THESIS

EVALUATING THE EFFECTS OF AN ESSENTIAL OIL FEED ADDITIVE ON PRODUCTION EFFICIENCY, CARCASS ATTRIBUTES, AND BEEF STEAK QUALITY IN FEEDLOT STEERS

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ABSTRACT

EVALUATING THE EFFECTS OF AN ESSENTIAL OIL FEED ADDITIVE ON PRODUCTION EFFICIENCY, CARCASS ATTRIBUTES, AND BEEF STEAK QUALITY IN FEEDLOT STEERS

The antimicrobial and antioxidant properties of essential oil (EO) present them as potential alternatives to conventional feed additives in feedlot cattle. Therefore, the objective of this research was to evaluate the effects of a dietary essential oil blend (EOB), administered alone or in combination with monensin (M) and tylosin (T), on finishing steer growth performance, carcass characteristics, and subsequent meat quality attributes of longissimus lumborum steaks. Four hundred cross-bred steers (initial BW 368.7 ± 11.0 kg) were utilized in a randomized complete block design and assigned to one of five dietary treatments: 1) Control (no additives); 2) EOB (3 g/d); 3) EOB+M+T; 4) M+T; or 5) EOB+M. Performance and carcass data were collected for all steers. Following harvest, two striploins per pen from a subset of pens (n=4/treatment) from the Control, EOB, and M+T treatments were collected, aged for 21 days, and analyzed for a suite of meat quality characteristics.

While the overall average daily gain (ADG) was similar ($P > 0.10$) among treatments for the entire feeding period, steers fed EOB-supplemented diets demonstrated periods of improved early growth. Steers fed Control, and EOB had greater ($P < 0.05$) overall dry matter intake (DMI) than those fed M+T. Feed efficiency was lowest ($P < 0.10$) for Control steers compared to all other treatments. Regarding carcass characteristics, steers supplemented with EOB had a greater

($P < 0.10$) dressing percentage (64.3%) compared to all other treatments. Conversely, the incidence of liver abscesses was lowest ($P < 0.10$) for steers fed M+T compared to Control, EOB, and EOB+M treatments. In the subsequent meat quality analysis, dietary treatments were similar ($P > 0.05$) on instrumental color, pH, lipid oxidation, metmyoglobin reducing activity, microbial populations, shear force, or fatty acid profiles of steaks aged for 21 days.

These results indicate that supplementing an EOB in finishing steer diets can improve early growth rates and dressing percentage, with feed conversion similar to ionophore-based treatments. However, the EOB did not reduce the incidence of liver abscesses. Furthermore, the EOB inclusion did not influence the quality or shelf-life characteristics of the final meat product. This suggests that essential oil blends may serve as a viable production enhancer in beef finishing systems without altering beef quality attributes. However, they may not fully replace the liver abscess control provided by tylosin.

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CHAPTER 1

Literature Review

Introduction

The beef industry faces a continuous challenge: balancing production efficiency and profitability with consumer demands for animal health and environmental responsibility. Antibiotics and ionophores, long-standing tools in feedlot cattle production, have come under scrutiny due to concerns about antibiotic resistance and potential negative environmental impacts. This growing pressure necessitates the exploration of alternative strategies.

Essential oil, a diverse group of plant-derived compounds, have emerged as promising candidates for replacing or supplementing traditional technologies. Their potential benefits are multifaceted, ranging from improved feed efficiency and the potential for reduced methane emissions to decreased liver abscesses (LA), a costly issue in feedlot cattle. However, the current research on EO presents a mixed picture, with some studies demonstrating positive effects while others show no significant differences compared to control groups.

This literature review delves into the existing body of research on EO in beef production. It will explore the characteristics of EO, their proposed mode of action, and the current state of scientific evidence regarding their effectiveness. By critically evaluating the strengths and weaknesses of current research, this review aims to identify knowledge gaps and pave the way for future studies.

Beef Production Systems

Beef products are a critical source of protein for human consumption and nutrition. The production of beef is a complex multi-tier supply chain from the birth of calves to the final production of retail products. In the United States, typically, there are three categories of beef producers: cow-calf operations, backgrounding operations, and feedlot operations.

Cow-calf operations specialize in raising breeding cows and their offspring. The calves are typically reared on their mother's milk until they attain a weight of approximately 200 kilograms. At this stage, the calves are weaned and sold to either backgrounding operations or directly to feedlot finishers. Backgrounding operations focus on grazing weaned calves for a period of one to three months, allowing them to acclimate to a forage-based diet. The number of backgrounded cattle in the U.S. varies from year to year, as backgrounding is a common practice in the beef industry. However, specific statistics for backgrounded cattle are not always reported separately. Instead, backgrounding falls under the broader category of beef cattle production, making it challenging to track exact numbers. Subsequently, these calves are sold to feedlot finishers, who intensively feed them grain-based diets to achieve slaughter weight, typically around 600 kilograms.

In the United States, in 2022, the calf crop was at 30,305,073 head from 622,162 cow/calf operations, with 7,889,279 of those calves coming from operations with 50 cows or less. In 2022, the National Agricultural Statistics Service (NASS) reported that there were 30,273 operations with animals on feed in the United States. In 2022, a total of 24,449,077 head were sold for slaughter (USDA - National Agricultural Statistics Service - 2022 Census of Agriculture - Volume 1, Chapter 1: U.S. National Level Data). Average feedlot capacity typically ranges in the thousands of head. However, some facilities significantly exceed this, with certain feedlots

housing over one hundred thousand cattle. Notably, the Five Rivers feedlot in Wellington, Arizona, once held a peak population of approximately 120,000 head. Moreover, the entire Five Rivers operation historically accommodated nearly one million head of cattle, emphasizing the potential for substantial animal concentrations within individual facilities (Ag Proud, 2024).

Cow-calf and feedlot operations are subject to high price variability caused by rapidly changing market conditions, such as weather factors that impact feed-cost changes, import competition, and competition from other protein sectors such as poultry, swine, and lamb, leading to extremely thin margins year over year. Intense market competition has compelled livestock producers to adopt innovative strategies to enhance production efficiency. Technological advancements, including steroid implants, growth-promoting antibiotics, and feed additives, have emerged as critical tools to optimize average daily gain (ADG), improve feed efficiency (FE), and reduce morbidity and mortality rates. These technologies are essential for maintaining profitability and ensuring the long-term sustainability of livestock production systems. Over the past five decades, the integration of these technologies into production management has become indispensable for industry success (Pulina et al., 2021).

Hormonal Implants

Since the USDA's initial approval of steroid implants for beef cattle in the 1950s, their utilization has become an integral component of beef production profitability. These implants can be used throughout the cattle lifecycle, from suckling calves to the finishing phase of beef production. In feedlots with less than 500 head, 41 percent implanted their cattle; in feedlots with between 1000 and 7,999 head, 73.9 percent implanted their cattle; and in feedlots with a capacity greater than 8,000 head, 89.8 percent implanted their cattle (USDA, 2011a). Underutilization of implanting programs could be a significant missed opportunity for producers as these programs

appear to offer the highest return on investment compared to other management tools (Mader, 1998).

Type of Implants

Anabolic steroids, often called hormone growth-promoting agents, have been used in the US since originally being approved by the FDA in 1954 and have proven safe and effective, although several countries do not allow the use of implants for various reasons. There are three basic categories of synthetic growth-promoting hormones used in all species of animals: estrogens, androgens, and progestins. Examples within the estrogen category include estradiol benzoate, approved in 1956; estradiol 17-beta, approved in 1991, and zeranol, approved in 1969. Within the category of androgens are compounds mimicking the growth-stimulating properties of testosterone. Trenbolone acetate (TBA), approved for use in implants since 1987, is the most commonly used androgen and has 10 to 50 times the anabolic activity of naturally occurring testosterone (Eng, 2000; Johnson et al., 2013; Beck et al., 2014). The third category of growth-promoting agents comprises progestins, which are synthetic hormones that mimic progesterone. Melengestrol acetate (MGA), approved by the FDA in 1968, is an example of a progestin employed in feedlot heifer management to suppress estrus and enhance growth efficiency (Johnson et al., 2013; Beck et al., 2022).

Given the approved use of hormonal implants throughout the bovine lifecycle, cattle destined for slaughter may receive multiple implantations prior to harvest. Implantation protocols vary but commonly involve between four and six applications, influencing growth performance and carcass characteristics (Mader, 1997). Due to the numerous combinations of the three primary compounds, producers have a complex set of choices regarding which drugs and reimplant combinations will lead to the most profitable outcome within their specific niche. Pre-

weaned calves, stockers, and finishing cattle all respond differently to different combinations of growth promotants.

Impact of Implants on Efficiency and Carcass Quality

Growth-promoting implants offer substantial economic returns to producers. Research indicates a favorable return on investment across various production stages. Previous research has shown that backgrounding programs can have (FE) gains of 5-10% and daily weight gains of 15-20% on moderate-energy diets. In finishing programs, where the cost of gain is critical, it's been reported that compared to non-implanted cattle, there is an 8% feed: gain (F: G) improvement, 6% greater feed intake, and 18% increase in ADG, but 14.5% fewer cattle are graded choice (Mader, 1998). An important consideration in finishing programs is the carry-over effect from previous implants from prior backgrounders on the animal's performance, with a large variation attributed to the choice of the sequence of previous implants (Duckett et al., 1996b; Mader, 1998).

While the evidence is overwhelming that implants have the potential to improve the profitability for producing gain and feed efficiency in cattle, it is important to acknowledge that implants have a potential impact on carcass quality, including marbling scores. The impact is complex and can vary depending on several factors, such as implant type, dosage, animal genetics, nutrition, and management practices. Lean et al. (2018) performed a meta-analysis on the impact of hormonal growth promotants on beef quality. This meta-analysis combined different combinations of growth-promoting compounds into one outcome. Utilizing 181 treatment comparisons, they observed that single implants only increased Warner-Bratzler shear force (WBSF), an indicator of meat tenderness, by 0.176 kg, and multiple implants increased

WBSF by 0.248 kg. In contrast to WBSF measurements, sensory panel evaluations did not indicate a negative association between hormone growth-promoting agents and meat tenderness.

Another meta-analysis from 2009 looked at 91 treatments from 51 different studies evaluating single implants versus non-implant controls (Wileman et al., 2009a). It showed an increase in average daily gain of 0.08 kg/day compared to controls in heifers, but there were no differences in (G: F) or dry matter intake (DMI) compared to nonimplanted controls. In steers, they reported a mean increase of 0.25 kg/d for ADG and an increase in DMI of 0.53 kg/d for implanted animals compared to non-implant controls, which led to a G: F improvement of 0.02. The same meta-analysis incorporated an economic model, estimating a $77/animal reduction in production costs for implanted animals compared to non-implanted controls fed identical rations (Wileman et al., 2009b). A study by Duckett et al. (1996) revealed the complexity of managing implants within the production value chain for the beef industry. When cattle were implanted, they showed an 18% increase in weight gain, a 6% increase in feed intake, and an 8% increase in feed efficiency compared to the non-implanted control group. However, this also led to a 14.5% decrease (from 74.0% to 59.5%) in the number of animals qualifying for the desirable "choice" grading. This decrease in marbling directly impacts market value and consumer preferences, highlighting the multifaceted nature of implant use in beef production (Duckett et al., 1996a).

