 1, 3, 7 and 14 days ageing time. In addition, monensin and virginiamycin residue analyzes were performed. Combination of additives reduced pH ($P < 0.05$) and produced a lighter meat ($P < 0.05$). Drip loss was not altered by diets, but cooking loss reduced ($P < 0.05$) with MO+AD at 14 days aging time. Meat was more tender ($P < 0.05$) from bulls fed MO+VM and MO+AD diets. The inclusion of additives did not alter ($P > 0.05$) the lipid oxidation, which was affected only by aging time ($P < 0.05$). Additives had no effect ($P > 0.05$) on meat antioxidant activity. No monensin and virginiamycin residues were observed in the meat. The inclusion of ionophores, antimicrobial compounds and Advantage Confinamento® in the diets of bulls finished on feedlot can improve color and texture of meat without leaving residue on the meat.

Keywords: additives; antibiotics, beef quality, cattle, ionophores, *Saccharomyces cerevisae*

INTRODUCTION

The industries in the beef sector, in addition to efforts to increase efficiency in the farm, are also currently concerned with the meat and by products qualities. The efficiency of the production system in the farms has increased significantly over the past 20 years in Brazil.[1] In the 1990s, Brazil had 170 million cattle and produced approximately 4.5 million tons of meat (2). In this decade, Brazil has approximately 210 – 220 million cattle and produces 10 million tons of meat (2). Thus, over the past 30 years, there was 25% of increase in the herd and 120% of increase in meat production (2). This increase in beef productivity in recent years was due to genetic gains (3,4), improvements in pastures (5), disease control and eradication, professional management of properties and implementation of more productive systems such as the pasture supplementation (6,7) and finishing cattle in feedlot (8).

The diets used in feedlot system are rich in carbohydrates (8,9). The rapid rate of degradation of carbohydrates in the rumen can increase the risk of metabolic disorders and ruminal acidosis (10). To avoid the risks of ruminal disorders, antimicrobial compounds such as ionophores (10, 11) and antibiotics (12) have been used in the diet of cattle finished in feedlot system. Thus, impacting ruminant metabolism by increasing energy metabolism efficiency, improving nitrogen metabolism, and reducing acidosis risk (13). These compounds change the ratio of fatty acid in the rumen, increasing propionic acid production and reducing of butyric and acetic acids production (14), providing more energy to the animal through increased glucose supply. Increased production of propionic acid from the rumen increases hepatic gluconeogenic flux (15). Changes in the flow of fatty acids in rumen and subsequently in the liver could have an effect on the composition of the meat (16–18).

On the other hand, due to the demands for the export of meat to several countries in the world and to meet the new desires of the internal population, the quality of the meat is being monitored by the industries. The use of antimicrobial compounds in the diets of cattle finished in feedlot is somewhat restricted by importing countries and domestic consumers due to the possibility of the presence of residues in the meat and risks to human health (19,20). In contrast, yeasts, such as *Saccharomyces cerevisiae*, has been widely used in the industry because it’s generally regarded as safe (GRAS) status, presenting health benefits of ruminants (21,22) and improving digestibility of food and maintaining animal performances of ruminants (23) becoming alternative supplements in animal feed.

However, studies concerning the effect of the combination between antimicrobials compounds and minerals trace and yeast have been focusing on animal performance, feed efficiency, and ruminal modulation (24,25), while those experiments that are focused on meat characteristics are still limited in beef cattle (26,27). Moreover, the results of these studies show that the addition of antimicrobials compounds in the diets of cattle finished in feedlot has little influence on the meat characteristics (28,29). Finally, there is a lack of knowledge regarding to the effect of the combinations among antimicrobials compounds with minerals trace and yeast on meat quality, lipid oxidation, and antioxidant activity for beef cattle.

Therefore, our hypothesis is that the inclusion of antimicrobial compounds (monensin or virginiamycin) in combination with a blend of minerals trace and yeast (Advantage Confinamento®) could alter meat quality and still if there is residues of these products in the meat.

This study was conducted to evaluate the inclusion of the combination of monensin and virginiamycin or monensin and a blend of minerals trace and yeast (Advantage Confinamento®) on meat quality of bulls finished in feedlot fed high-grain diet.

MATERIAL AND METHODS

Ethic committee, location, animals, and diets

This study was approved by the Department of Animal Production and Research Ethic Committee at the State University of Maringá, Brazil (protocol nº 1103290719).

The experiment was conducted at the Rosa & Pedro Sector (Experimental Farm) at the State University of Maringá, Maringá city, Paraná state, Brazil south. The meat analysis were realized in the Laboratory of Quality Analysis of Animal Products, Maringá State University.

A total of 24 crossbred bulls (European *vs.* Nellore), 24 ± 3.2 months of age and with mean body weight (BW) of 385.5 ± 3.84 kg were used in a completely randomized design experimental. The bulls were weighed at the beginning of the experiment and assigned to individual pens (10 m^2), concreted floor and partially covered. They had free access to clean water throughout the experiment.

Bulls were randomly distributed into four diets according to initial BW, with an acclimation period of 14 days. The basal diet comprised 850 g/kg corn cracked and soybean meal and 150 g/kg corn silage, and it was offered *ad libitum* for 84 days. The basal diet was the same for all animals, formulated to have the same amount of nitrogen and energy (Table 1) according to BR CORTE (30). Four experimental diets were used as follows: CONT – without additives, MONE – inclusion of 30 mg of monensin/kg of dry matter (DM), MO+VM – inclusion of 30 mg of monensin/kg DM + 30 mg of virginiamycin/kg DM, MO+AD – inclusion of 30 mg of monensin/kg DM + 3.0 g of Advantage Confinamento®/100 kg BW. The Advantage Confinamento® is a blend of organic minerals trace and live yeast (*Saccharomyces cerevisae).*

Meat sampling

At day 84 in the feedlot, the bulls were weighed after 16 h of fasting and transported to a commercial slaughterhouse (Campo Mourão city, Paraná state, Brazil south). The transport was less than 80 km and the stock density of trucks were 0.8 ± 0.2 bulls/m^2. The bulls were stunned with a captive-bolt pistol and bled through exsanguinations by cutting the neck vessels. Then, the carcasses were divided medially from the sternum and spine, and the half-carcasses were washed, identified and stored in a chilling chamber at 4° C for 24 h.

The *Longissimus thoracis* (LT) muscle was excised from the left half of the carcass from the 6^{th} to the last lumbar vertebra and transported to laboratory of Meat Quality of Maringá State University. The LT was sliced into steaks in 2.5 cm thick, weighed, vacuum-packed (99% vacuum, Sulpack SVC 620) in polyamide/polyethylene pouches (120 µm; 1 $cm^3/m^2/24$ h O_2 permeability and 3 $cm^3/m^2/24$ h CO_2 permeability, at 5° C and 75% relative humidity; 3 $g/m^2/24$ h water vapor transmission rate at 38° C and 100% relative humidity; 97°C Vicat softening temperature; 1.3 g dart drop strength), and aged for either 1, 3, 7 and 14 days at 4°C.

Monensin and virginiamycin analyses

The samples of meat were sent to the Agro Safety Laboratory (Piracicaba city, São Paulo state, Brazil) and analyzed for monensin and virginiamycin presence according to Kaklamanos et al. (2013) (31). An amount of 2 g sample was mixed with 5 ml of the extraction solution acetonitrile/ethanol/water (80:10:10, v/v/v) in 1% of formic acid, vortexed for 1minute. Then, 0.2 g of NaCl and 1 g of $MgSO_4$ were added and stirred vigorously for 1 minute. Next, this content was centrifuged at 3,100 rpm for 10 minutes to better separate the liquid from the solid phase. A 1-ml aliquot of the supernatant was transferred to a tube of 15 mL of C18 column, stirred for 1 minute and centrifuged at 3,100 rpm for 10 minutes. The extract was filtered through a 0.22 µm nylon filter and then analyzed in the LC/MS/MS system set in the full scan mode. The Accela LC system (Thermo Scientific, San Jose, CA, USA) coupled to an Orbitrap model QE Exactive high resolution mass spectrometer equipped with a Phenomenex Sinergy analytical column (150 mm × 2.0 mm, 4 µm) was used to perform the separation of analytes. The mobile phase was composed of eluent A (0.1% formic acid in distilled water; v/v) and eluent B (0.1% formic acidin acetonitrile; v/v). A gradient mode was applied starting with 5% eluent B increasing to 90% for 2 minutes and maintained for 16.5 minutes, after which it immediately reduced to 5% eluent B, with a rebalancing time of 5 minutes. The column and the automatic sampler were maintained at a constant temperature of 40 ºC. A 2-µl aliquot of the sample extract was injected into the chromatographic system for reading and the data were processed using Xcalibur 4.3. Software (Thermo Scientific, San Jose, CA, USA).

Meat quality

The pHmeter (Hanna instruments model HI99163, Romaria, Brazil) was used to measure the pH and it was calibrated using buffer solutions of known pH (4.0 and 7.0, respectively) prior the measurements. The electrode was inserted into the LT muscle at the days 1, 3, 7, and 14 after slaughter.

The parameters L* (lightness), a* (redness) and b* (yellowness) were measured using the CIELab system with a Minolta CR-400 Chroma meter (Japan) (with a 10° view angle, D65 illuminant, 8 mm of aperture with a close cone). Each sample was exposed to oxygen for 30 minutes before reading. Six measurements at randomly selected points were recorded per sample.

Meat drip loss was measured using steaks of each animal collected 24 h *post mortem*, placed in a bag, and kept at 4° C. After 24 h, samples were removed from the bag, dried with absorbent paper, and weighed. Results were expressed as a percentage of the initial weight (32).

The quantification of weight loss during cooking was performed by recording the sample weights before and after cooking. For this, the samples were wrapped in aluminum foil and cooked in a grill (Grill Philco Jumbo Inox, Philco SA, Brazil; previous heat up to 200° C) until reach an internal temperature of 72º C. The sample was then removed from the grill and kept in the environmental temperature to cool until reach 25° C and then the samples were weighed again.