Given the complexity of multiple combinations of steroid implants, opportunities for reimplanting at different stages of growth, and the difference in value in the finished carcass, responsible use of implants is crucial. Understanding the diverse effects of each compound type, adhering to withdrawal periods, and choosing implants based on individual animal needs and production goals are key to maximizing benefits while minimizing potential drawbacks.

Ionophores

Ionophores represent another tool for enhancing beef production profitability. These compounds function as ion transporters within cellular membranes. Li et al. (2022) reported that ionophores encompass both synthetic and natural compounds. These molecules exhibit antimicrobial and antiparasitic properties, demonstrating efficacy against fungal and viral pathogens. Some commercial products that highlight the diverse nature of this class of compounds include drugs such as amphotericin B, bedaquiline, and ivermectin, as well as the livestock products monensin and lasalocid (Li et al., 2022).

Ionophores are molecules produced by various strains of *Streptomyces*. These molecules, encompassing both natural and synthetic variants, possess a unique ability to transport ions across cell membranes. Their efficacy likely stems from their selective impact on bacterial populations and their ability to selectively modulate the rumen microbiome, influencing the composition and activity of various bacterial populations (Bergen and Bates, 1984; Li et al., 2022; Ekinci et al., 2023). Gram-positive bacteria, which rely heavily on substrate-level phosphorylation, are particularly susceptible to inhibition by ionophores, while gram-negative organisms with fumarate reductase can survive, leading to an enrichment of the gram-negative population in the rumen (Bergen and Bates, 1984). The ionophores, by selectively inhibiting the growth of gram-positive organisms, alter the ratio of volatile fatty acids (VFA) produced, the main source of energy for the ruminant, by changing the propionate to acetate ratio. Propionate is the only VFA capable of being converted to glucose in the ruminant. The other important VFAs, acetate and butyrate, have to be made by the breakdown of amino acids (Russell, 2002).

Monensin

Monensin sodium ($C_{36}H_{61}O_{11}Na$), a naturally occurring ionophore produced by the bacterium *Streptomyces cinnamonensis*, first discovered in 1967 (Agtarap et al., 1967; Haney and Hoehn, 1967), belongs to a broader class of carboxylic polyether ionophores, and was initially recognized for its efficacy in controlling coccidiosis in poultry. Monensin's potential for improving feed efficiency in ruminants was later identified in 1972 (Duffield et al., 2012). This discovery of monensin's benefit in ruminants led to its registration in December 1975 under the trade name Rumensin® (Elanco Products Co., Indianapolis, IN) for use in feedlot cattle. This marked a significant shift in the application of monensin sodium, highlighting its broader utility within the livestock industry (Tedeschi et al., 2003). The biologically active nature of monensin alters rumen fermentation patterns, leading to an increase in the molar proportion of propionic acid which leads to a decline in the molar proportion of acetate and butyrate. This change in the rumen microbial environment ultimately contributing to improved production characteristics (Haney and Hoehn, 1967; Perry et al., 1976; Boling et al., 1977).

A meta-analysis of 64 research papers, encompassing 169 individual trials, investigated the impact of monensin on FE in growing and finishing cattle (Duffield et al., 2012). This analysis revealed that monensin supplementation improved FE by reducing feed intake by 0.53 kg of feed per kg of body weight gain. Monensin also reduced DMI by 0.27 kg and increased ADG by 0.029 kg/d. There was considerable heterogeneity within the outcomes for FE. The main factor contributing to heterogeneity was the decade in which the study was done. Studies show a decline in FE improvement from monensin over time. In 1988, monensin led to an average improvement of 8.1%. By 2010, this benefit had decreased to a range of only 2.3% to 3.5%. This is thought to be the result of later studies having higher FE in the control group, and

not as much improvement was achieved on top of these already high FE numbers. This could also be due to other management factors impacting FE, particularly in US feed production (Duffield et al., 2012).

Lasalocid

Lasalocid was approved in 1976 under the trade name Avatec for use in poultry. Lasalocid, produced by *Streptomyces lasaliensis,* has a broader ion specificity than monensin and was approved for use in cattle to control coccidiosis disease in 1979 (Fitzgerald and Mansfield, 1979; Mahtal et al., 2020). Beyond controlling coccidia, lasalocid was approved to improve weight gain in grazing cattle in 1984 (Andersen and Horn, 1987; Ekinci et al., 2023). A meta-analysis conducted in 2016 using 31 studies with 61 comparisons showed that lasalocid improved ADG by 40 g/d and improved F: G by 410 g/kg in beef cattle (Golder and Lean, 2016). Fourteen studies with 25 comparisons showed that lasalocid increased hot carcass weight (HCW) by 4.73 kg but not dressing percentage, marbling score, or mean fat cover (Golder and Lean, 2016).

Out of more than 120 identified polyether carboxylic ionophores, only six, including monensin, have received approval for use in livestock. According to the USDA NAHMS report, 90.5 percent of feedlots include ionophores in their diets (USDA, 2011a). This statistic is consistent for all sizes and regions of feedlots (USDA, 2011a; Golder and Lean, 2016). In feedlot/finishing cattle where fermentable carbohydrates are normally fed as a large portion of the diet, ionophores generally depress feed intake, but ADG is not depressed; therefore, FE is improved. Mature cattle, including cows and stockers primarily consuming roughage diets rich in β-1,4-linked carbohydrates, exhibit increased ADG while maintaining dry matter intake. This combination results in improved FE. Other improvements that have been seen include a decrease

in protein degradation, leading to improved nitrogen metabolism and a decrease in feedlot disorders, especially lactic acidosis (chronic) and bloat (Raun et al., 1976; Bergen and Bates, 1984).

A 2016 survey by Samuelson et al. (2016) investigated feedlot nutritionist practices in operations housing over 1,000 head of cattle. The survey revealed that 91.5% of the feedlots utilized ionophores in their feeding regimen, with monensin being the primary choice (USDA, 2011b; Samuelson et al., 2016). A comprehensive Web of Science database search using "monensin" AND "beef" as keywords (May 10, 2024) identified 521 research articles. Due to the extensive body of research available on monensin, it has been established as a standard reference point for evaluating the effectiveness of new feed additives in beef cattle. Studies frequently compare new products to monensin rather than simply a control group without additives.

While monensin offers several benefits in cattle production, it's crucial to note its potential toxicity at high concentrations. Monensin's LD50 (lethal dose at which 50% of the population dies) falls between 20 and 80 mg/kg BW, significantly lower than the 50-150 mg/kg BW LD50 of lasalocid. Therefore, strict adherence to recommended dosages and responsible feeding practices is essential when using monensin to avoid potential adverse effects on animals (Ekinci et al., 2023).

Liver Abscess

One area that impacts beef producers' and packers' profitability in the United States is the loss of liver condemnation due to LA, as well as the decrease in the performance of cattle affected by this problem. The 2016 National Beef Quality Audit (NBQA) found that 17.8% of all carcasses sampled had LA (Eastwood et al., 2017). It is highly probable that the reported 17.8%

hides the real impact of liver problems in production from both a carcass value and a production inefficiency. Nagaraja et al. (1996) reported that feedlot incidence averaged 12% to 32%, but it might be as high as 90% or 95% in some operations (Nagaraja et al., 1996; Nagaraja and Lechtenberg, 2007). Underlying the reported LA rate in total is the stratification due to differences in cattle breeds. Holstein steers have a higher incidence rate, with rates as high as 40 to 60 % common (Amachawadi and Nagaraja, 2016a). This is higher than traditional beef breeds. Beef on Dairy (Beef X Dairy) cattle, a terminology used for crossing beef bulls with Holstein dairy cows to produce a beefier grade of dairy beef, is thought to be intermediate in liver condemnations between beef breeds and dairy breeds. In 2022, Lawrence et al. (2022) reported that Beef X Dairy had an LA incident rate of 39% versus 26% in native fed steers and 22% heifers. The same study reported that Canadian-origin cattle had about the same LA rate as the US at 25%, and Mexican-origin cattle had a lower rate at 17% (Lawrence, 2022). Due to numerous factors in the dairy industry, Beef X Dairy is increasing in the fed cattle population, with possibly as many as an additional 4 to 5 million more Beef X Dairy than five years ago headed to feedlots in the near future (Phelix, 2023). The higher frequency of LA in the Beef X Dairy concerns the packer and the producer, as the packer will pass the condemnation loss on to the producer's feedlots (Amachawadi and Nagaraja, 2016; Foraker et al., 2022).

The LA is primarily believed to be due to aggressive feeding programs utilizing a high level of grain concentrates versus forages, with an increasing percentage of livers affected by abscesses as the percentage of forage drops in the ration (Brent, 1976). In addition, it is believed that grains with a high level of starch fermentation contribute more to LA than slower-fermenting grains (Nagaraja and Lechtenberg, 2007). The rapid fermentation and increasing level of concentrates can lead to an increase in lactic acid-producing bacteria, leading to rumenitis,

allowing pyogenic microorganisms to gain access to the liver, most frequently by portal vein, but also via the hepatic artery, the bile duct, and by direct extension (Fulton et al., 1979; Nagaraja and Lechtenberg, 2007). While only detected at slaughter, liver abscesses are thought to occur and heal throughout the animal's lifetime, leaving small scars on the liver.

Liver abscesses are usually polymicrobial infections, with anaerobes being the most common organisms. The predominant etiologic agent is *Fusobacterium necrophorum,* a gram-negative anaerobic bacterium that uses lactate as a primary substrate (Nagaraja and Lechtenberg, 2007). In a study in 2017, researchers looking at the LA of crossbred cattle and Holsteins reported that 100% of the cultures grew *F. necrophorum*. The second most prevalent organism was a gram-positive, facultative anaerobe, *Trueperella pyogenes,* which was found in 49.8% of the cultured abscesses. *T pyogenes* is also often found in cattle infections, including mastitis, pyometra, arthritis, and foot abscess. The third most prevalent species was *Salmonella*, which brings up the human health aspect of LA (Amachawadi et al., 2017).

During post-mortem inspection at slaughter, liver abscesses are evaluated and classified using a scoring system that differentiates them by count and dimension. Although the scoring system lacks perfect consistency, the most frequently used grading scheme is:

0 = No abscesses are present. The liver is normal.

A- = One or two small abscesses or inactive abscess scars are present.

A = One to two large abscesses or multiple small abscesses. Some scoring systems describe this as two to four well-organized abscesses, less than 2.5 cm (1 inch) in diameter, that are present.

A+ = Multiple large abscesses. In another scoring system, one or more large, active abscesses or multiple small abscesses are present along with inflamed portions of the diaphragm, frequently adhered to the surface of the liver, and must be trimmed to separate the liver from the carcass.

A+AD = Liver adhered to the gastrointestinal tract or diaphragm, or both

A+OP = Open Liver abscess (Nagaraja et al., 1996; Brown and Lawrence, 2010)

Using the scoring system makes it possible to estimate losses due to LA. To maximize profits, producers aim for rapid cattle growth using high-energy diets. However, this strategy must be carefully managed to prevent health problems like LA that can negatively affect ADG and carcass quality. Ultimately, poor carcass quality translates to lower returns being passed on to producers.