The texture of the previously cooked steaks was measured using a texturometer (TAXT Plus Texture Technologies Corp., Godalming, Surrey, UK) equipped with a Warner- Bratzler blade. The instrument was set to a speed of 2 mm/s, distance target was 30 mm and trigger was 10 g, following to the protocol proposed by Honikel (1998)(32). The samples were cut perpendicular to muscle fibers into rectangular pieces of 1 cm^2.

The lipid oxidation was measured as malonaldehyde (MDA) equivalent with the thiobarbituric acid (TBARS) (33). Samples (5 g) and TCA solution (10 mL; 7.5% TCA, 0.1% gallic acid and 0.1% EDTA) were mixed, homogenized (Ultra Turrax), and then centrifuged (4000 rpm; 4° C; 15 min). The supernatant was filtered (Whatman filter paper: 0.16 mm thickness, 20-25 sec. filtration speed, 4-12 Â µm particle retention) and mixed with TBA reagent (1:1 v/v). The mixture was boiled for 15 minutes at 100° C, cooled, and the absorbance was measured at 532 nm using a spectrophotometer (Evolution TM 300, Thermo Fisher Scientific, UK). The results were expressed as mg MDA/kg of meat.

For analysis of antioxidants, meat samples were homogenized with methanol (1: 1 w/v) using an Ultra-Turrax (IKA® - T10, USA). Then, the samples were centrifuged (4.000 rpm, 15 min) and filtered using filter paper (gram mage 80 g/m^2, thickness 205 m, pores 14 m). It is recommended to perform at least two antioxidant methods due, mainly, to differences in the chemistry of the assay and the type of molecules detected (33,34). Therefore, in this study the antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) assays.

For DPPH, samples extract (150 µL) were mixed with a DPPH methanolic solution (60 µM) (2850 µL). Absorbance was read at 515 nm after 30 min in dark conditions(35), with some modifications(33). The antioxidant activity was calculated according to the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (1 - (A_{sample\ t} / A_{sample\ t\ =\ 0}) \times 100$$

where: A_{sample} t is the absorbance of the sample at 30 min and, A_{sample} t = 0 is the absorbance of the sample at time zero.

The ABTS assay was evaluated according to Re at al. (1999)(36). The $ABTS^{+}$ was formed by the interaction of 7 mM ABTS (5 mL) with 140 mM potassium persulfate (88 µL) for 16 h at room temperature in dark conditions. Extract (40 µL) were mixed with $ABTS^{+}$ solution (1960 µL) and the absorbance was measured after 6 min at 734 nm. The antioxidant activity (%) was calculated according to the following equation:

$$\text{ABTS radical scavenging activity (\%)} = (1 - (A_{\text{sample t}} / A_{\text{sample t}} = 0)\ \text{x}100$$

where: A_{sample} t is the absorbance of the sample at 6 min and, A_{sample} t = 0 is the absorbance of the sample at time zero.

Statistical analyses

All parameters were tested for normality (Shapiro–Wilk test), and had normal distribution. The analysis of variance (ANOVA) was evaluated using the SAS statistical software (Statistical Analysis System, ver. 9.4, Proc GLM) was applied.

For drip loss, statistical model was as follows:

$$Y_{\text{ijk}} = \mu + D\text{i} + a_{\text{k}} + e\text{i}_{\text{k}}$$

where Y_{ijk} is the dependent variable, µ is the overall mean, D_{i} is the fixed effect of the diet, a_{k} is the random effect of the animal and $e\text{i}_{\text{k}}$ is the random error.

For the other parameters, the statistical model was:

$$Y_{\text{ijk}} + \mu + D_{\text{i}} + A_{\text{j}} + a_{\text{k}} + (D_{\text{i}} \cdot \text{X} A_{\text{j}}) + e_{\text{ijk}}$$

where Y_{ijk} is the dependent variable, µis the overall mean, D_{i} is thefixed effect of the diet, A_{j} is the fixed effect of aging, a_{k} is the random effect of the bulls, ($D_{\text{i}}\text{X}\cdot A_{\text{j}}$) is the interaction effect of diet and aging, and e_{ijk} is the random error.

In the two models, the mean and standard error of the mean were calculated. Differences between means were evaluated using Tukey's test ($P < 0.05$).

Results and discussion

Monensin and virginiamycin analyses

The great success story of antibiotics and ionophores is in danger due to emerging resistance to these compounds (20). It is well accepted that the need studies to confront the growing problem of antibiotics and ionophores in animal feed in relation to possible residues in meat. In this study, no residues were observed in the meat of cattle fed the inclusion of 30 mg of monensin/kg DM or 30 mg of virginiamycin/kg DM in the diet of bulls finished in feedlot for 84 days.

Meat pH

Meat pH from bulls fed MONE and MO+AD diets were lower ($P < 0.05$) than meat pH from bulls fed CONT and MO+VM diets until day seven of aging (Table 2). The meat pH from bulls fed MO+AD diet showed a synergistic positive effect, since it resulted in lower meat pH (value below 5.8 for seven days meat aging); while meat pH from bulls fed CONT diet was 6.1. Thus, the addition of MO+AD resulted in a meat pH to values closer to the ideal pH equal to 5.6 (37). According to some studies, the addition of monensin or virginiamycin separately in the diet of cattle finished in feedlot did not alter the meat pH (26,38).

The aging time reduced ($P < 0.05$) the meat pH of all diets from first to seven days; reducing from 6.0 to 5.7. At 14 day of aging, there was an increase ($P < 0.05$) in the pH of the meat in all treatments (Table 2). The treatments MONE and MO+VM showed the lowest values at 14 days of aging ($P < 0.05$). Thus, the aging time up to seven days reduces the meat pH, but a longer period (14 days) increased it. This increase in late storage may be caused by the growth of spoilage bacteria leading to the accumulation of alkaline components (e.g., ammonia and trimethylamine). Similar results were obtained by Herrera-Mendez et al. (2006)(39).

The observed meat pH in this study (5.9), although acceptable, is slightly high. Under normal conditions, meat pH varies from 5.5 to 5.8 (9,40). The relatively high pH in this experiment could be explained, in part, by animal genetics. In general, Zebu cattle show a higher pH after slaughter due to aggressive behavior during transport and handling before slaughter (16,41,42), in similar conditions, observed a meat pH above 6.2 for crossbred cattle (Angus *x*. Nellore) finished in feedlot.

Meat colour

The L* parameter (lightness) measures the colour variation in the meat from light to dark (43). Higher value means lighter meat and lower value means darker meat. The L* ideal value in red meat is from 38 to 40 points (37).

With one, three and seven ageing days, values L* were greater (P < 0.05) for meat from bulls fed MONE and MO+AD diets (Table 2). Lower L* values were observed in meat from bulls fed CONT and MO+VM diets, therefore darker meat. This might be explained by high pH values (44) found in this study. Thus, according L* values, the meat that were most attractive to the consumer before and three and seven days of ageing were of meat from bulls fed MONE and MO+AD diets, which values were close to 40 (Table 2). On the last evaluation day (day 14), the L* values were similar (P > 0.05) for meat from bulls fed all diets (Table 2). Those values were much lower (value below 35.8), characterizing a darker meat, therefore, unattractive to the consumer.

The a* value measures the red colour of the meat (43). Thus, a higher a* value means a redder meat, therefore, more attractive to the consumer (37). Monensin, virginiamycin and Advantage Confinamento® inclusion in the diets did not alter (P > 0.05) a* values in meat of bulls (Table 2).

The ageing time lead to an increase (P < 0.001) in a* values in the meat from bullsfed all diets (Table 2). In this context, ageing makes the meat more attractive to the consumer, since a higher a* values represents a more reddish colour. The a* values increased from 12.7 before ageing to 14.5 on the fourteenth day of ageing.

The a* values in the meat is very variable compared to the L* values. The a* values, for example, can vary from 15 to 25 in beef (37). However, under similar conditions of feeding, handling and genetic groups of bulls, a* values ranged from 11 (45) to 18 (46,47), with intermediate values from 14 to 17 (40,48). Thus, a* values observed in this experiment are close to the values observed for crossbred bulls (European vs. Zebus) under similar feeding and handling conditions.

The b* values characterizes yellow color in meat (43). Higher b* values characterizes meat that is more yellowish, less dark and therefore more attractive to the consumer.

With the exception of meat from bulls fed with CONT diet, ageing increased the b* value from first day (12.3) to seventh day (13.4) (Table 2) thus, making the meat more attractive to consumers. On the third and seventh ageing, the b* values was higher (P < 0.05) for the meat from bulls fed with additives addition in the diets, but without differences among diets with additives (Table 2). However, on the fourteenth day, the value of b* declined to a value similar to that observed before ageing (12.6). The parameter b* shows that ageing up to seven days makes the meat more attractive to the consumer; however, a longer ageing time (14 days) has no attractive benefit for the meat.

In general, meat b* value from bulls finished in feedlot and slaughtered close to 24 months and fed with high grain diets for a period of 90 days varies from 10 to 14 (40,45,48). Thus, the values observed in this experiment for the b* parameter are close within normal values.

Drip loss and cooking loss

At 24 h after slaughter, monensin, virginiamycin and Advantage Confinamento® inclusion in the diets did not alter ($P > 0.05$) drip loss compared to CONT (Table 3). Drip losses were low and varied from 1.4 to 1.8%. Lactic acid formation post-mortem is responsible for the drop in pH and the consequent decreased ability of the meat to retain water (45). With the high pH obtained in this study, it was possible to retain water in the cells having low losses due to capillary forces (gravity). Drip losses 24 hours after slaughter were similar than those reported by Monteschio et al. (2017)(45) and Ornaghi et al. (2020)(40) for meat from crossbred bulls finished in feedlots and fed a high-grain diet.

Before ageing, meat cooking losses were similar ($P > 0.05$) for meat from bulls fed all diets (Table 3). At three days of aging, cooking losses were lower ($P < 0.05$) for meat from bulls fed CONT diet. At seventh day of ageing, meat cooking losses were lower ($P < 0.05$) from bulls fed CONT and MONE. At the contrary, in the last ageing time (14 days), cooking losses were lower ($P < 0.05$) for meat from bulls fed MO+AD diets (18.9%).