Several studies have examined whether LA is associated with cattle performance. In a large study utilizing 3,570 yearling steers, investigators found an LA incidence rate of 16.35% but no difference in daily gains in steers with LA versus no LA (Harman et al., 1989). In a study published in 1975 (Brown et al., 1975) combining data from 6 different studies looking at LA in non-antibiotic-treated cattle, having an A+ graded LA reduced the weight gain by 12.7% while A- or A graded livers showed no detrimental effect on weight gain.

Carcass Loss Due to LA

The economic impact of LA on both producers and packers is substantial, primarily due to carcass and offal losses. These losses outweigh the effects on slaughter weight and feed efficiency. A recent study by Lawrence et al. (2022) quantified the economic consequences of LA in cattle. Using an average weight of 13.44 lbs. per liver (at $0.60/lb. for beef liver), LA would

result in a loss of $8.06 per incidence of liver condemnation. Adhesions cause a more significant loss to the animal carcass in A+ abscesses because it results in the loss of the outside skirt, which is currently worth $9.63 per lb. with an average weight of 7.2 lb. per animal, resulting in a loss of $69.33 per animal with skirt adherence. (USDA Agricultural Marketing Service, 2024). In addition to these losses per animal, animals with an A+ liver score on average had 31 fewer pounds of HCW, which includes 6.75 pounds of diaphragm loss. A 31-pound reduction in HCW at a current price of $2.98 per pound (USDA Livestock, Poultry & Grain Mkt News, 2024) results in a financial loss of $161.84 per affected animal. Additionally, open liver abscesses, which necessitate condemnation of the entire visceral mass (including liver, gallbladder, heart, lungs, trachea, esophagus, stomach, small intestine, large intestine, spleen, pancreas, and bladder), incur an estimated additional loss of $50 per animal (Lawrence, 2022)

It has been known since 1958 that antibiotics can decrease the incidence of LA in beef cattle. A database review (Lawrence, 2022) reported that cattle with no in-feed antibiotic control had 44.7% abscessed livers, whereas cattle fed with in-feed antibiotics had 12.8% abscessed livers. Six antibiotics have been approved in the US for preventing LA: bacitracin, methylene disalicylate, chlortetracycline (CTC), oxytetracycline, tylosin, and virginiamycin (Nagaraja and Lechtenberg, 2007). Of these, tylosin with the brand name Tylan (Elanco Animal Health, Greenfield, IN, United States) is the most used product. In 2011, tylosin was estimated to be used on 71.2% of all US feedlots (USDA, 2011b; Cazer et al., 2020a). Originally, tylosin, a macrolide antibiotic, was isolated in 1955 as a fermentation product of *Streptomyces fradiae*, and was used for the treatment of gram-positive bacteria in respiratory disease in poultry, such as *Mycoplasma gallisepticum* and swine dysentery (Palmer and Brown, 1967). Even though the main bacteria, *F.*

necrophorum, found in LA are gram-negative*,* tylosin has been found to reduce the incidence level of abscesses by 40% to 70% (Nagaraja and Lechtenberg, 2007).

An early study using 20 mg of CTC per kg of final feed demonstrated a decrease in overall condemned livers from 67% for controls to 54% for the treated animals (Wieser et al., 1966). Brown et al. (1975) compared the treatments of CTC and tylosin, compared to no treatment controls, for the prevention of LA in feedlot cattle using continuous treatment. There was a reduction in moderately abscessed (A) livers with LA rate 23% for CTC and 8.9% for tylosin compared to 28% for controls. The A+ livers, which are the class that causes the most economical performance and cutting floor loss, were 11.9% for CTC and 2.7% tylosin compared to 18.3% for controls (Brown et al., 1975). These authors also found that cattle with A+ livers had reduced gain of 12.7%, while cattle with A or A- graded livers had no detrimental effect on weight gains. In 1994, a summary of 40 trials showed a 73% reduction in LA due to tylosin use. In addition to the reduction in LA, tylosin-treated cattle gained 2.1% faster and had 2.6% more F: G than untreated control animals (Vogel and Laudert, 1994; Nagaraja and Lechtenberg, 2007). Recent studies across five U.S. regions have also confirmed tylosin's effectiveness in reducing LA. These studies found that tylosin decreased the LA prevalence from 11.6% in untreated cattle to 6.3% in those treated with tylosin (Weinroth et al., 2019).

Tylosin isn't the sole solution for reducing LA. A study compared the effects of increased roughage to tylosin on LA prevalence. While tylosin reduced LA from 19.2% to 13% in cattle fed a normal 7% cornstalk diet, increasing roughage to 13% or 19% cornstalks decreased LA to 11.8% and 14.4%, respectively. However, despite the benefit of reducing LA, higher roughage levels resulted in lower BW, ADG, and FE (Word et al., 2024).

While tylosin is effective in treating LA, it is in the family of macrolides, which are a medically important class of antibiotics for humans. Even though tylosin is not used in human medicine, other macrolides, such as azithromycin and erythromycin, treat *Campylobacter* and *Salmonella* infections in humans. Tylosin can select bacteria resistant to all macrolides because of the common resistant mechanism conferred by ribosomal methylation (Cazer et al., 2020b). The U.S. Food and Drug Administration's "judicious use" policy implemented in 2016 resulted in the withdrawal of the growth promotion label claims of tylosin in the United States (FDA, 2024a). However, tylosin is approved for continuous use in cattle at 60–90 mg/head/day to reduce the incidence of LA. (FDA, 2024b) In 2017, sales of medically important antibiotics, specifically macrolides, of which tylosin is the main antibiotic, were 274,479 kg for use in cattle. However, many people feel that tylosin may be at risk for future use in beef cattle production.

There has been an increasing and growing marketplace for meat produced with no-antibiotic-ever meat labels and organic meat production. Finding non-antibiotic alternatives to tylosin for LA control, as well as other modern technologies such as monensin and hormonal implant technology, is a high priority for many companies and researchers (Wileman et al., 2009b). One aspect of tylosin that is a difficult hurdle is its extremely low cost for the producer. Using Tylan for the 180- to 200-day finishing period costs between $2.00 and 2.40, or around $0.01 to $0.015 per head per day. Other additives, such as yeast culture, cost much more and are often $0.05 to $0.10 per head per day. So, finding a product that is less expensive than tylosin and shows the same level of effectiveness is a rigorous research path and a big commercial opportunity.

Essential oil

One alternative candidate product for replacing tylosin and other production technologies is essential oil (EO). Essential oil were used in medicine for centuries before modern science. The term was originally derived from "*Quinta essential,*" coined by Paracelsus von Hobenheim 1443-1541, a Swiss medical pioneer, although now we understand EO are not actually essential for nutrition or oils but are oily in appearance (Burt, 2004; Hart et al., 2008).

Essential oil are a category of compounds derived from plants, produced as secondary metabolites (not involved in plant production or reproduction) and often involved in plant protection. The EO are typically extracted from plant material by the process of steam distillation (Hart et al., 2008). Structurally, EO can be classified as alcohol, ester, or aldehyde derivatives (Greathead, 2003). The plant might produce the compounds in the roots, flowers, twigs, bark, seeds, petals, leaves, fruit, or stems (Burt, 2004; Hart et al., 2008). The concentration level within the plant varies with the stage or speed of plant growth, maturity, plant health, plant injury, the circadian clock, and moisture or is regulated by daylight (Dudareva et al., 2004; Benchaar et al., 2008).

Essential oil are aromatic hydrophobic liquids, which are thought to allow them to penetrate the lipid layer of bacteria and accumulate in the lipidic bilayer of the cell. This disturbs the cell's osmotic pressure (Florou-Paneri et al., 2019) leading to a breakdown in the membrane integrity and ion transport. Once sufficient leakage has occurred, the cell will collapse, causing cell death, or the energy expended to increase the ion pumps causes a slowing of the growth, causing a shift in the bacterial population, such as increasing propionate versus acetate producers (Griffin et al., 1999; Burt, 2004; Akram et al., 2021)

In 1958, researchers, after testing 45 different oils and 95 combinations of oils, found cinnamon, origanum red, and eucalyptus to have the greatest antibacterial activity in vitro (Maruzzella and Henry, 1958). They found that the antibacterial action was greater against gram-positive bacteria than against gram-negative (Chao et al., 2000). Gram-negative bacteria possess a complex outer wall with both hydrophilic and hydrophobic properties, making them more resistant to the penetration of EO. However, EO rich in terpenoids and phenolic compounds, such as those found in thyme and oregano, including carvacrol, have demonstrated effectiveness against bacteria like *Salmonella and E. coli* in laboratory conditions. (Burt, 2004; Peñalver et al., 2005; Akram et al., 2021).

Many EO compounds have been studied in vitro for their antimicrobial effect and effect on rumen fermentation. The main plant-derived EO compounds that have been investigated for showing antibacterial impact are cilantro, coriander, cinnamon, oregano, rosemary, sage, clove, and thyme (Burt, 2004; Cobellis et al., 2016). One goal of EO use in beef production is to utilize the antibacterial properties of EO compounds to manipulate the rumen fermentation microbial ecology (Calsamiglia et al., 2007a). Modifying the microbial population to favor propionate production versus acetate would increase the energy derived from rations. In addition, reducing methane production and increasing the efficiency of protein utilization would improve production and have a beneficial environmental effect (Calsamiglia et al., 2007b; He et al., 2015).

The EO mode of action is considered broad, as many molecules are present within many EO compounds. This may also vary depending on the source of the compound. For instance, the common name oregano is not specific to the species it represents. Oregano is known for its antimicrobial, antioxidant, and anti-inflammatory properties (Zamuner et al., 2023). Oregano's

antibacterial activity is likely due to a synergistic effect between thymol and carvacrol rather than the action of a single molecule. Thymol is a potent antibacterial agent, and its effectiveness is amplified when combined with carvacrol, as found in oregano. The source, though, alters the makeup of the compound. For example, oregano from Alamos, Mexico, contained 22.37% p-cymene, 21.39% thymol, 6.69% g-terpinene, and 16.7% iso-aromandrene, while oregano from Sonora, Mexico, contained 14.25% p-cymene, 15.11% thymol, 4.23% g-terpinene, and 0.62% iso-aromandrene. Oregano from Tunisia contained 46% p-cymene, 18% thymol, 16% g-terpinene, and 15% carvacrol (Rodriguez-Garcia et al., 2016). Research has demonstrated that oregano, with varying concentrations of carvacrol and thymol, inhibited a wide range of pathogens, including *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, and many others. This evidence suggests that oregano's antibacterial power stems primarily from the combined action of thymol and carvacrol, as well as the ratio of their mix, rather than from either compound alone (Lambert et al., 2001; Rodriguez-Garcia et al., 2016).