The greatest cooking losses ($P < 0.01$), regardless of diets, were observed before ageing (day 1), close to 31.3% in comparison with the other ageing days (Table 3). Thus, the greatest loss of water by cooking occurred before the meat ageing.

During ageing, meat water loss is expected as a consequence of changes in muscular fibers caused by rigor mortis and modifications of myofibrillar structure (49). It is known that cooking denatures the muscle proteins, which directly influences the structural characteristics (50). The changes lead to substantial cooking loss in the range of 20 to 30% (40,46,47). However, the amount of cooking loss is dependent on cooking method, cooking time and end-point temperature (50).

Meat texture

The texture measured as Warner-Bratzler shear force (kgf) on the first day of evaluation was lower ($P < 0.001$) for the meat from bulls fed CONT diet in comparison meat texture from bulls fed with additives addition in the diets (Table 4). In the third and seventh days of evaluations, shear force values from bulls fed MONE diet was higher when compared to shear force values from bulls fed other three diets. On the last evaluation day (14 days), shear force values from bulls fed MO+VM or MO+AD diets was lower ($P < 0.05$) when compared to shear force values from meat of bulls fed CONT and MONE diets. In the general context of the four days of evaluations, the shear force values coincided with a tougher texture for the meat from bulls fed MONE diet in comparison to meat from bulls fed other diets. In this way, MO+VM or MO+AD addition improved meat texture.

Before ageing, the mean shear force was close 8.9 kgf; which is not considered a tender meat (shear force > 5kgf is considered meat tough) (51). Meat aging time linearly improved the meat texture from bulls fed with four diets. The shear force values in this study varied from 8.9 kgf before ageing to 4.96 kgf 14 days after ageing. In this way, ageing is a process that improves meat tenderness (52). Bulls with Zebu genetic, as Nellore bulls used in this study have less tender meat compared to bulls with European genetic (51) due to the calpain-calpastatin complex (53), which inhibits calpain activity that is responsible for the degradation of myofibrillar proteins during rigor mortis. This proteolysis is an important process in the establishment of tenderness (54). Several studies carried out with crossbred bulls between zebu and European, finished in feedlot, with age close to 24 months showed a shear force varying from 9 to 4 kgf during the ageing period for 14 days (40,45,55).

Lipid oxidation

Monensin, virginiamycin and Advantage Confinamento® inclusion in the diets of bulls finished in feedlot did not alter ($P > 0.05$) lipid oxidation (Table 5). Thus, none of the dietary treatments altered the meat's protection against lipid oxidation.

On the other hand, lipid oxidation increased with meat ageing (Table 5). The TBARS values increased from 0.37 mg MDA/kg of fresh meat at the first day to 0.71 mg MDA/kg of fresh meat on the fourteenth day of ageing. The ageing time is the factor that has the greatest effect on lipid oxidation due to the presence of oxygen that accelerates the oxidation process (56).

Before ageing, the TBARS values was high (0.37 mg MDA/kg fresh meat) when compared to the values observed by other authors (45–47,57). This higher value could be explained between the time of collecting of meat and the time for carrying out the analyzes in the laboratory as observed in other studies (40,45). In meat, even if it is vacuum-packed and frozen at -20º C, soon after the storage period oxidation of the lipids process begins (58). The TBARS values were close to 0.7 mg MDA/kg fresh meat at the end of the ageing period (14 days). MDA/kg values of fresh meat above 2.0 mg makes it rancid with rejection to human consumption (59). In this experiment, these values were well below; therefore, without harmful effect on meat consumption.

Antioxidant activity

The meat antioxidant activity was measured using two methodologies: DPPH and ABTS (Table 5). The inclusion of monensin, virginiamycin, and Advantage Confinamento® ($P > 0.05$) does not cause either a positive or negative effect on meat antioxidant activity, unlike the inclusion of vegetable oils that significantly increases antioxidant activity (45,60).

Likewise, the ageing time did not influence ($P > 0.05$) the antioxidant activity in meat measured by DPPH methodology (Table 5). Thus, the aging time does not have a negative effect on meat antioxidant activity from bulls finished in feedlot and fed antibiotic, ionophores and trace mineral. However, when the antioxidant activity was measured using the ABTS methodology, the values were on the 7th day of evaluation was lower ($P <0.05$) for the meat from bulls fed CONT diet in comparison meat from bulls fed with additives addition in the diets (Table 5). Moreover, an increase in antioxidant activity was observed during ageing time, the values increased from 43.4% before aging to 66.5% at 14th days aging.

Although the MO+AD treatment contains some antioxidant compounds in its composition, the amount provided by the 84-day period of feedlot was not sufficient to influence the antioxidant activity of the meat. Among these compounds are mannans and glucomannans have antioxidant activity (Liu, Huang & LV, 2018). Also, present in its composition is the selenium organic, with function antioxidant related to the antioxidant enzyme glutathione peroxidase acting in delaying postmortem oxidation reactions in muscle tissue (Mahan et al., 2014).

Conclusion

The inclusion of antimicrobials compound and minerals trace + yeast in the cattle diets does not show harmful effects on meat quality and improved some parameters, such as the colour of meat. In particular, the combination monensin and blend of minerals trace and yeast (Advantage Confinamento®) reduced cooking losses by improving meat texture making them a promising natural alternative in animal feed.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

Thais study was partiallyfundedbythe Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Moreover, the authors thank the Alltech do Brasil (Maringá city, Paraná State, Brazil) to support partially financial this study. The trade names or commercial products in this publication are mentioned solely for the purpose of providing specific information and do not imply recommendations or endorsement by the Department of Animal Science, Universidade Estadual de Maringá, Maringá, Paraná, Brazil.

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Table 1. Ingredients of experimental diets (DM basis)

Ingredients	**Diets**			
	CONT[1]	**MONE**[2]	**MO+VM**[3]	**MO+AD**[4]
Corn silage	17.108	17.469	16.761	16.723
Corn cracked	74.374	73.493	74.239	74.461
Soybean meal	7.007	7.155	6.865	6.849
Limestone	0.410	0.418	0.401	0.400
Urea	0.432	0.441	0.423	0.423
Mineral salt	0.669	0.683	0.655	0.654
Monensin	0.000	0.341	0.328	0.327
Virginiamycin	0.000	0.000	0.328	0.000
Advantage Confinamento®[5]	0.000	0.000	0.000	0.163

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mgof monensin/kg of dry matter(DM); [3]MO+VM – Inclusion of 30 mg of monensin/kg of DM+ 30 mgof virginiamycin/kg of DM; [4]MO+AD – Inclusion of 30 mgof monensin/kg DM+ 3.0 gof Advantage Confinamento®/100 kg of BW. [5]Levels: iron - 4,000 mg/kg, magnesium - 3,000 mg/kg, sodium - 21,300 mg/kg, sulfur – 1,600 mg/kg, zinc - 10,000 mg/kg, copper – 3,340 mg/kg, manganese - 6,650 mg/kg, iodine - 420 mg/kg, selenium - 75 mg/kg, cobalt - 88 mg/kg, chromium - 330 mg/kg, glucomannans - 65,160 mg/kg, mannans - 50,670 mg/kg, *Saccharomyces*/1026 - 1 x10^8 CFU/g, *Saccharomyces*/8417 - 1 x10^8 CFU/g.

Table 2. Effect of additives inclusion in the diet in pH and meat colour from crossbred bulls finished in feedlot

Parameters	**Diets**					
Days	**CONT[1]**	**MONE[2]**	**MO+VM[3]**	**MO+AD[4]**	**SEM[5]**	**P - value**
			pH			
1	6.09aA	5.92bA	6.05aA	5.90bA	0.033	0.002
3	5.90aB	5.71bB	5.86aB	5.80bB	0.028	0.004
7	5.70aC	5.63bC	5.78aB	5.68bC	0.026	0.017
14	6.24aA	6.02bA	6.02bA	6.33aA	0.060	0.021
SEM[5]	0.052	0.027	0.055	0.032		
P - value	0.009	0.014	0.013	0.006		
			L*			
1	38.14bB	39.86aB	37.52bB	39.73aB	0.206	0.001
3	39.78cA	42.75aA	40.54bA	41.87aA	0.233	0.001
7	38.02bB	38.99aB	38.01bB	40.19aB	0.282	0.017
14	36.01C	35.67C	35.43C	36.09C	0.258	0.790
SEM[5]	0.317	0.269	0.287	0.275		
P - value	0.003	0.001	0.001	0.001		
			a*			
1	12.94B	13.27B	12.82B	11.85B	0.193	0.057
3	12.75B	13.05B	13.71AB	13.75A	0.140	0.055
7	14.00A	13.80B	14.02A	13.57A	0.165	0.757
14	14.31A	15.38A	13.88AB	14.33A	0.193	0.041
SEM[5]	0.217	0.161	0.158	0.184		
P - value	0.023	0.001	0.031	0.001		
			b*			
1	12.04	12.99B	12.14B	12.00C	0.163	0.1026
3	13.03b	13.93abA	13.97aA	14.58aA	0.132	0.0004
7	12.94b	13.42abA	13.34abA	13.84aAB	0.123	0.0775
14	12.34	12.82B	12.16B	12.94B	0.172	0.3186
SEM[5]	0.181	0.128	0.161	0.148		
P - value	0.1590	0.0091	0.001	<0001		

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mgof monensin/kg of dry matter(DM); [3]MO+VM – Inclusion of 30 mgof monensin/kg of DM+ 30 mgof virginiamycin/kg of DM; [4]MO+AD – Inclusion of 30 mgof monensin/kg DM+ 3.0 gof Advantage Confinamento®/ 100 kg of BW. [5]SEM - Standard error of means; Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different Tukey's test (P<0.05).