EO as a Feed Additive for Beef Cattle

Several research trials have been undertaken to test the effectiveness of EO on beef production. One of the earlier trials occurred in 2009, utilizing 468 crossbred yearling steers in 5 treatments with 10 pens per treatment. The researchers tested two products: an EO product (EOM) containing thymol, eugenol, vanillin, guaiacol, and limonene, and an experimental product (EXP) containing guaiacol, linalool, and alpha-pinene. They were compared against a diet of monensin and tylosin, EO and tylosin, and a control with no additive. The experiments were conducted both in vivo and in vitro. In the in *vivo* experiments, there were no significant changes for the treatment compared to the control, except a decrease in DMI (11.4 kg/d for monensin tylosin versus 12.1 kg/d for the control) and G: F (0.153 for the EOM and tylosin,

0.156 for the tylosin versus 0.145 for the control treatment). The only significant change in carcass characteristics was an improvement in yield grade for the EOM and tylosin treatment (2.7) compared to the control (2.1). The EOM treatment reduced the total number of LA by 11.6%. When combined with tylosin, the results were even more pronounced, with a 20% decrease in total abscesses. The tylosin monensin treatment showed significant results, with a 22.2% decrease in total abscesses, all of which were A- (1 or 2 small abscesses or abscess scars). In contrast, the control group had 8.9% A+ abscesses (one or more large or active abscesses, with or without adhesions), while the EOM group had 6.9%, the EXP group had 9.2%, and the EOM + tylosin group had 2.9%. There were no significant changes seen in fermentation and digestion patterns within the experimental model. However, the fistulated cattle had depressed intake patterns, which could account for the results even seen in the treatment, including monensin, which typically reduces intake and acetate to propionate ratios (Meyer et al., 2009).

A meta-analysis (Khiaosa-ard and Zebeli, 2013) examined the effects of EO on performance and rumen fermentation in dairy, small ruminants, and beef cattle. While they observed a decrease in methane production, potentially beneficial for performance, they attributed this to a reduced acetate-to-propionate ratio. Increased propionate is linked to better ADG in beef cattle. However, due to limited studies, they could not report performance data for beef. The analysis did reveal improved milk protein content and percentage in dairy cattle, possibly resulting from the decreased acetate-to-propionate ratio. A more recent study (Torres et al., 2021) compared EO against diets containing monensin on beef performance values and hepatic abscess formation. Using ten peer-reviewed publications with 27 different treatments, they found no significant change in BW, carcass weight, ADG, and FE with EO supplementation compared to monensin treatment. In addition, there was a significant increase in the EO carcass

dressing percentage, subcutaneous fat thickness, and ribeye area. Although the use of EO in this study looked promising, when the LA rate was assessed, it suggested a problem compared to monensin. The percentage of cattle with LA increased by 84.9% when monensin was replaced with EO, and the risk ratio increased from 1.07 to 3.97 (P=0.28).

The most recent meta-analysis covers papers from 2010 to 2023 and analyzed a blend of various EO, primarily consisting of cinnamaldehyde, eugenol, thymol, capsaicin, and anethol. These studies were done in 10 different countries, and experimental designs categorically were divided into EO dose (≤400 mg/kg and >400 mg/kg), experimental period (≤90 days and >90 days), and diet concentrate level (≤70% and >70%). These subgroups were explored to determine the impact of the weighted mean difference (WMD). The meta-analysis found improved DMI, final body weight (FBW), HCW, FE, and *longissimus dorsi* muscle area, but no difference in digestibility, all of which had a high level of heterogeneity except FE. Utilizing subgroup analysis, the authors reported no differences in the primary active compound (EO) used in the trials on any parameters. The main subgroups impacting the differences in WMD for utilizing EO found in the heterogeneity testing were that the essential oil dose impacted DMI, backfat thickness, total VFAs, and acetate level. The length of the supplementation period also impacted FBW and ADG, while the diet concentrate level impacted DMI, FBW, and ADG (Orzuna-Orzuna et al., 2022).

Many things might contribute to the inconsistent results with EO supplementation, such as the varying composition of the compounds in the sample EO, differences in pH of the rumen compared to in vitro applications, the impact of rumen bacteria on the breakdown of the EO, the impact of the EO on DMI, impacts on other ration digestibility and adaptation of the rumen bacteria over a longer feeding period than would happen in an in *vitro* setting (Burt, 2004;

Beauchemin and McGinn, 2006; Akram et al., 2021). There may also be an interaction with other feeding protocols such as hormonal implants, monensin, or antibiotics such as tylosin in the ration.

Ideally, due to the change in acceptance of synthetic technologies in beef production, finding a natural EO that would replace some or all of the other technologies, such as tylosin or monensin, would be highly desirable. Recently, EO compounds such as cinnamon, oregano, and thyme have consistently shown antibacterial activity in vitro. In *vivo,* results are much more inconsistent (Burt, 2004; Akram et al., 2021). The purpose of this trial is to evaluate the impact of an EO containing oregano, thyme, cinnamon, and Actifibe prebiotic (Prosper EO, Ralco Agriculture, Marshall, MN; PEO) on feedlot performance and carcass characteristics compared to monensin and tylosin supplementation.

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CHAPTER 2: An Essential Oil Blend Fed To Feedlot Steers On Growth Performance and Carcass Characteristics

Introduction

Antibiotics are known to reduce mortality, morbidity, and liver abscesses in finishing feedlot cattle (Scott et al., 2017). However, antibiotic resistance concerns have resulted in the reduction/elimination of the sub-therapeutic feeding to livestock to prevent diseases (Russell et al., 2003; Salazar et al., 2019, Cangiano et al., 2020). Natural ingredients/products/additives are actively being studied as stand-alone or combination alternatives to antibiotics for use as growth promoters to maintain or improve growth performance while reassuring the public that animal health is not being compromised (Kowalski et al., 2009; Reddy et al., 2020; Arne and IIgaza, 2021). Many of these additives can be antimicrobial, antiviral, antifungal, and antioxidant (Rivaroli et al., 2016; Froehlich et al., 2020, Swedzinski et al., 2020).

A viable alternative option to the use of antibiotics may be essential oil (EO), which inhibits rumen Gram-positive bacteria more than Gram-negative *Escherichia coli* growth through alteration of electron transport chains (Marino et al., 2001; Nazzaro et al., 2013). Essential oil is the distillation of extracts of plant metabolites, also known as plant essence (Calsamiglia et al., 2007; Benchaar et al., 2008; Frohlich et al., 2017). Miguel et al. (2010) reported that EO demonstrated antibacterial, anti-viral, anti-fungal, insecticidal, and herbicidal activities by causing conformation changes in the cell membrane to become less impermeable (Calsamiglia et al., 2007; Benchaar et al., 2008) along with having antioxidant characteristics (Burt et al., 2005, Lejonklev et al., 2016, Paraskevakis, 2018).

A unique proprietary EO blend (EOB; Aspire; Ralco Inc., Marshall, MN) consisting of cinnamaldehyde, oregano, and thymol has been developed based upon innovative product

research and development. Cinnamaldehyde, another potent and often used EO, demonstrates added antibacterial properties by inhibiting protein binding and enzymatic activity of glucose-producing bacteria. This stops the use of energy for bacterial growth and keeps that energy for efficient production (Wendakoon and Sakaguchi, 1995).

Oregano demonstrates strong antimicrobial and antioxidant activities due to carvacrol and/or thymol (Burt et al., 2005, Lejonklev et al., 2016, Paraskevakis, 2018), while Bampidis et al. (2006) reported that oregano may be as effective as neomycin due to their phenolic structures composed of oxygenated cyclic hydrocarbons (Burt et al., 2005, Lejonklev et al., 2016, Paraskevakis, 2018). Ultee et al., (2002) showed that these hydroxyl groups of phenols may provide ionophore-like properties due to the transmembrane carrier of monovalent cations and protons. Thymol, a phenolic compound, has been shown to reduce ammonia N concentrations and increase amino acids *in vitro,* inhibiting deamination (Borchers, 1965, Broderick and Balthrop, 1979).

Additionally, thymol has strong antimicrobial properties against both gram-positive and negative bacteria as it can permeate membranes (Dorman and Deans, 2000). Thymol in combination with other EO has been shown to increase total VFA production when measured *in vitro* (Castillejos et al., 2008).

A limited number of *in vivo* studies have evaluated various EO products for efficacy in improving livestock performance and rumen microbial fermentation by feedlot cattle (Meyer et al., 2009; Pukrop et al., 2019; Araujo et al., 2019). There are more than 1500 known EO with several that may have benefits for feeding livestock (Florou-Paneri et al., 2019). Certain characteristics of specific EO may be able to replace growth-promoting products (i.e., ionophores) and reduce antibiotic usage in the feedlot, and are being researched. However, there

is a paucity of literature evaluating the long-term *in vivo* feeding of EO on growth performance (Kolling et al., 2018). In vitro data by Zhou et al. (2020) reported that the addition of an oregano/cobalt lactate blend shifted ruminal fermentation to greater volatile fatty acid (VFA) concentrations with a greater molar percentage of propionate, combined with a 16% reduction in methane output. Calsamiglia et al., (2007) demonstrated that certain EO, in singular use, reduce ammonia N, methane, and the VFA acetate and may result in higher levels of propionate and butyrate concentrations. These ruminal fermentation shifts would be similar to shifting ruminal fermentation with the ionophore monensin (Burt et al., 2005; Duffield et al., 2012; Schären et al., 2017; Menezes et al., 2022).

The experimental hypothesis is that a proprietary EOB, used as a natural feed additive, can serve as an antibiotic alternative to monensin and tylosin, achieving improved growth performance, feed conversion, and carcass characteristics while reducing liver abscesses.

Materials and Methods

Animal and Receiving Procedures

Before the initiation of this experiment, all animal care, handling, and procedures described herein were approved by the Colorado State University Institutional Animal Care and Use Committee (Approval # 3824). The steers were managed, cared for, and fed following the guidelines published in the 4th edition "Guide for the Care and Use of Agricultural Animals in Research and Teaching" published by ADSA-ASAS-PSA (2020).

Four hundred and fifty-two crossbred steers (initial BW 812.6 ± 23.8 lbs.) were utilized from 2 separate cattle sources. Each group was transported to the Colorado State University Agriculture, Research, Development, and Education Center (ARDEC) in Fort Collins, Colorado. After arrival, steers were individually weighed, identified with a unique ear tag, and breed type

was assigned to each steer based on hair color and phenotype (red, black, or black-white face, etc.), and a frame score was assigned to each steer. Each steer was vaccinated with Bovishield Gold (Zoetis, Parsippany, NJ) and 7-Way Ultrachoice (Zoetis, Parsippany, NJ), given Noromectin (NorBrook Labs, Lenexa, KS), drenched with Synanthic (Boehringer Ingelheim, St. Joseph, MO) for parasite control, and implanted with Revalor – XS (200 mg Trenbolone Acetate and 40 mg Estradiol, Merck Animal Health, DeSoto, KS). Following initial BW weighing and processing, steers were provided *ad libitum* access to long-stem grass hay and water and were housed (10 animals per pen) overnight.

Randomization of steers in this experiment was like that described by (Caldera et al., 2017 and Budde et al., 2019). Briefly, steers were ranked by BW within cattle source, and individuals whose BW was ± 2 SD from the mean BW were eliminated from further consideration. Steers exhibiting white coat color or those found to be bulls, heifers, or displaying symptoms of illness or lameness were excluded. The remaining steers were blocked by BW and assigned a random number from 1 to 1,000 within a block using the random number function in Excel 2007 (Microsoft Corporation, Redmond, WA). Steers with the lowest random numbers were eliminated from the experiment, reducing the number of remaining steers to 400. The 400 eligible steers were ranked by BW within cattle source and divided into 8 BW block replicates, each consisting of 50 steers. Within each BW block replicate, steers were ranked by BW and randomly assigned to 1 of 5 pens. By following this randomization schedule, 8 BW block replicates with 2 cattle sources, each containing 5 pens with 10 steers per pen, were assembled for each of the 5 treatments in the experiment. On day 0, steers were individually weighed prior to being fed, visual ear tags identifying treatment and animal ID within a pen replicate were

inserted into the right ear of each steer, cattle were placed into their respective treatment pens, and the experiment was initiated.

Pens were checked daily to ensure that the cattle were in the correct assigned pen, that all cattle had *ad libitum* access to feed and water, and that all gates were secure. In addition, all cattle were monitored daily for illness and lameness. Steers exhibiting symptoms of distress were removed from the pen, and rectal body temperatures were recorded. Steers with rectal body temperatures greater than 39.4°C were treated utilizing the appropriate treatment protocols and immediately returned to their home pen. If illness or lameness persisted in a specific steer, the steer was removed from the experiment. If a steer was removed from the experiment, the steer was weighed, the feed in the feed bunk was weighed and placed back into the feed bunk, a feed sample was obtained for DM determination, and the feed delivery was adjusted accordingly for that pen the next day.

Rations

Steers were fed a series of step-up rations for adjusting to the final finishing ration based on steam-flaked corn (Table 1). Ration changes during the step-up program were simultaneous across all treatments, and cattle reached the finishing diet by d 29 of the experiment. Finishing rations were formulated to meet or exceed NASEM (2016) nutrient requirements for growing and finishing beef cattle. Each experimental ration was delivered once daily in the morning (0700h) in amounts to allow cattle *ad libitum* access to feed for a 24 h period. During inclement weather or when excessive amounts of feed remained in the feed bunk, feed was removed from the feed bunks, weighed, and subsampled. Subsamples were analyzed for DM and used to calculate the DM weight of the orts for a given week. This value was then subtracted from the

total DM delivered to a given pen of cattle to calculate dry matter intake (DMI) for a given period.

Feed samples of each experimental ration were collected weekly and stored at -20°C. At the end of each month, weekly treatment samples were subsampled and composited into monthly composites for use in ration analysis. The experimental treatments consisted of: 1) Control: no added EOB, M, or T; 2) EOB: EOB was added to the Control ration at 3 g/d to provide 0.30 g/d of the proprietary EOB (cinnamaldehyde, oregano, and thymol; Ralco Nutrition, Marshall, MN); 3) EOB+M+T: the control ration containing EOB, M, and T to provide 3 g/d of EOB, 38.5 g/metric ton of M (Monovet, Huvepharma Inc., Peachtree, GA), 7.7 g/metric ton of T (Tylovet, Huvepharma Inc., Peachtree, GA); 4) R+T: the control ration containing M and T at the same inclusion rates; and 5) EOB+M: the control ration containing EOB and M at the same inclusion rates. The experimental treatments were blended into equal amounts of wheat midds and soy hulls, fat, and the respective feed additives and pelleted (Ralco Nutrition, Marshall, MN) that were blended into the TMR at mixing time.

Weighing and Carcass Data Collection

Steers were weighed individually on d -1 and 0, every 28 d, and on 2 consecutive days prior to slaughter. Equal numbers of pen replicates per treatment were transported to a commercial abattoir (JBS USA, Greeley, CO) on days 160, 174, and 182. Carcass data and liver (Brink et al., 1990; Hicks, 2014; Elanco, 2019) and lung score (Bryant et al., 1999; Schneider et al., 2009) were collected by Diamond T Livestock Services Inc. (Yuma, CO). Hot carcass weights (HCW) were determined at the time of slaughter, and carcasses were chilled for approximately 36 h before carcass data were obtained. Carcass data collected included dressing

percentage (DP), LM area, adjusted subcutaneous adipose tissue thickness (USDA, 1989), KPH, marbling score, quality grade, and calculated USDA yield grade (YG).

Feed Analysis

At the end of the experiment, feed samples (TMR, concentrate mix, and individual ingredients) were thawed and composited by month. The monthly composited experimental TMR samples were sent to SDK Laboratories, Inc. (Hutchinson, KS) for DM and nutrient analysis and subsequent DMI calculations. Feed samples were analyzed for nutrient concentrations following standard AOAC International methods (2019): DM (935.29), crude protein (CP**;** 990.03), non-protein nitrogen, i.e. urea (967.07), neutral detergent fiber (NDF) with amylase (2002.04), acid detergent fiber (ADF; 973.18), Ca (985.01), P (985.01), Mg (985.01), K (985.01), S (923.01), Fe (985.01), Cu (985.01), Zn (985.01), Mn (985.01), Mo (996.16), and Se (996.15). The NE_m and NE_g concentrations were calculated according to NASEM (2016) equations.

Statistical Analysis

All data were checked for normality and outliers using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) before any statistical analyses were conducted. The box and whisker plots and the Shapiro-Wilk Test were used to verify that the remaining data were normally distributed ($P > 0.15$). Data points exceeding 3 standard deviations above or below the mean were removed from the data set prior to analysis. Body weights (initial, interim, and final) were assessed on a pen mean BW basis using a restricted maximum likelihood-based, mixed-effects model, repeated-measures analysis (PROC MIXED; SAS Institute Inc., Cary, NC) for a randomized complete block design (RCBD; Steel and Torrie, 1980) having 5 treatments. The statistical model analyzed was: $Y_{ijk} = \mu + Block_i + T_j + Day_k + (T_j \times Day_k) + e_{ijk}$

Where Y_{ijk} = dependent variable, μ = overall mean, $Block_i$ = block $_{(i=1 \text{ to } 8)}$, T_j = treatment $_{(j=1 \text{ to } 5)}$, Day_k = day $_{(k= 0, 28, 56, 84, 112, 140, \text{ and } 168)}$, T_j x Day_k = interaction of treatment by day, and e_{ijk} = residual random error. Pen was the experimental unit, and cattle source (1 or 2) was found to be nonsignificant ($P > 0.50$) and excluded from the model. Treatment, day, and the interaction of treatment by day were considered fixed effects, while block was considered random. The experimental day was considered a repeated measurement in time, having an autoregressive covariance structure. When the F-test for treatment was determined significant ($P < 0.15$), least squares adjusted means were separated by the PDIFF statement, which tests least squares means using the least significant difference method (LSD). Differences among treatments were considered significant at $P < 0.05$ and trends at $0.05 < P \leq 0.10$.

Results and Discussion

Ration Nutrient Composition

The analyzed TMR nutrient compositions of starter, step 1, step 2, and finisher are given in Table 2. A statistical analysis of the experimental finisher TMR samples collected during the course of the experiment indicated no significant differences ($P > 0.75$) among the major nutrients, i.e, DM, CP, etc.; therefore, these values were averaged across all TMR finisher samples to reduce the table size. The addition of the experimental pellet containing the experimental treatments resulted in similar ($P > 0.10$) nutrient compositions across treatments. The nutrient composition would be expected to meet or exceed the nutrient requirements of growing beef steers (NASEM, 2016).

Feedlot Performance

Overall morbidity rates throughout the experiment were low. Twelve steers (2 Control; 7 EOB; 1 EOB+M+T, 0 M+T; and 2 EOB+M) and their individual data were excluded from data

analysis for reasons unrelated to treatment (death, chronic bloat, excessive lameness, etc.). Five steers were treated for respiratory disease and recovered, with data used for statistical analysis.

The treatment by time interaction was nonsignificant ($P > 0.15$) for growth performance. As expected, time was a significant source of variation ($P < 0.01$) for growth performance parameters, but it will not be discussed further (Table 3). Initial, final, and 28 d BW were similar ($P > 0.10$) for steers fed all treatments. Several studies have been reported evaluating similar and different EO fed to growing feedlot steers. Meyer et al., (2009) reported that cattle fed an EO blend (thymol, eugenol, vanillin, guaiacol & limonene) demonstrated similar BW gains compared with those fed control or monensin plus tylosin. Three recently reported studies (Rivaroli et al., 2017; Pukrop et al., 2019; Araujo et al., 2019) reported similar BW for feedlot bulls or steers when fed EO based on a blend of oregano, garlic, lemon, rosemary, thyme, eucalyptus, and orange, a proprietary blend, or a carvacrol, cinnamaldehyde, and capsaicin blend. In contrast, Valero et al., (2014) reported improved BW gains when feedlot bulls were fed an EO blend of ricinoleic acid, anacardic acid, cardanol, and cardol compared with steers fed without EO (i.e. control). Thus, growth performances can be obtained when feeding an EO blend that contains the appropriate EO, but more research work is needed to better define the individual EO types and amounts to include in a blend.

The overall experimental ADG was similar ($P > 0.10$) for steers fed all treatments (Table 3). However, during the 0 – 28 d period, steers fed EOB+M demonstrated greater ($P < 0.10$) ADG compared with steers fed the remaining treatments. After the 2nd weighing period of 0 – 56 d, steers fed EOB demonstrated greater ($P < 0.10$) ADG compared with steers fed EOB+M+T and M+T, with steers fed Control and EOB+M intermediately being similar or different. After the 3rd weighing period of the 0 - 84 d, steers fed Control and EOB demonstrated greater ($P <$

0.10) ADG compared with steers fed the remaining treatments. These data demonstrate that feeding EOB improved early feedlot growth rates, but after 84 d, there appears to be little benefit to feeding EOB. Given that EOB characteristics are speculated to improve feedlot steer performance during receiving and early feedlot phases by reducing stress, disease challenges, and getting steers on feed. Several studies (Benchaar et. al., 2005; Pukrop et al., 2019; Araujo et al., 2019, Meyer et al. 2009 and Rivaroli et al. 2017) using various EO products have reported no improvement during the early phase or overall feedlot ADG. In contrast, Valero et al. (2014) reported an ADG improvement when feeding an EO product to feedlot bulls, while Chaves et al. (2008) reported that cinnamaldehyde can increase ADG in small ruminants. Given the large differences among the various EO blends reported, it is difficult to summarize performance responses across experiments.

Feed Intake and Efficiency

Except during the 0 – 28 d period, the subsequent five 28-d periods, and overall experimental DMI were greater ($P < 0.10$) for steers fed Control compared with steers fed R+T, consuming the lesser DMI, with steers fed the remaining treatments being intermediate and either similar or different (Table 3). During the first 0-28 d period, steers fed EOB+M demonstrated greater DMI compared with steers fed the remaining treatments. The speculation is that the combination of EOB as a flavor with M could get receiving steers on feed sooner, thereby reducing stress and sick pulls. A reduction in DMI is commonly observed with the addition of monensin (Duffield et al., 2012) and tylosin (Potter et al., 1985) in the ration. Araujo et al. (2019) reported that steers fed a combination of EO and M demonstrated a trend for lower DMI compared with steers fed M+T. In agreement, Meyer et al. (2009) reported that an EO with M or with T decreased DMI. These 2 studies may suggest an antagonism between certain EO and

M or T reducing DMI. Several studies (Valero et al., 2014; Pukrop et al., 2019; Rivaroli et al., 2017) have reported similar DMI among treatments when feedlot steers or bulls were fed various EO blends.