Table 3. Effect of additives inclusion in the diet in dripping losses (24 h after slaughter) and cooking losses (%) from crossbred bulls finished in feedlot

	Diets					
Days	**CONT[1]**	**MONE[2]**	**MO+VM[3]**	**MO+AD[4]**	**SEM[5]**	**P - value**
		Dripping losses (%)				
1	1.52	1.43	1.80	1.52	0.132	0.827
		Cooking losses (%)				
1	29.35A	31.64A	32.51A	31.66A	0.492	0.100
3	18.89bB	23.37aB	23.12aB	23.74aB	0.173	0.001
7	22.92bB	21.52bB	25.08aB	25.93aB	0.142	0.001
14	20.63bB	26.1aB	21.99bB	18.89cC	0.150	0.001
SEM[5]	0.283	0.547	0.336	0.425		
P - value	0.0002	<0.001	<0.001	0.0032		

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mgof monensin/kg of dry matter(DM); [3]MO+VM – Inclusion of 30 mgof monensin/kg of DM+ 30 mgof virginiamycin/kg of DM; [4]MO+AD – Inclusion of 30 mgof monensin/kg DM+ 3.0 gof Advantage Confinamento®/ 100 kg of BW. [5]SEM - Standard error of means; Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different Tukey's test (P<0.05).

Table 4. Effect of additives inclusion in the diet on meat texture (kgf) from crossbred bulls finished in feedlot

	Diets					
Days	**CONT[1]**	**MONE[2]**	**MO+VM[3]**	**MO+AD[4]**	**SEM[5]**	**P - value**
1	7.16bA	9.84aA	9.12aA	9.42aA	0.423	0.001
3	6.15bAB	8.35aAB	5.83bB	6.72bB	0.339	0.005
7	5.69bA	7.13aA	5.33bB	5.15bB	0.288	0.082
14	5.36aB	5.53aA	4.85bC	3.86bC	0.245	0.001
SEM[5]	0.367	0.448	0.325	0.270		
P - value	0.0114	<0.001	<0.001	<0.001		

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mgof monensin/kg of dry matter(DM); [3]MO+VM – Inclusion of 30 mgof monensin/kg of DM+ 30 mgof virginiamycin/kg of DM; [4]MO+AD – Inclusion of 30 mgof monensin/kg DM+ 3.0 gof Advantage Confinamento®/ 100 kg of BW. [5]SEM - Standard error of means; Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different Tukey's test ($P<0.05$).

Table 5. Effect of additives inclusion in the diet on meat lipid oxidation (mg/malonaldehyde/kg^{-1} of meat) and antioxidant activity from crossbred bulls finished in feedlot

Days	**Diets**					
	CONT[1]	**MONE[2]**	**MO+VM[3]**	**MO+AD[4]**	**SEM[5]**	**P - value**
			MDA			
1	0.35C	0.40B	0.37C	0.36C	0.015	0.729
3	0.47B	0.43B	0.51B	0.49B	0.027	0.805
7	0.49B	0.46B	0.54B	0.50B	0.025	0.775
14	0.70A	0.74A	0.70A	0.68A	0.036	0.891
SEM[5]	0.034	0.040	0.031	0.033		
P - value	0.0002	0.0027	0.0020	0.0012		
			DPPH (%)			
1	22.93	21.36	25.04	21.57	0.966	0.541
3	25.72	19.47	27.25	25.53	1.149	0.080
7	23.93	21.63	27.33	22.95	0.723	0.076
14	22.14	23.78	24.80	22.36	0.824	0.659
SEM[5]	1.310	1.262	0.847	0.736		
P - value	0.320	0.080	0.607	0.060		
			ABTS (%)			
1	43.40C	45.09C	46.54C	47.40B	1.519	0.823
3	47.25B	50.56CB	51.54BC	44.43B	1.244	0.162
7	55.04ABb	63.74ABa	63.53Aa	61.63Aa	1.038	0.003
14	62.64A	66.50A	63.10AB	63.19A	1.833	0.888
SEM[5]	1.899	2.231	2.064	2.152		
P - value	<0.001	0.004	0.009	<0.001		

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mgof monensin/kg of dry matter(DM); [3]MO+VM – Inclusion of 30 mgof monensin/kg of DM+ 30 mgof virginiamycin/kg of DM; [4]MO+AD – Inclusion of 30 mgof monensin/kg DM+ 3.0 gof Advantage Confinamento®/ 100 kg of BW. [5]SEM - Standard error of means; Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different Tukey's test (P<0.05)

CAPÍTULO V

(Journal of Sensory Studies)

Meat acceptability of crossbred bulls fed a high-grain feedlot diet with antimicrobial compounds and a blend of organic minerals trace and yeast

ABSTRACT

The meat acceptability from 24 crossbred bulls (24 ± 3.2 months of age; body weight (BW) of 385.5 ± 3.84 kg) were evaluated. The animals were fed the diets: CONT – without additives; MONE – inclusion of 30 mg of monensin/kg of dry matter intake (DMI); MO+VM – inclusion of 30 mg of monensin + 30 mg of virginiamycin/kg DMI; MO+AD – inclusion of 30 mg of monensin/kg DMI + 3.0 g of Advantage Confinamento®/100 kg BW. The Advantage Confinamento® is composed by organic trace minerals and live yeast Saccharomyces cerevisae. There were no differences in meat mineral levels among treatments ($P > 0.05$). Meat aged for 7 days was better accepted by consumers and meat from bulls fed MO+AD diet resulted in the highest willingness to buy. Diet did not alter meat odor, flavor, and overall acceptability. Visual acceptability was not influenced by diets until 5 days of display and decreased with display time.

PRACTICAL APPLICATIONS

With the ban on the use of antimicrobials in animal feed in some countries, natural alternatives with similar functions are being sought by the industry. Changes in animal production interfere with the quality of the meat and consequently with the acceptability by the consumer, who is increasingly demanding in terms of quality, animal welfare and sustainability. The acceptability of the final product by the consumer must be well known. These data indicate inclusion of additives in the diet, especially monensin and a natural product based on organic trace minerals and live yeast, may be beneficial to meat acceptability and consumer willingness to buy.

Keywords: cattle, minerals trace, natural additives, *Saccharomyces cerevisae*, sensorial

INTRODUCTION

Cattle finished in feedlot generally receive high-grain diets as an energy source (Monteschio et al., 2017; Ornaghi et al., 2017; Rivaroli et al., 2016). However, the rapid degradation rate of these diets increases the risk of metabolic disorders and acidosis in the rumen (Giger-Reverdin et al., 2002; González et al., 2012). Thus, several antimicrobials have been used to prevent these risks and increase animal performance and improve feed efficiency (Monteschio et al., 2017; Prado et al., 2016; Rivaroli et al., 2016; Torrecilhas et al., 2021) by beef producers. However, antimicrobial use in order to minimize the risk of metabolic disorders and acidosis in the rumen, such the ionophores and antibiotics, can lead to antibiotic resistance, representing a threat to animal and human health (Cameron & McAllister, 2016; Russell & Houlihan, 2003; Schäberle & Hack, 2014).

Results of studies show that the addition of minerals traces and yeast in the diet of cattle finished in feedlot has influence on meat characteristics (Cozzi et al., 2011; Geng et al., 2018; Silva et al., 2020). However, studies on the meat acceptability of cattle by consumers are scarce in the literature.

Diet supplementation with yeast can improve the productive performance of ruminants because it modulates the microbial ecosystem, improves digestion of fiber, presenting a gain rate similar to antibiotics (Shen et al., 2018, 2019), as well as improve their immune system and intestinal health (Zheng et al., 2018). In addition, some minerals traces has antioxidant function (Silva et al., 2020).

Beyond the effect on the animal performance it is important to study the acceptability of the final product (meat) by the consumer market, increasingly demanding, in relation to tenderness, flavour, colour and aspects related to health, at the time of purchase (Guerrero et al., 2018; Vital et al., 2018).

The hypothesis of this study was that the inclusion of ionophores (monensin) or antibiotics (virginiamycin) in combination with a minerals traces and yeast could alter the sensory acceptability of meat by consumers.

This study was realized to evaluate the use of a blend of organic minerals trace and yeast as an alternative to antimicrobial compound on meat acceptability from bulls finished in feedlot fed high-grain diet.

MATERIAL AND METHODS

Ethic committee, local, animals, and diets

This experiment was approved by the Department of Animal Production and Research Ethic Committee at the State University of Maringá, and it followed the guiding principles of biomedical research with animals (CAAE: 44460020.3.0000.0104 protocol number).

This study was conducted at the Rosa & Pedro Sector of the Experimental Farm Station at the State University of Maringá (UEM) Maringá city, Paraná state, Brazil south. The sensorial evaluation was performed at the Meat Quality Laboratory at the UEM.

A total of 24 crossbred bulls (*Bos taurus x* Nellore) at 24 ± 3.2 months of age and weighting mean initial body weight of 385.5 ± 3.84 kg were used in a completely randomized design. The bulls were weighed at the beginning of the experiment and assigned randomly to 10 m^2 individual pens, partially covered and with concrete floors and water drinkers.

The bulls were distributed into four diets according to initial body weight. The basal diet comprised 850 g/kg concentrate and 150 g/kg corn silage that was offered *ad libitum* for 84 days. The basal diet was the same for all animals and it was formulated to be isonitrogenous and isoenergetic according to Valadares Filho et al. (2016) for an ADG of 1.8 kg/day. The four experimental diets were as follows: CONT – without additives, MONE – inclusion of 30 mg of monensin/kg of DM, MO+VM – inclusion of 30 mg of monensin/kg DM + 30 mg of virginiamycin/kg DM, MO+AD – inclusion of 30 mg of monensin/kg DM + 3.0 g of Advantage Confinamento®/100 kg/BW. The Advantage Confinamento® is a blend of organic minerals traces and live yeast (*Saccharomyces cerevisae*) with guarantee levels of: glucomannans (65.16 g/kg), mannans (50.67 g/kg), zinc as zinc proteinate (10 g/kg), manganese as manganese proteinate (6.65 g/kg), iron as iron proteinate (4 g/kg), copper as copper proteinate (3.34 g/kg), iodine as potassium iodate (0.42 g/kg), chromium as chromium-enriched yeast

(0.33 g/kg), cobalt as cobalt proteinate (88 mg/kg), selenium as selenium-enriched yeast (75 mg/kg), *Saccharomyces cerevisae* strain 1026 (1 x 10^8 UFC/g), and *Saccharomyces cerevisae* strain 8417 (1 x 10^8 UFC/g).