Feed efficiency for the time periods 0-112 d, 0-140 d, 0-168 d, and overall experimental feed efficiency was lowest for steers fed Control compared with steers fed the remaining treatments. The improvement in feed efficiency was similar among the feed additives EOB, M, and T when fed alone or in combination. Duffield et al. (2012) in their meta-analysis reported that monensin improves feed efficiency by shifting ruminal fermentation to increase propionate supply, inhibiting Gram-positive ruminal bacteria, and reducing bloat and acidosis. Zhou et al. (2020) reported a shift in ruminal fermentation to more propionate when supplementing EO (similar EO composition). Thus, feeding steers EOB can shift ruminal fermentation to increase propionate supply to improve feed efficiency compared with steers fed Control. In contrast, Schären et al. (2017) reported that feeding a different EO blend did not improve ruminal propionate concentrations. The literature is not consistent on EO improving propionate concentrations and feed efficiency, with some studies reporting improvements (Valero et al., 2014; Meyer et al., 2009; Meschiatti et al., 2019), while other studies reported no improvements (Rivaroli et al. 2017; Araujo et al., 2019; Pukrop et al., 2019). Thus, DMI and feed efficiency improvements could be obtained if the specific EO blend contains the appropriate individual EO, but more research work is needed to better define the individual EO types and amounts to include in a blend.

Carcass Measurements

The steers were harvested in 3 separate lots at 160, 174, and 182 d based on reaching a pre-set body condition score with equal reps across treatments being harvested at the same time.

Hot carcass weights were similar ($P > 0.10$) for steers fed all treatments (Table 4). However, steers fed EOB demonstrated greater dressing percentages compared with steers fed the remaining treatments. The increase in dressing percentage for steers fed EOB resulted in an additional 13 kg of retail carcass weight. The remaining measured carcass parameters were similar ($P > 0.10$) for steers fed all treatments. Several studies have reported no impact of feeding various EO blends on HCW, dressing percentage, or other carcass (meat) characteristics (Pukrop et al. 2019; Araujo et al. 2019; Meyer et al. 2009; Rivaroli et al. 2017; Valero et al., 2014)

Liver abscesses were lowest ($P < 0.10$) for steers fed M+T compared with steers fed Control, EOB, and EOB+M, with steers fed EOB+M+T being intermediate and similar ($P > 0.10$) to steers fed M+T (Table 4). The EOB+M+T combination demonstrated some impact on liver abscesses compared to steers fed Control, but the increase in liver abscesses compared with steers fed M+T could indicate a potential antagonism among these three feed additives. The EOB antimicrobial characteristics did not reduce liver abscesses unless blended with other feed additives, i.e. M or T. Previous EO research (Araujo et al., 2019; Pukrop et al., 2019) reported that steers fed an EO in combination with M or T resulted in similar liver abscesses. In contrast, Meyer et al. (2009) reported that steers fed an EO blend observed decreased liver abscesses compared with steers fed tylosin. Given the differences in individual EO among these various EO blends, future research work is needed to find, if possible, an appropriate blend of specific EO to reduce liver abscesses.

Conclusions

There is potential for EO to replace the sub-therapeutic antibiotic feeding to either enhance (early feedlot) or maintain overall feedlot growth performance and feed conversions.

However, the appropriate EO blends of specific EO have not been identified to date to reduce liver abscesses when feeding a high-grain ration. However, cattle feeders evaluating alternatives to feeding antibiotics, the feeding of a proprietary EOB during the finishing phase demonstrated improved early growth rates, overall feed conversions, and carcass dressing percentages. But the incidence of liver abscess is still a problem to be solved. This study demonstrates that cattle feeders do have viable alternatives to antibiotics. Subsequent analyses were conducted on steaks from a subset of these steers to evaluate the effects of these dietary treatments on final meat quality, as detailed in Chapter 2.

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Table 1. Ingredient composition of basal rations fed from starter to finisher.

	Ration			
Ingredient	Starter	Step 1	Step 2	Finisher
	% of Mix			
Steam-flaked corn	36.6	49.2	66.5	72.1
Corn silage	46.6	36.0	18.5	13.5
Dried distillers grains	13.5	9.5	8.5	6.80
Liquid supplement[1]	3.3	3.3	3.3	4.0
Treatment supplement2	0.0	2.0	3.2	3.6

[1]Liquid molasses base supplement containing (DM basis): 92% crude protein, 0.53 Mcal/kg NE_m, 0.33 Mcal/kg NE_g, 14.5% Ca ($CaCO_3$), 7.0% salt (NaCl), 1.14% potassium (KCl), 73332.6 IU/kg Vitamin A, and 880.0 IU/kg Vitamin E.
[2]Treament supplement consisted without or with experimental feed additives.

Table 2. Nutrient composition of basal rations fed from starter to finisher.

	Ration			
Nutrient	Starter	Step 1	Step 2	Finisher
Dry matter, % as-fed	48.4	53.3	64.0	68.3
Crude protein, %	14.18	13.3	13.2	13.4
Non-protein nitrogen, %	2.91	2.91	2.91	3.32
Acid detergent fiber, %	17.26	14.0	8.54	7.74
Neutral detergent fiber, %	28.64	23.33	16.20	14.55
Ether extract, %	2.98	3.21	3.51	3.94
NE_m, Mcal,kg[1]	1.92	1.93	2.0	2.21
NE_g, Mcal/kg[2]	1.28	1.34	1.40	1.49
Calcium, %	0.61	0.63	0.64	0.68
Phosphorus, %	0.32	0.29	0.28	0.39
Magnesium, %	0.19	0.17	0.14	0.15
Potassium, %	0.92	0.78	0.60	0.61
Sulfur, %	0.22	0.19	0.17	0.22
Cobalt, mg/kg	0.48	0.46	0.45	0.49
Copper, mg/kg	20.30	19.69	18.99	19.20
Iron, mg/kg	84.69	70.62	52.21	45.45
Manganese, mg/kg	54.59	50.50	44.81	49.76
Molybdenum, mg.kg	0.50	0.45	0.35	0.33
Selenium, mg/kg	0.42	0.38	0.37	0.40
Zinc, mg/kg	85.53	81.37	77.48	87.16

[1]$NE_m = \{[1.37 \times (TDN \times 0.0361)] - [0.0138 \times (TDN \times 0.0361) \times (TDN \times 0.0361)] + [0.0105 \times (TDN \times 0.0361) \times (TDN \times 0.0361) - 1.12]\} \div 2.205$.

[2]$NE_g = \{[1.42 \times (TDN \times 0.0361)] - [0.174 \times (TDN \times 0.0361) \times (TDN \times 0.0361)] + [0.0122 \times (TDN \times 0.0361) \times (TDN \times 0.0361) \times (TDN \times 0.0361) - 1.65]\} \div 2.205$.

Table 3. Feedlot finishing performance by steers fed a ration containing a proprietary essential oil blend (EOB), monensin (M), or tylosin (T) alone or in combination.

	Treatment						
Item	Control[1]	EOB	EOB+M+T	M+T	EOB+M	SEM	$P <$[1]
Pens/treatment	8	8	8	8	8	---	---
BW, kg							
Initial	369.1	369.3	368.2	367.9	369.4	11.0	0.99
28d	411.0	441.1	413.3	412.2	420.4	9.2	0.96
56d	468.8	473.7	466.6	462.2	469.4	10.8	0.97
84d	530.1	534.7	521.5	517.1	524.3	12.0	0.86
112d	579.3	584.5	575.1	569.9	577.7	28.8	0.96
140d	624.6	630.9	621.2	618.9	624.1	12.9	0.98
168d	659.8	652.6	648.0	640.0	655.4	13.6	0.87
Final	673.9	672.1	669.9	659.8	671.7	11.9	0.93
ADG, kg/d							
0-28d	1.49	1.61	1.61	1.59	1..82	0.11	0.12
0-56d	$1.54^{a,b}$	1.86^{a}	$1.75^{b,c}$	$1.68^{b,c}$	$1.72^{a,b}$	0.06	0.05
0-84d	1.92^{a}	1.97^{a}	1.82^{b}	1.78^{b}	1.85^{b}	0.07	0.02
0-112d	1.98	1.92	1.84	1.81	1.86	0.06	0.38
0-140d	1.82	1.89	1.81	1.80	1.82	0.08	0.66
0-168d	1.80	1.75	1.74	1.69	1.76	0.12	0.48
Overall	1.75	1.75	1.73	1.68	1.73	0.10	0.78
DMI, kg/d							
0-28d	$7.5^{b,c}$	7.7^{c}	$7.3^{b,c}$	7.1^{b}	8.0^{a}	0.28	0.006
0-56d	9.3^{a}	$9.1^{a,c}$	$8.7^{b,c,d}$	8.4^{b}	$8.9^{a,d}$	0.27	0.007
0-84d	10.2^{a}	$9.9^{a,c}$	$9.5^{b,c}$	9.1^{b}	$9.5^{b,c}$	0.30	0.007
0-112d	10.5^{a}	$10.6^{a,c}$	$9.7^{b,c}$	9.4^{b}	$9.7^{b,c}$	0.32	0.01
0-140d	10.6^{a}	$10.3^{a,c}$	$9.9^{b,c}$	9.6^{b}	$9.9^{b,c}$	0.37	0.03
0-168d	10.7^{a}	$10.3^{a,c}$	$10.0^{b,c}$	9.7^{b}	$10.0^{b,c}$	0.41	0.03
Overall	10.6^{a}	$10.2^{a,c}$	$10.0^{a,c}$	$9.6^{b,c}$	$10.0^{b,c}$	0.50	0.05
Feed efficiency, gain/feed							
0-28d	0.200	0.212	0.222	0.223	0.230	0.012	0.20
0-56d	0.193	0.205	0.201	0.202	0.201	0.005	0.32
0-84d	0.188	0.200	0.192	0.195	0.195	0.004	0.30
0-112d	0.179	0.190	0.189	0.191	0.191	0.003	0.08
0-140d	0.173^{a}	0.182^{b}	0.183^{b}	0.187^{b}	0.185^{b}	0.003	0.04
0-168d	0.170	0.175	0.174	0.177	0.180	0.003	0.27
Overall	0.164^{b}	0.171^{a}	0.173^{a}	0.174^{a}	0.173^{a}	0.003	0.05

[1]Probability of F test for treatment.
[a,b,c]Means containing different superscripts differ, $P \leq 0.05$.

Table 4. Carcass measurements from steers fed a ration containing a proprietary essential oil blend (EOB), monensin (M), or tylosin (T) alone or in combination.

	Treatment						
Measurement	Control[a]	EOB	EOB+M+T	M+T	EOB+M	SEM	$P <$[1]
Replicates/treatment	8	8	8	8	8	---	---
Hot carcass weight, kg.	408.8	413.8	407.2	400.7	404.5	14.9	0.21
Dressing percentage[2]	63.2^{x}	64.3^{w}	63.4^{x}	63.3^{x,y}	62.7^{y}	0.25	0.02
Marbling score[3]	639.2	601.4	635.2	637.4	634.3	12.2	0.18
Preliminary YG, %	3.34	3.32	3.22	3.26	3.24	0.04	0.19
Fat thickness, cm.	1.36	1.35	1.25	1.28	1.28	0.05	0.22
Ribeye area, cm.	90.4	90.9	91.0	89.3	88.4	1.92	0.21
USDA YG	2.67	2.71	2.54	2.65	2.54	0.09	0.50
Calculated YG[4]	3.17	3.17	3.01	3.08	3.15	0.08	0.51
Liver abscess present, %[i]	59.9^{w}	53.7^{w,y}	35.3^{x,y,z}	26.3^{x,z}	43.9^{w,z}	6.5	0.01
Lung score, % with lung lesion[j]	45.9	33.7	30.7	28.8	38.1	5.4	0.20

[1]Probability of F test for treatment.
[2]Final live body weight pencil-shrunk by 4% prior to dressing percentage calculation.
[3]Slightly Abundant=800, Moderate=700, Modest=600, Small=500, Slight=400.
[4]Calculated YG = 2.50 + (6.35 × fat thickness, cm) + (0.2 × KPH, %) + (0.0017 × HCW, kg) – (2.06 × LM area, cm^2).

CHAPTER 3: Meat Quality and Shelf-Life Characteristics of Steaks from Steers Fed an Essential Oil Blend

Introduction

Enhancing beef production efficiency and profitability represents a crucial objective for the beef industry, directly impacting global food security and the ability to meet the increasing protein demands of a growing population. Producers employ various strategies to achieve these improvements (e.g., average daily gain and feed efficiency), including feeding of established compounds such as monensin and tylosin (Samuelson et al., 2016; Cazer et al., 2020). An alternative to M and T is EO, a category of compounds derived from plants produced as secondary metabolites (not involved in plant production or reproduction), and are often involved in plant protection (Calsamiglia et al., 2007; He et al., 2015). The EO is typically extracted from plant material by the process of steam distillation (Hart et al., 2008). The EO mode of action is broad, and they are known for antimicrobial, antioxidant, and anti-inflammatory properties (Zamuner et al., 2023) that can impact production attributes and meat quality. When deciding on the selection of a feed additive, a beef producer must consider not only the production aspects of the product but also the consumers' differential choice for the end meat product.

Appearance/color, texture, and flavor are the three most important quality attributes that influence consumer acceptance of meat (Min and Ahn, 2005). Studies have shown that EO feeding can improve meat quality aspects, which are critical components for consumer choice (Amaral et al., 2018). Specifically, previous research exploring the effects of feeding EO to cattle have demonstrated that meat quality attributes such as color stability, cook loss, color, and lipid oxidation are impacted by EO (Yang et al., 2010; Monteschio et al., 2019; He et al., 2024). Therefore, the objective of this study was to examine the impact of a feed additive consisting of a

proprietary blend of oregano, thyme, and cinnamon (Prosper EO, Ralco Agriculture, Marshall, MN; PEO) on meat quality attributes compared to a control or a combination of M and T in finishing cattle.

Materials and Methods

This research, involving the care and handling of animals, was approved by the Colorado State University Institutional Animal Care and Use Committee (Approval # 3824), with all procedures following the standards set forth in the 4th edition of the "Guide for the Care and Use of Agricultural Animals in Research and Teaching" (ADSA-ASAS-PSA, 2020). Details regarding the experimental design, animal management, growth performance, and carcass characteristics for the steers used in this study have been previously published (Poppy et al., 2025). A total of four hundred and fifty-two crossbred steers (initial BW 368.6 ± 10.8 lbs.) from two distinct cattle sources were employed in this study. These animals, the same cohort as those detailed in Chapter 2, were raised under conventional feedlot conditions, receiving standard industry diets and management protocols. Samples for meat quality analyses were obtained post-harvest from a USDA-inspected commercial facility. Only Control, PEO, and M+T were sampled for meat quality evaluation. Two striploins (longissimus lumborum; LL; IMPS #180) were collected per pen, and four pens per treatment (n = 4) were sampled. Striploins were aged for 21 days from the date of harvest in vacuum packaging, in darkness at 2 ± 1ºC. After the aging period, the striploins were fabricated into 2.54 cm-thick steaks. One steak each from each loin was assigned to either Warner Bratzler Shear Force (WBSF), trained sensory panels, fatty acid analysis, and seven additional steaks were designated for simulated retail display. Steaks designated for shear force, trained sensory, and fatty acid analysis were vacuum packaged and frozen at -20ºC until analysis. Steaks designated for the retail display and shelf-life evaluation were packaged on a soaker pad lined with

polystyrene foam tray, wrapped in polyvinyl chloride film (oxygen transmission = 23,250 mL x m2 x d-1, 72 gauge: Resinite Packaging Films, Borden, Inc., North Andover, MA), and placed into a retail display case. On each retail display day, instrumental color, pH, thiobarbituric acid reactive substances (TBARS), metmyoglobin-reducing activity (MRA), and microbiological evaluation were conducted.

Instrumental Color

The instrumental color, *L** (lightness), *a**(redness), *b** values (yellowness), of steaks were measured daily using a portable spectrophotometer (Illuminant A, 2.54-cm aperture, 10° standard observer; HunterLab MiniScan LabScan EZ4500, Hunter Labs, Reston, VA) during the 7-day retail display. The spectrophotometer was calibrated with white and black tiles and covered with polyvinyl chloride film (PVC) before each use. A total of three readings of CIE *L** (lightness), *a**(redness), *b** values (yellowness) were collected and averaged for each steak.

pH Evaluation

Five ± 0.05 g of each LL steak was excised from each sample and homogenized for 1 min using a Pro Scientific PRO250 homogenizer (Pro Scientific, Oxford, CT). The homogenized sample was vortexed for 10 seconds, and pH was measured using an Orion Star A211 pH meter (Thermo Fisher Scientific, Waltham, MA), equipped with an Orion 8157BNUMD Ross Ultra pH/ATC triode (Thermo Fisher Scientific, Waltham, MA). The pH meter was calibrated before use daily using pH 4.0, 7.0, and 10.0 buffers (Thermo Fisher Scientific, Chelmsford, MA).

Thiobarbituric Acid Reactive Substances (TBARS)

Five ± 0.05 g of cryo-pulverized LL sample were weighed into a 50 mL conical tube. A 22.5 mL 11% trichloroacetic acid (TCA) solution was added to each sample and homogenized for

1 min. The supernatant was filtered through Whatman #1 filter paper (CAT No. 1001-090, China). Two mL of filtrate were mixed with 2 mL of 20 mM thiobarbituric acid (TBA) solution in a 15 mL tube, vortexed, and incubated in the dark at 20°C for 20 hours. The absorbance of the aliquot was read at 532 nm using a UV-1800 Shimadzu UV spectrophotometer. Duplicates were averaged, and a standard curve was used to determine the concentration of malondialdehyde (MDA) in the samples. The values represent the amount of MDA in mg/kg of meat.

Metmyoglobin Reducing Activity (MRA)

A 5 cm x 5 cm x 1.5 cm square LL sample was submerged in a 0.3% sodium nitrite solution for 20 min. After that, samples were blotted dry and vacuum packaged individually. (3 mil standard barrier nylon/PE vacuum pouch, Prime Source vacuum pouches, USA). Immediately after packaging, sample reflectance spectra were measured in three different locations with a Hunter Lab Mini scan EX4500. The vacuum-packaged samples were then incubated for 2 h at 30°C. After the 2 h incubation, reflectance spectra from 700 to 400 nm were collected as described previously. The percentage of surface metmyoglobin (pre-incubation as well as post-incubation) was calculated based on K/S ratios according to established formulas (King et al., 2023) and was used to calculate MRA.

Microbiological Evaluation

A 4 × 4 cm^2 sample aseptically excised using a sterile disposable scalpel from the center of each steak was used to determine aerobic plate count (APC) and lactic acid bacteria (LAB) count on each day of retail display. The excised samples were placed into a sterile Whirl-Pak filter-separation bag (710 mL; Nasco, Modesto, CA) with 50 mL of maximum recovery diluent (MRD; Acumedia-Neogen, Lansing, MI). The samples were then mechanically pummeled for 2 min for bacterial detachment. The stomached samples were serially diluted in MRD and appropriate

dilutions were plated, in duplicate, onto tryptic soy agar (TSA; Acumedia-Neogen, Lansing, MI) to enumerate APC, and a pour plate overlay method was used to culture LAB. Specifically, 1 mL of appropriate sample dilutions was mixed in 10 mL of molten (<45°C) Lactobacilli MRS Agar (Becton, Dickinson, and Company [BD], Sparks, MD), followed by an overlay of molten Lactobacilli MRS Agar to generate an anaerobic environment. All plates were incubated at 25°C, and colonies were counted after 72 ± 1 hours of incubation. Colony count data were then converted to log CFU/cm^2.

Warner-Bratzler Shear Force (WBSF) and Cook loss

The LL steaks were trimmed of excess external fat and cooked to a targeted internal temperature of 71°C in a Rational SCC WE 61 commercial cooking appliance (Rational AG, Landsberg am Lech, Germany), set to 200°C and 0% humidity. Additionally, cooking loss was determined by the difference between the initial weight of the raw steaks and the final weight of the cooked steaks. WBSF was measured using a Warner-Bratzler shear force tester (G-R Electric Manufacturing Company LLC., Fort Wayne, IN). The steaks were cored into 1-inch-thick cylindrical samples. Cores were placed on the Warner-Bratzler shear head, perpendicular to the muscle fibers. The machine was operated in displacement control mode, and the peak shear force was recorded. This procedure was repeated for a total of 7 cores from each steak. The peak shear force values for each steak were averaged to obtain a single WBSF value for each sample.

Crude Fat

The crude fat content of meat samples was determined using the Folch method (Folch et al. 1957). Initially, 1 ± 0.05 g of tissue was weighed into a 30-ml Pyrex glass tube. Twenty mL of chloroform:methanol (2:1) solution was added to each tube, and the samples were homogenized for 30 seconds at a speed setting of 5 using a Pro Scientific PRO250 homogenizer. The samples

were then agitated on an orbital shaker (Burrell Model 75, Burrell Corp., Pittsburgh, PA) for 20 minutes at room temperature. Four mL of sodium chloride solution (0.9%) was added to the samples, covered with parafilm, and stored overnight at 4°C to allow for phase separation. The next day, the organic layer (lipid extract) was removed and transferred to a pre-weighed scintillation vial (Duran Wheaton Kimble no. 986541). Vials were then placed in a NE-VAP 112 nitrogen evaporator with OA-SS heating system (Organomation Associates Inc. Berlin, MA) until dry (approximately 30 min.) The samples were air dried for 1 hour under the hood vent to remove all traces of chloroform. The samples were then placed in the Dry Matter Oven (Lab-Line Imperial II Radiant Heat Oven, Lab-Line Instrument, Inc., Melrose Park, Illinois, USA) at 60°C for at least 16 hours. After the samples were dry, they were weighed to determine the weight of the dried fat.