Meat sampling

At day 84 of feedlot, the bulls were slaughtered following the usual practices of the Brazilian beef industry. Afterwards, the carcasses were divided medially from the sternum to spine, resulting in two similar half carcasses. Then, they were washed, identified, and stored in a chilling chamber at 4° C, where remained for 24 h.

After the 24-h period, 24 samples of the *Longissimus thoracis* (LT) muscle was collected from the left half carcass between the 6^{th} and 13^{th} ribs and transported in ice to the Meat Quality Laboratory at the Maringá University. The LT muscle was sliced into steaks (2.5 cm thick), weighed, vacuum-packed (99% vacuum, Sulpack SVC 620) in polyamide/polyethylene pouches (120 μm; 1 cm3/m^2 for 24 h O_2 permeability and 3 cm^3/m^2 for 24 h CO_2 permeability at 4° C and 75% relative humidity; 3 g/m^2 for 24 h water vapor transmission rate at 38° C and 100% relative humidity; 97° C Vicat softening temperature; 1.3 g dart drop strength and was aged during 1 or 7 days at 4º C. After, samples were frozen and stored (−20° C) until the moment of the sensorial analysis.

Mineral analysis of meat

For the quantification of minerals in the meat, the ash content was performed according to AOAC (2005). After this procedure, 5.0 mL of HCl (1:1 v/v) was added in the ash and the mixture was heated in a hot block acid digestion system at 250º C until the volume is reduced to approximately 1.0 ml. The solution was finally cooled down to room temperature and diluted to 100 mL using ultrapure water. The concentrations of potassium, phosphorus, calcium, magnesium, zinc, iron, manganese, copper, chromium, and cobalt were measured by inductively Agilent 4200 microwave plasma-atomic emission spectrometer (MP-AES; Agilent Technologies, Santa Clara, CA) equipped with an Inert One Neb nebulizer and a double-pass class cyclonic spray chamber

(Agilent Technologies).

Microbiological analysis of meat

Before the meat freezing for further sensory evaluation by consumers, microbiological analyzes were performed on the samples for all treatments. Coliforms at 45° C were quantified as colony forming units per gram (UFC/g), Salmonella spp. expressed as presence and absence at 25/g and the counting of Sulphite Reducing Bacteria following the ISO 15213: 2003 methodology.

Questionnaire

Prior to consumer testing, a supplementary questionnaire was applied in order to gather more information about consumption habits, buy preferences, or the willingness to buy beef. It included closed questions with multiple choices (Table 1).

Sensory acceptability

In order to assess the acceptability of the meat from the different diets tested, a consumer test was performed in a private room (adequately adapted to perform a sensory test at the UEM). Consumers were randomly selected at the UEM (students, employers, and visitors) being the sample representative of according to Brazilian national profile (IBGE), divided by gender and age interval (from 18 to 24 years, from 25 to 39 years, from 40 to 54 years, and more than 55 years old). The socio-economic data, the level of education, and family income were also collected.

The 150 participants in the test were divided in 15 sessions, attending 10 people in each session. The consumers evaluated four samples identified with a three-digit code, corresponding to the four different diets CONT, MONE, MO+VM, and MO+AD and D1 and D7. Samples were provided to consumers one at a time, following a randomized design to avoid any order or carryover effects (Macfie et al., 1989). For beef preparation, each steak was covered with aluminum foil and cooked in a pre-heated grill at 200° C (Philco Grill Jumbo Inox, PHILCO S.A., Brazil) until an internal temperature reached 75° C. Each steak was then cut (2 × 2 cm cubes), wrapped in aluminum foil, and kept at 50° C until consumer evaluation (10 s after cooking). Consumers were asked to eat unsalted toasted bread and rinse their mouth with water before evaluating each sample, including the first one. Consumers were only informed that they would be evaluating beef. All consumers were asked to taste the meat samples and evaluate the acceptability of four attributes: odour, flavour, tenderness, and overall acceptability; this was achieved using a structured hedonic nine-point scale ranging from 1 = dislike extremely to 9 = like extremely, where a medium level was not included according to methodologies described by Font-i-Furnols et al. (2008). After evaluation acceptability test, as well as willingness to buy or not the meat was asked using a two-point scale ranging from 1 = I would certainly would not buy to 2 = I would certainly buy.

Visual evaluation

Display and photography

The meat samples were thawing at refrigerator (+ 4º C) for 12 h. After, the meat samples were exposed in a display vitrine, simulating typical Brazilian market conditions for selling meat on a tray in stores for visual evaluation. LT steak (2.5-cm) of each animal was then packaged in an individual polystyrene trays wrapped with a film (Goodyear®, Americana, São Paulo, Brazil), and placed in a refrigerated and illuminated display (4º C, fluorescent lamp, 380 lux, 12 h/day). The total time of display for steaks was 15 days.

In order to produce images in standardized conditions, photographs were taken according to Passetti et al. (2017, 2019). The over-wrapped steaks were photographed every two days through the fifteenth days of evaluation, using a NIKON D3100 digital camera mounted on a photographic stand containing two D65 fluorescent light tubes as the standard illuminant. An additional grey-colour cardboard was used to cover the entrance of the cabinet to provide lighting evenly distributed across the sample and also to avoid exposure to external light. The camera was fixed perpendicularly 45 cm to the surface of the meat sample. Following preliminary experiments to fix meat samples for appearing entirely in photos, the camera parameters were chosen: manual mode; shutter speed, 1/20; aperture size, F5.3; ISO, 1600; focal distance 40 mm. Images were stored and transferred to computer as JPEG file. A GretagMacbeth mini Colour-Checker (Colour-confidence, Birmingham, UK) which contains 24 coloured patches was photographed with each meat sample for checking the colour reproduction capability (Passetti et al., 2017).

Photography evaluation

The meat evaluation was performed using 40 evaluators and images photographed every two days for 15 days. This methodology was adopted and the space of two days was used since it does not interfere in the variation of the results obtained (Passetti et al., 2017, 2019).

At the beginning of the analyses, evaluators were instructed to evaluate the acceptance of meat color, disregarding other aspects as size, purge, marbling, and subcutaneous fat. For visual analyses was determined that 5 s was sufficient time to evaluate one photograph and after every 40 evaluation, a 1 minute break was taken to avoid fatigue (Passetti et al., 2019). The analyses occurred in a comfortable place in the conference room of the Animal Science Department of State University of Maringá, where evaluators were distributed on individual chairs in such a way, they could not have direct contact with each other. At the beginning of the analyses, evaluators were instructed to evaluate the acceptance of meat colour, disregarding other aspects as size, purge, marbling and subcutaneous fat. Photography’s were projected individually in the room by a multimedia device (Epson Powerlite S27 2700 – UHE lamps 200 watts). Consumers were instructed to evaluate the acceptability of colour on a hedonic scale from1 (“I dislike it extremely”) to 9 (“I like it extremely”), where a medium level (5) was not included according to methodologies described by Font-i-Furnols et al. (2008).

A total of 192 photography’s (24 steaks × 8 time points) in which the days of display and bulls were presented and asked the same question on acceptability in randomized fashion (Passetti et al., 2017).

Statistical analysis

Quantification of minerals in the meat and acceptability of the sensory attributes was assessed via analysis of variance using general lineal model (GLM) procedures, with SPSS v.23.0 (IBM SPSS Statistics; SPSS Inc., Chicago, IL, USA) for Windows. Differences between means were evaluated using Tukey's test ($P < 0.05$). For acceptability of the sensory attributes the diets and ageing time were considered as fixed effects and the consumer as random effect on the sensorial test; session was considered as block effect.

Answers related to habits and preferences of consumption were analyzed by the frequency of response. Hierarchical cluster analysis was used to determine the different segments of consumers, according to the overall acceptability using XLSTAT (v. 20.0). The number of clusters was selected by a dendrogram. Principal Components Analysis (PCA) was used to verify the relationships between diets and the acceptably attributes and it was showed in a graphic. Visual appraisal tests were assessed via ANOVA, using GLM procedures, with SPSS (v.23.0) for Windows. Visual attributes were evaluated considering diet and ageing time of display as fixed effects.

RESULTS AND DISCUSSION

Mineral analysis of meat

The presence of chromium and cobalt were not detected in the meat by the analytical method used in any of the samples analyzed. Besides that, no differences ($P > 0.05$) were detected in meat from bulls fed to diets for the potassium, phosphorus, calcium, magnesium, zinc, iron, manganese, and copper concentrations (Table 2).

Doornenbal & Murray (1982) observed that the contents of Cu, Fe, Zn, and Mg in the *Longissimus dorsi* muscle were different from those in the semimembranosus muscle. In contrast, Rossi et al. (2020) evaluated the supplementation of beef cattle with selenium, zinc, copper and manganese against inorganics and found higher levels of organic minerals in meat. In this study, the meat was quantitatively the most enriched of potassium followed by phosphorus, calcium and magnesium as observed by Barge et al. (2005) and Doornenbal & Murray (1982).

Microbial analysis of meat

All samples of microbiological analyses showed coliforms values at 45º C and 35º C < 3 NMP, coagulase positive staphylococcus < 1 x 10^2 UFC/g, absence of salmonella spp in 25 g, sulfate reduction clostridium counts < 10 UFC/g, in accordance with Brazilian regulation (RDC no 12.2001) as observed by Carvalho et al. (2017). Thus, the samples were released for sensorial analysis procedure.

Socio-demographic characteristics

The survey application increases the information about to consumers' preferences, meat habits buying and consumption (Table 1). For beef testing, consumer sample was composed by 46% men and 54% women. Moreover, the majority of consumers are under 40 years old (80%), which represents the Brazilian population.

The majority personal incomes per month among consumers varied from 2 to 6 minimum income (1,030 Brazilian Real or 220 US$) (50%). Consumer with personal income above 10 minimum income represented only 11.6% of population (10,030 Brazilian Real or 2,000 US$). The majority of the consumers answered that they had high school complete (56.0%) or were finishing high school (34.7%). Vital et al. (2018) verified values close to this study, at the similar condition using the universities populations. These results may not represent the reality of Brazilian population, but a portion of the population, because the study was developed in Maringá city, Paraná state, Brazil south where the population has a higher income compared to the country mean. Besides that, the consumers were professors, researchers, students, university professionals, and the university visitors.

Consumption habits of consumers

The majority of personals consume beef from two to four times per week (56.7%) or more than five times per week (26.0%) (Table 3). These beef consumption habits were expected; the high consumption of beef has already been reported in other studies performed in Brazil (Carvalho et al., 2014; Kirinus et al., 2013). Beef is a traditional product in the Brazilian habit, and with the current socio-economic changes and the increase on the population purchasing power is reflected as an increase in meat (beef and poultry) consumption decreasing other basic food such as carbohydrates (Kirinus et al., 2013). Guerrero et al. (2018) reported a beef consumption frequency of 35% (more than five times a week) and 44% from 3 to 5 times a week in a study carried out in the south region of Brazil. Likewise, Vital et al. (2018) observed a consumption frequency beef above 5 times per week or more (35%) and 49% for beef consumption from 2 to 5 times a week in a study conducted in a region close to the region of this study. These frequencies are in agreement with the national official consumption data (42 kg of beef/people/year) from ANUALPEC (2020).

However, pork is consumed once a week (37.1%) and lamb once a month (75.0%). Also, the frequency of fish consumption is low (13.8% once a week) and close to lamb consumption. The high frequency of poultry consumption (2–4 times/week – 48%) or (5 times/week or more – 15%) could be explained by its low price (lower than other meats) (Carvalho et al., 2014). In addition to the lower price of chicken meat, another important factor in the consumer's decision is the belief that this meat is leaner, with less cholesterol and, therefore, healthier (Fernández-Ginés et al., 2005). This belief is the result of some studies in human medicine that affirm this hypothesis (Mir et al., 2017).

Purchasing habits of consumers

The majority of consumers chose to buy the meat in a supermarket (68.0%), followed by a butcher (31.3%), with a lower percentage of consumers purchasing the meat from specialized boutiques (0.7%) (Table 4). According Behrens et al. (2010), the increase of purchasing meat in supermarkets is consequence of the changes on food habits and modern life style, which demands time saving efforts and reduce time availability. Supermarkets help people in purchasing different kinds of products at the same establishment. At contrary, the minority of Brazilian consumers prefer to buy meat in specialized boutiques (0.7%). In Brazil, the low acquisition of beef in specialized boutiques is related to the high price of beef in this market. The high price of meat in specialized boutiques is due to the origin of the meat (better quality), presentation of specialized cuts, packaging and appearance of the meat, among others. A similar tendency of this study had been previously reported in Brazil by Kirinus et al. (2013) and Mazzuchetti & Batalha (2004). In an essay realized in the same location, Vital et al. (2018) observed that consumers prefer to buy meat in a supermarket (67.8%), butcher (31.1%) and specialized meat boutique(1.1%). Similar results were observed by Eiras et al. (2017) with 53.3, 44.0 and 2.80% the consumers buying meat in supermarket, butcher and specialized meat boutique, respectively.

Participants in this essay preferred buying fresh meat (80.0%) than other packaging forms (14.0% in tray and 6.0% in vacuum-packed). Eiras et al. (2017) and Vital et al. (2018) observed that the preference for the purchase marketing mode was for fresh meat (84.0%), vacuum (6.8%) and on a tray (3.7%). Nevertheless, buying meat directly on trays is an increasing habit due to it being easier to freeze and store (Velho et al., 2009).

Myoglobin, the protein responsible for meat colour, occurs in three forms: deoxymyoglobin (purplish-red colour), oxymyoglobin (cherry-red colour, in fresh meat), and metmyoglobin (brown colour, spoiled or non-fresh meat (Mancini & Hunt, 2005).

Consumers considered colour (52.0%) to be one of the most important attributes that influence meat acquisition, followed by price (40.0%), showing that other factors (animal age, breed and others) have a low importance in the purchase preference (8.0% or less), which is in according with other studies (Eiras et al., 2017; Monteschio et al., 2020; Velho et al., 2009). For comparison, European consumers considers other attributes such as meat origin or animal feeding system to be more important than the colour and price (Realini et al., 2013).

Regarding the gender preferred, meat from heifers (19.3%) followed by steers (13.4%) was preferred compared with that from cows (0.0%) or bulls (2.0%). However, for majority participants the animal gender has little importance in the preference of buying meat (65.3%). Other studies (Eiras et al., 2017; Guerrero et al., 2018) have shown that the majority of the Brazilian population prefers heifer meat. Heifer meat, compared to other bovine gender, is more tender and, therefore, more accepted by the

Brazilian population (Guerrero et al., 2018; Rotta et al., 2009).

The traditional Brazilian beef production system (pasture) was accepted by 43.3% consumers; while the same percentage (43.3%) considered that the production system has little importance on the choice of meat at the purchase time. The feedlot (13.4%) was the least preferred production system. The beef produced in the pasture system has a higher concentration of unsaturated fatty acids due feeding system (tropical forage) and age (< 30 mo) (Maggioni et al., 2009; Padre et al., 2007; Rotta et al., 2009). Therefore, this meat has greater acceptance by Brazilian consumers, although this meat is less tender.

In this study, consumers were asked whether they would eat the meat of animals supplemented with antibiotics in the diet; 70.0% of the consumers answered yes and 30.0% answered no. These results indicated that concerns about antibiotic supplementation were held by only a minority of participants. Some studies published in the literature mention that the addition of ionophores or antibiotics could leave residues in the meat of cattle (Cameron & McAllister, 2016; Russell & Houlihan, 2003; Schäberle & Hack, 2014). Therefore, these compounds should not be included in animal diets (OJEU, 2003). However, in the present study, we carried out the analysis of virginiamycin and monensin residues in the meat, but no residues were detected (unpublished data).

Sensorial evaluation of meat

There was no interaction (P > 0.05) among diets and ageing time on sensory evaluation by the consumers. Thus, the diet and ageing time effects are presented and discussed as main effects.

Diet effect

The inclusion of additives in the diets did not alter (P > 0.05) meat odour, flavour, and overall acceptability (Table 5). The scores (scale from 1 to 9) varied little among diets to odour (from 6.33 to 6.60), flavour (from 6.53 to 6.69), and overall acceptability (from 6.50 to 6.74). However, the observed scores show that the meat acceptability was above 6.5; this score can be considered to be the threshold level of acceptability (Font-i-Furnols & Guerrero, 2014; Polkinghorne & Thompson, 2010). In this way, meat from four diets could be considered of good acceptability as observed in other studies (Monteschio et al., 2020; Ornaghi et al., 2020; Torrecilhas et al., 2021). These results show that the use of additives in the diet did not affect meat overall acceptability.

The tenderness is extremely important for meat acceptability (Guerrero et al., 2018). Tenderness was similar (P > 0.05) to meat from bulls fed CONT, MONE and MO+VM diets and lower (P < 0.001) than meat from bulls fed MO+AD diet (Table 5). Wang et al. (2020) found that feeding of crossbred steers with monensin + tylosin; essential oils and benzoic acid did not improve tenderness for LT muscle.

Ageing effect

Ageing time did not modify (P > 0.09) meat odour (Table 5). However, the mean scores assigned by consumers for the flavour, tenderness and overall acceptability were higher (P < 0.001) for meat aged for seven days than those day 1. These results lead us to infer that ageing improves consumer meat acceptability, which has also been observed by Eiras et al. (2017). Ageing time influences on flavour development precursors, usually improving the acceptability up to a certain ageing time threshold (seven 10 days), after which off-flavours can develop (Monsón et al., 2005). Consumers can focus on the flavour when the meat is tender. Ageing is an endogenous process of degradation of the myofibrils and connective tissues integrity and starts immediately after slaughter (Kemp & Parr, 2012). However, in ageing process, it takes about 7 days before meat attributes are effectively improved via enzymatic activity (Kemp & Parr, 2012). Thus, despite of Brazilian consumers prefer to buy fresh products, meat aged from young bulls for 7 days improved consumers acceptability, as observed by other authors (Monteschio et al., 2020; Ornaghi et al., 2020; Torrecilhas et al., 2021).

Principal components analyses

Information about diets and meat ageing preferences of the consumers is graphically presented in Figure 1.

The first two axes explained 92.25% of the total variance. Attributes of odour, flavour, tenderness, and overall acceptability and willingness to buy are situated on the right side of the graph, closely located to the diets with additives addition with seven days of ageing. Meat before ageing (D1) is located on the left side of the graph (CONT, MONE and MO+AD), inversely related to acceptability attributes and willingness to buy. Tenderness, flavour, and overall acceptability and willingness to buy are located in the same quadrant for all diets aged for seven days, indicating the preference for meat ageing. Flavour, tenderness, and overall acceptability and willingness to buy are located in the upper right quadrant and associated with an ageing time of seven days, especially with the MO+VM and MO+AD diets. In this way, the result in the PCA analysis is clear that the inclusion of the different additives significantly improved the acceptability of meat by consumers as observed by other authors with the addition of other additives in the diet of cattle finished in feedlots (Monteschio et al., 2020; Ornaghi et al., 2020; Torrecilhas et al., 2021). Thus, additives and their combinations in the diet has no injuries effect on meat acceptability of cattle finished in feedlot and fed high-grain diet.

Visual evaluation of meat

Consumers are able to distinguish meat colour from different animal, breed, diets, gender, feeding, finishing system among other factors before and after ageing (Brugiapaglia & Destefanis, 2009; Passetti et al., 2019).

The inclusion of MONE or MO+VM or MO+AD in the diet of bulls finished in feedlot influenced ($P > 0.001$) visual evaluation of the meat during the 15 days of exposure in a display (Table 6). In the first seven days of evaluation when the meat was considered desirable for human consumption (score above 5.0), the meat from bulls fed the inclusion of MONE diet or MO+AD diet had the highest ($P < 0.001$) evaluation scores in comparison to the CONT and MO+VM diets. Which can be explained by the instrumental evaluation of the colour of the meat, was observed the ageing time led to a reduction in L* values in meat from bulls fed CONT and MO+VM diets, characterizing a darker meat, therefore, unattractive to the consumer (unpublished data). From the 7 days, when the evaluation scores were below 5, meat was considered undesirable for the human consumption, for all diets.

Ageing time has a determining effect on evolution of consumers' perception of meat quality (Passetti et al., 2017, 2019). Throughout the display period (15 days), consumer acceptance for meat appearance decreased (Table 6). The visual acceptability was not influenced until 5 days of display for all diets. On the seventh day, the mean value was 5.7; thus, above 5.0, which is considered the limit score for the willingness to buy meat. However, in the ninth day, score mean was 4.6. In general, the acceptability limit score for beef on display is between the sixth and eighth day (Passetti et al., 2017, 2019; Prado et al., 2015). At the fifteenth day (evaluation last day), the score for visual acceptability was above 2. In general, after eighth days, meat obtain score below 3 (Eiras et al., 2017; Prado et al., 2015).

Oxidation is a natural process which reduces the shelf life of meat and is linked to the deterioration of the product due to the oxidation-reduction of myoglobin, which is changed from oxy to metmyoglobin (Mancini & Hunt, 2005). The low deterioration observed (TBARS value below, data no showed) in the current study may be related to meat origin. Cattle finished in pasture system have higher carotenoid contents, which decrease meat deterioration (Realini et al., 2004). The bulls used in the present study were raised on pastures for 20 months; the increased carotenoid levels may have prevented a more rapid deterioration of the meat (Descalzo & Sancho, 2008).

The gradual decline in visual evaluation was expected because oxidative processes are a natural cause of meat deterioration (Vital et al., 2016), which is particularly relevant for meat from concentrate-fed animals (Prado et al., 2015; Warren et al., 2008). The maximum time of practical exposure of meat was seven days. After nine days, all of scores attributed by appraisers were below 5, which can be considered inappropriate for human consumption.

For the meat from all diets, a quadratic equation ($R^2 > 0.99$) of the visual scores were observed (Table 7). The shelf life of the meat was longer for the CONT diet (9.4 days), shorter for the MO+VM diet (6.8 days) and intermediate for the MONE and MO+AD diets (8.3 and 8.9 days, respectively).

Willingness to buy

For willingness to buy, it was applied to evaluators a question on hedonic scale from 1 (I certainly would not buy) to 2 (I certainly would buy).

The meat of bulls fed CONT diet resulted in a lower ($P < 0.07$) willingness to buy score (Table 8) day 1 when compared to meat from the bulls fed supplemented other diets. Still, at day 1, the meat from the bulls supplemented with additives had similar ($P > 0.05$) scores.

From the third to the seventh day, the meat from MONE and MO + AD diets showed higher ($P < 0.01$) values of intention to buy. As of the ninth day, the purchase intention was greater for the CONT diet. However, on the 11 days, the meat from bulls fed MO + AD diet also showed greater willingness to buy by consumers. On the 15th day, there was no effect of the diets.

However, the purchase intention was below 1.5 points (considered the limit point for the purchase intention of the meat) on the seventh day for the meat of animals fed with CONT and MO+VM diets and on the ninth day for meat of animals fed with MONE and MO + AD diets. Thus, supplementation with monensin or monensin + Advantage Confinamento® increased the shelf life of meat.

In relation to the display time, the willingness to buy the meat decreased over time, which was expected due to the deterioration of the meat (Vital et al., 2016). Until the seventh day of exposure the meat from bulls fed MONE and MO+AD diets had high purchase acceptability, therefore, there was no rejection for the purchase of meat is this period. Monteschio et al. (2020) reported a similar result when evaluating the meat of heifers received a diet supplemented with natural additives.

CONCLUSION

Knowledge of the interrelationships of the meat's attractiveness attributes, such as colour, visual assessment and purchase intention with the meat quality attributes (odour, flavour, tenderness and overall acceptability) is an important factor for the buyer's decision at the time of purchase. In this study, it was observed that supplementation of bulls finished in feedlot and fed diets supplemented with antibiotics or a combination of antibiotics with minerals traces + yeast had small effects on meat acceptability. Yet, when there was an effect, they were more positive than negative. In this way, a combination of organic minerals with conventional additives is suitable for supplementation of cattle finished in feedlot and fed with high-grain diets.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was partially funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Moreover, the authors thank the Alltech do Brasil (Maringá city, Paraná State, Brazil) to support partially financial this study. The trade names or commercial products in this publication are mentioned solely for the purpose of providing specific information and do not imply recommendations or endorsement by the Department of Animal Science, Universidade Estadual de Maringá, Maringá, Paraná, Brazil.

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Table 1

Socio-demographic characteristics of the consumers (n = 150 consumers)

Age	Beef consumers		
	Total population	Men	Women
Years	%	%	%
< 24	48.7	42.0	54.3
25-39	31.3	27.5	34.6
40-54	10.7	17.4	4.9
> 55	9.3	13.1	6.2
Total, %	100	46	54
Total, n	150	69	81
Socio-economic		Beef consumers, %	
Familiar income:	Until 2MW	18.0	
Minimum wage, MW[a]	From 2 to 6 MW	50.0	
	From 6 to 10 MW	20.7	
	Higher than 10 MW	11.3	
Education level	Primary School incomplete	0.7	
	Primary School complete	1.3	
	Secondary School incomplete	0.7	
	Secondary School complete	6.6	
	High School incomplete	34.7	
	High School complete	56.0	

[a]MW: minimum income (2020): 1,045 Brazilian Real = 205,00 US$.

Table 2

Levels of minerals (ppm)in meat from crossbred bulls fed with or without additives inclusion in feedlot

Minerals	Diets				SEM[5]	P - value
	CONT[1]	MONE[2]	MO+VM[3]	MO+AD[4]		
Potassium	3180.83	3180.00	3192.08	3022.50	0.052	0.9568
Phosphorus	1817.08	1922.50	1915.83	1877.50	0.048	0.9283
Calcium	242.08	234.42	275.00	245.83	0.112	0.3102
Magnesium	210.00	240.00	250.00	232.50	0.047	0.6534
Zinc	61.08	51.67	63.33	61.42	3.342	0.6356
Iron	30.17	24.42	31.25	25.17	1.807	0.4567
Manganese	1.83	1.75	2.00	1.67	0.119	0.7930
Copper	3.25	3.75	3.75	3.75	0.352	0.9549

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mg of monensin/kg of dry matter (DM); [3]MO+VM – Inclusion of 30 mg of monensin/kg of DM + 30 mg of virginiamycin/kg of DM; [4]MO + AD – Inclusion of 30 mg of monensin/kg DM+ 3.0 g of Advantage Confinamento®/100 kg of BW. [5]SEM – Standard error of means.

Table 3

Consumers habits of consumption (n = 150 consumers)

Frequency of consumption	Answers	% Beef consumers
Beef	1 time/month	2.0
	2 times/month	3.3
	1 time/week	12.0
	2–4 times/week	56.7
	> 5 times/week	26.0
Pork	1 time/month	17.4
	2 times/month	28.0
	1 time/week	37.1
	2–4 times/week	15.2
	> 5 times/week	2.3
Poultry	1 time/month	4.0
	2 times/month	10
	1 time/week	23.3
	2–4 times/week	48.0
	> 5 times/week	14.7
Lamb	1 time/month	75.0
	2 times/month	10.5
	1 time/week	10.5
	2–4 times/week	2.6
	> 5 times/week	1.4
Fish	1 time/month	67.7
	2 times/month	10.8
	1 time/week	13.8
	2–4 times/week	7.7
	> 5 times/week	0.0

Table 4

Consumer preferences and habits of purchase (n = 150 consumers)

	Answers	% Beef consumers
Place where buy beef	Butcher	31.3
	Supermarket	68.0
	Meat boutique	0.7
How do you prefer to buy beef?	Fresh cut	80.0
	Vacuum packed	6.0
	On tray	14.0
The most important factor when buy beef	Price	40.0
	Colour	52.0
	Animal age	1.3
	Breed	0.0
	Other	6.7
Which beef cattle category do you prefer?	Steers	13.4
	Bulls	2.0
	Heifers	19.3
	Cows	0.0
	Little importance	65.3
What system of beef cattle production do you prefer?	Pasture	43.3
	Feedlot	13.4
	Little importance	43.3
Would you eat the meat of an animal that was fed a diet supplemented with antibiotics?	Yes	70.0
	No	30.0

Table 5

Effect of additives inclusion on consumer acceptability of attributes of meat from crossbred bulls finished in feedlot aged for 1 and 7 days (n = 150 consumers)

Attributes	Diets					Ageing time			P-value		
	CONT[1]	MONE[2]	MO+VM[3]	MO+AD[4]	SEM[5]	1	7	SEM[5]	Diets, D	Ageing, A	D x A
Odour	6.59	6.47	6.46	6.33	0.050	6.39	6.53	0.048	0.121	0.098	0.060
Flavour	6.60	6.69	6.68	6.52	0.047	6.46	6.78	0.049	0.455	0.001	0.351
Tenderness	6.68ab	6.99a	6.70a	6.32b	0.061	6.33	7.02	0.053	0.001	0.001	0.258
Overall	6.70	6.74	6.73	6.50	0.053	6.47	6.87	0.047	0.242	0.001	0.211

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mg of monensin/kg of dry matter (DM); [3]MO+VM – Inclusion of 30 mg of monensin/kg of DM + 30 mg of virginiamycin/kg of DM; [4]MO + AD – Inclusion of 30 mg of monensin/kg DM + 3.0 g of Advantage Confinamento®/100 kg of BW. [5]SEM – Standard error of means; Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different ($P < 0.05$). Means followed by lowercase letters in the same row are different ($P < 0.05$). Means followed by uppercase letters in the same column are different ($P < 0.05$). Scale 1-9: 1 = dislike it extremely to 9 = like it extremely.

Table 6

Effect of additives inclusion and ageing time on visual appraisal of attributes of meat from crossbred bulls finished in feedlot during display time (n = 40 consumers)

Days	Diets				SEM[5]	P – value
	CONT[1]	MONE[2]	MO+VM[3]	MO+AD[4]		
1	6.44bA	7.11aA	7.06aA	6.86aA	0.053	0.001
3	6.54bA	6.96aA	6.37bA	6.95aA	0.051	0.001
5	6.45bA	6.92aA	6.25bA	6.95aA	0.052	0.001
7	5.57bB	5.96aB	5.25bB	5.96aB	0.063	0.001
9	4.52aB	4.27bC	4.00cC	4.52aC	0.071	0.001
11	3.98aC	3.70aC	3.07bD	4.05aC	0.065	0.001
13	3.31aC	2.59bD	2.66bD	3.20aD	0.060	0.001
15	2.25abD	2.13abD	1.98bE	2.43aD	0.051	0.016
SEM[5]	0.052	0.058	0.070	0.066		
P – value	0.001	0.001	0.001	0.001		

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mg of monensin/kg of dry matter (DM); [3]MO+VM – Inclusion of 30 mg of monensin/kg of DM + 30 mg of virginiamycin/kg of DM; [4]MO + AD – Inclusion of 30 mg of monensin/kg DM + 3.0 g of Advantage Confinamento®/100 kg of BW. [5]SEM – Standard error of means; Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different (P < 0.05). Means followed by lowercase letters in the same row are different (P < 0.05). Means followed by uppercase letters in the same column are different (P < 0.05). Scale 1-9: 1 = dislike it extremely to 9 = like it extremely.

Table 7

Regression analysis of the visual acceptability of meat from crossbred bulls finished in feedlot with or without additives inclusion

Diets	Random		P – value	R^2
	Days	Equation		
CONT[1]	9.37	$Y= -0.023x^2+0.055x+6.504$	0.001	0.982
MONE[2]	8.29	$Y= -0.011x^2-0.230x+7.665$	0.001	0.972
MO+VM[3]	6.77	$Y= -0.001x^2-0.370x+7.551$	0.001	0.988
MO+AD[4]	8.92	$Y= -0.013x^2-0.148x+7.358$	0.001	0.973

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mg of monensin/kg of dry matter (DM); [3]MO+VM – Inclusion of 30 mg of monensin/kg of DM+ 30 mg of virginiamycin/kg of DM; [4]MO + AD – Inclusion of 30 mg of monensin/kg DM+ 3.0 g of Advantage Confinamento®/100 kg of BW.

Table 8

Willingness to buy of meat from crossbred bulls finished in feedlot during display time (n = 40 consumers)

Days	Diets				SEM[5]	P – value
	CONT[1]	MONE[2]	MO+VM[3]	MO+AD[4]		
1	1.68bA	1.78aA	1.81aA	1.76aA	0.054	0.007
3	1.71bA	1.84aA	1.70bB	1.82aA	0.051	0.001
5	1.67bA	1.80aA	1.69bB	1.81aA	0.052	0.001
7	1.46bcB	1.58abB	1.37cC	1.68aA	0.063	0.001
9	1.48aB	1.21bC	1.22bD	1.30bB	0.071	0.001
11	1.26aC	1.12bCD	1.12bDE	1.27aBC	0.065	0.001
13	1.12aD	1.07abD	1.05bE	1.11abCD	0.061	0.009
15	1.03D	1.04D	1.04E	1.07D	0.051	0.288
SEM[5]	0.055	0.058	0.057	0.057		
P – value	0.001	0.001	0.001	0.001		

[1]CONT – No additives added; [2]MONE – Inclusion of 30 mg of monensin/kg of dry matter (DM); [3]MO+VM – Inclusion of 30 mg of monensin/kg of DM + 30 mg of virginiamycin/kg of DM; [4]MO + AD – Inclusion of 30 mg of monensin/kg DM + 3.0 g of Advantage Confinamento®/100 kg of BW. [5]SEM – Standard error of means; Means followed by lowercase letters in the same line are different. Means followed by capital letters in the same column are different Tukey's test ($P < 0.05$).

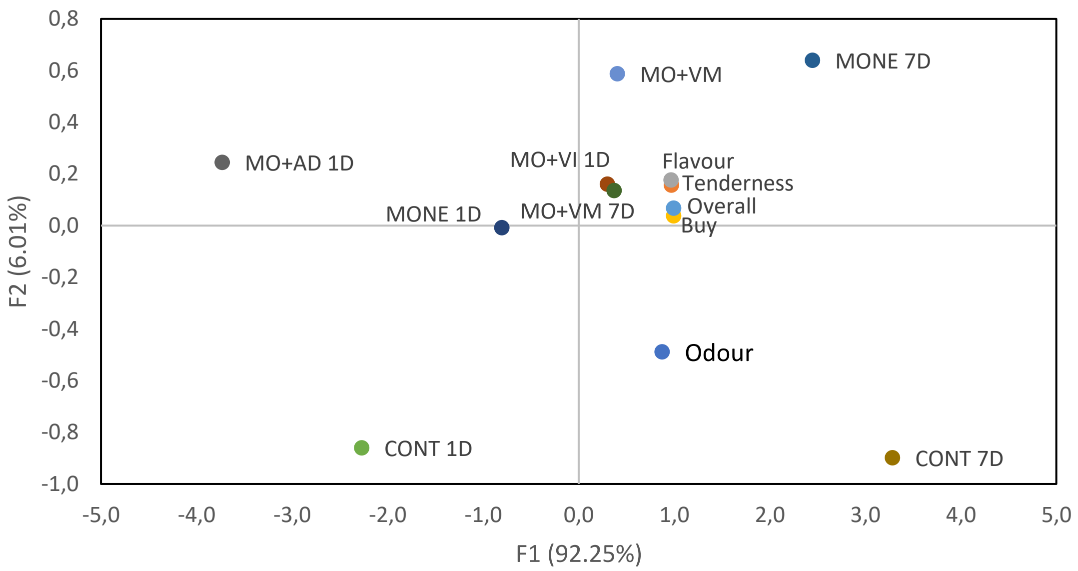

Figure 1

Principal component analysis of the scores for odour, flavour, tenderness, overall and willingness to buy acceptability of meat from crossbred bulls finished in feedlot during display aged 1 or 7 days. CONT – No additives added; MONE – Inclusion of 30 mg of monensin/kg of dry matter (DM); MO+VM – Inclusion of 30 mg of monensin/kg of DM + 30 mg of virginiamycin/kg of DM; MO + AD – Inclusion of 30 mg of monensin/kg DM + 3.0 g of Advantage Confinamento®/100 kg of BW.

CONSIDERAÇÕES FINAIS

Ionóforos e antibióticos, como monesina e virginiamicina são utilizados na suplementação de bovinos com o objetivo de melhorar o desempenho animal e eficiência alimentar, por sua capacidade de modular a fermentação ruminal. No entanto, mais recentemente, alguns países baniram o uso ionóforos na alimentação animal. Assim, alternativas são buscadas, mais uma vez, para substituir essas substâncias. Vários produtos considerados não invasivos à saúde estão sendo objetivo de estudo de pesquisadores como, por exemplo, extratos de própolis, extratos vegetais, óleos essenciais e, também, as leveduras e microminerais orgânicos. Estes estudos comprovam que a inclusão destes compostos naturais aumentam o desempenho, eficência alimentar e bem-estar animal. No entanto, além deste fatores importantes, torna-se necessário realizar estudos sobre os atributos intrinsícos e a percepção visual e sensorial da carne produzida com estes compostos naturais pelos consumidores. Na realidade, a inclusão destes compostos deveria, no mínimo, manter o tempo de prateleira próximo dos observados com o sistema atual de produção e não despertar rejeição pelos consumidores da carne bovina produzida com a adição destes novos compostos na dieta animal. Nesse sentido, foram testadas a adição de monensina, monensina + virginiamicina e monensina + uma mistura de microminerais orgânicos e levedura viva (*Saccharomyces cerevisiae*) sobre os atributos intrinsícos e a aceitabilidade da carne bovina pelos consumidores. No geral, a inclusão dos aditivos, durante 84 dias, na dieta de bovinos jovens terminados em confinamento, reduziu o pH e aumentou os valores de L*. Houve redução na perda por cozimento aos 14 dias na carne do tratamento MO+AD. A carne dos bovinos alimentados com as dietas MO+VM e MO+AD foi mais macia. A oxidação lipídica foi afetada apenas pelo tempo de maturação. Não foi encontrado resíduos de monensina e virginiamicina na carne. No teste sensorial, em que 150 consumidores provaram pedaços de carne de todos os tratamentos e avaliaram de acordo com sua preferência atribuindo notas para as características sensoriais, observou-se maior disposição de compra da carne do tratamento MO+AD. Além disso, a carne maturada por sete dias foi melhor aceita pelos consumidores. No teste de aceitabilidade visual, 40 consumidores receberam fotografias dos bifes, de forma aleatória, e também atribuíram notas sobre a coloração da carne. A inclusão dos aditivos não afetou a avaliação da cor até cinco dias de exposição. As notas atribuídas diminuíram com o tempo de exposição até o décimo quinto dia. Os aditivos, sobretudo a associação da monensina com uma mistura de microminerais orgânicos e levedura, apresentam completo potencial de ação, pois, apresentam efeitos sobre o produto final que vão refletir em melhor aceitabilidade pelo consumidor. Em conclusão, é possível substituir, na sua totalidade ou parte dela, os compostos antimicrobianos ainda usados na dieta de bovinos terminados em confinamento pela combinação destes com novos compostos que não tem rejeição pelos consumidores e conferem resultados semelhantes ou, até mesmo superior, em comparação aos compostos ou moléculas recorrentemente usados na terminação de bovinos em confinamento por um período de 90 dias.