Methylation of Crude Fat

One mL of 0.5 N potassium hydroxide (KOH) in methanol (MeOH) was added to each 20 mL scintillation vial (Duran Wheaton Kimble no. 986541) containing the dried-down lipid product from the previous step. The vials were tightly capped and heated in a 70°C water bath (Fisher Scientific Isotemp 215, Pittsburg PA) for 10 minutes. Then, 1 mL of 14% boron trifluoride-methanol solution (Aldrich B1252) was added to each vial. The vials were tightly capped and heated again in a 70°C water bath for 30 min. While the vials were heating, 30 ml glass tubes were prepared by adding 800 mg of sodium sulfate (Na_2SO4) to each tube. After the vials reached room temperature, 2 mL of high-performance liquid chromatography (HPLC) grade hexane was added to each vial (no.986541). Two mL of saturated sodium chloride (NaCl) were added to each vial, capped, and vortexed for 1 minute. Once the solution got separated into three layers, the upper layer (hexane layer) was pipetted off each vial and transferred to a 30 ml glass tube containing 800 mg Na_2SO_4. Further, 2 mL of hexane was added to the scintillation vial containing the saturated

NaCl and was vortexed for 10 seconds. The hexane layer was, again, transferred from the scintillation vial to the 30 ml glass tube containing Na_2SO_4. The hexane was evaporated completely from the 30 ml glass tube using the NE-VAP 112 nitrogen evaporator.

Gas Chromatography

The dried fatty acid methyl esters (FAMEs) were reconstituted with 1mL of hexane and analyzed by gas chromatography (GC). The GC system consisted of an Agilent 6890 Series gas chromatograph equipped with a flame ionization detector (FID) and a 100 m x 0.25 mm (i.d.), fused silica capillary column (SP-2560, 0.2 µm film thickness, Supelco). Triacylglycerol of tridecanoic acid (13:0, 1.0 mg) was used as the internal standard. The oven temperature was maintained at 175°C for 40 min, and then increased to 240°C at a rate of 10°C/min. The injector and FID temperatures were both set to 245°C. Helium was used as the carrier gas at a split ratio of 50:1 and a constant flow rate of 0.8 mL/min. Fatty acid peaks were recorded and integrated using GC ChemStation software (version A.09.03, Agilent Technologies). Retention times were compared with known FAME standards to identify individual fatty acids (Nu-Chek Prep, Inc., Elysian, MN, and Matreya Inc., Pleasant Gap, PA).

Statistical Analysis

One strip loin from the control group was missed during the collection in the commercial processing facility, resulting in a total of 23 samples. Data for pH, TBARS, shear force, and microbiological analyses were averaged by pen number and analyzed in a complete randomized design, with treatment and day, and their interaction as the fixed effects. Means were tested using an analysis of variance (ANOVA) with Tukey's correction and separated with the emmeans package in R (4.2.1). Statistical significance was set at $\alpha = 0.05$.

Results and Discussion

Instrumental color measurements (L^*, a^*, b^*) of longissimus lumborum steaks are shown in Table 5. No significant interaction between dietary treatment and display day ($P > 0.05$) or any main effect of treatment ($P > 0.05$) was observed. However, display day significantly affected color parameters ($P < 0.05$), indicating expected discoloration over time. The pH values of longissimus lumborum steaks from cattle fed the control, PEO, and M+T diets are presented in Table 6. No significant differences in pH were observed among the treatments ($P > 0.05$). Specifically, the mean pH values on day 0 were 5.47 for the control, 5.46 for PEO, and 5.48 for M+T. Furthermore, pH remained stable across the retail display period, with no significant changes observed over time ($P > 0.05$).

Lipid oxidation, measured as TBARS (Table 7), had no significant interaction between dietary treatment and display day ($P > 0.05$). However, a significant effect of display day on TBARS values was observed ($P < 0.05$). A potential treatment effect was also noted ($P = 0.050$; Table 8). Despite this borderline significance, post-hoc mean separation revealed no significant differences in TBARS values among the control, PEO, and M+T diets ($P > 0.05$). The observed differences, while statistically significant, were practically negligible. Metmyoglobin-reducing activity (MRA; Table 9) showed no significant differences among steaks from cattle fed the control, PEO, and M+T diets ($P > 0.05$). However, as anticipated, MRA significantly decreased during the retail display period ($P < 0.05$), indicating a reduction in the steak's ability to maintain its red color over time.

The aerobic plate count (APC) and lactic acid bacteria count (LABC) of the longissimus lumborum steaks are presented in Table 10. Statistical analysis revealed no significant interaction between dietary treatment and display day ($P > 0.05$) and no significant treatment effect ($P > 0.05$) for either APC or LABC. This suggests that the PEO and M+T dietary treatments did not

significantly influence bacterial growth on the beef steaks during the retail display period. However, as expected, APC and LABC increased significantly with increasing retail display days ($P < 0.05$), indicating a natural progression of microbial growth over time.

The dietary treatments did not significantly affect shear force and cook loss ($P > 0.05$; Table 11). This indicates that the treatments did not alter the tenderness or water-holding capacity of the steaks. The percentage of crude fat in the steaks, as shown in Table 12, exhibited no statistically significant differences among the control, PEO, and M+T treatments ($P > 0.05$). Specifically, the mean crude fat percentages were 6.61% for the control, 7.77% for PEO, and 6.48% for M+T. The fatty acid composition of the steaks is presented in Table 13. There was no difference ($P > 0.05$) in the fatty acid composition between the treatments. Generally, C18:1, C16:0, and C18:0 were the most abundant fatty acids in all the treatments.

In the current experiment, neither the treatment with PEO nor M+T affected lipid oxidation, pH, or instrument color data. Conversely, a study examining cinnamon EO supplementation in young bulls at 450 mg/kg and 880 mg/kg reported a significant quadratic relationship (P = 0.027) with lightness (L^*), suggesting a non-linear response (Torrecilhas et al., 2021). Furthermore, the 880 mg/kg treatment exhibited a trend ($P = 0.089$) towards lower yellowness (b^*) values (Torrecilhas et al., 2021). This was thought to have been due to an increase in pH, resulting in reduced muscle fiber shrinkage and decreased light scattering. However, these findings contrast with the outcomes of our current study, where cinnamon EO supplementation did not significantly affect pH. Additionally, while the aforementioned study observed changes in specific color components (L^* and b^*), our results did not indicate an overall significant change in instrumental color. A meta-analysis (Orzuna-Orzuna et al., 2022) examining 34 different peer-reviewed studies on different EO compounds and dosages found no

change in meat instrument color or pH, but did find a decrease in shear force, cook loss, and malonaldehyde (MDA) due to EO in general. The reduction in shear force with aging could be due to a delay in the oxidation of the meat, as seen in a different study on young feedlot bulls fed an EO blend of oregano, garlic, lemon, rosemary, thyme, eucalyptus, and sweet orange at 0, 3.5, and 7 g/animal/day (Rivaroli et al., 2016). These authors reported that the dosage at 3.5 g/animal/day decreased lipid oxidation seen in MDA/kg muscle but not at the 7 g/animal/day level. There was no change in the TBARs, as was seen in this study as well.

Overall, the meat quality attributes in the current study were not influenced by the addition of EO. The findings of this experiment are consistent with previous research that has shown that EO such as thyme, oregano, and cinnamon, as well as M+T, do not have a significant impact on the quality or shelf life of beef steaks. One key limitation in the experimental design was that because the product formulation was proprietary, the actual dosage of thymol, carvacrol (one of the active ingredients in oregano), cinnamon, and oregano was unknown. It is possible that any one of the EO molecules could have a minimally or maximally limited impact on the meat product. Although each of the compounds has strong anti-oxidative and antimicrobial attributes (Burt, 2004; Calsamiglia et al., 2007; Starcevic et al., 2015), little is known about their dosage titration, interaction with other compounds, or with specific base diets. More experimentation, looking at dosage and interactions of compounds, could uncover the answer to these questions. Another limitation of this experiment is that the sample size was relatively small. Further research with a larger sample size is needed to confirm the findings of this experiment. Furthermore, this experiment was conducted in a controlled setting. The effects of feeding cattle PEO or M+T on beef steaks' quality and shelf life may differ under commercial conditions.

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Table 5. Instrumental lightness (*L**), redness (*a**), and yellowness (*b**; ± standard error) of longissimus lumborum steaks from cattle fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets over a 7-d simulated retail display period (2.2°C).

	Day	0	1	2	3	4	5	6
Control[1]	*L**	40.7±0.1^{a}	35.3±0.07^{c,d}	34.0±0.08^{d}	39.4±0.12^{a,b}	40.4±0.10^{a,b}	33.9±0.11^{d}	38.1±0.10^{b,c}
	*a**	29.4±0.20^{b}	31.7±0.28^{a}	33.3±0.50^{a}	26.2±0.27^{c}	23.6±0.16^{d}	28.5±0.39^{b}	20.1±0.28^{e}
	*b**	23.7±0.18^{c}	27.9±0.40^{a,b}	30.1±0.37^{a}	22.3±0.23^{c}	20.3±0.16^{d}	27.0±0.31^{b}	17.5±0.21^{d}
PEO[2]	*L**	41.7±0.07^{a}	36.9±0.08^{c,d}	36.1±0.08^{d}	40.9±0.10^{a,b}	40.8±0.10^{a,b}	34.1±0.10^{d}	38.8±0.12^{b,c}
	*a**	29.8±0.26^{b}	32.5±0.19^{a}	31.6±0.23^{a}	26.9±0.24^{c}	24.0±0.11^{d}	29.2±0.25^{b}	21.4±0.34^{e}
	*b**	24.1±0.32^{c}	29.0±0.21^{a,b}	28.9±0.20^{a}	22.7±0.29^{c}	20.6±0.06^{d}	27.5±0.16^{b}	18.9±0.20^{d}
M+T[3]	*L**	43.4±0.05^{a}	38.1±0.09^{c,d}	38.0±0.07^{d}	43.2±0.09^{a,b}	42.6±0.07^{a,b}	35.9±0.09^{d}	40.1±0.08^{b,c}
	*a**	29.1±0.19^{b}	31.2±0.29^{a}	31.7±0.40^{a}	26.9±0.32^{c}	23.2±0.20^{d}	29.2±0.40^{b}	22.3±0.22^{e}
	*b**	23.3±0.20^{c}	27.4±0.38^{a,b}	28.9±0.40^{a}	22.3±0.23^{c}	19.9±0.14^{d}	27.2±0.33^{b}	19.3±0.19^{d}

$^{a-e}$ Means without common superscripts in a row differ (P<0.05)

[1]Control: no added Prosper essential oil, monensin, or tylosin.

[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.

[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.

Table 6. Mean pH (± standard error) of longissimus lumborum steaks from cattle fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets over a 7-d simulated retail display period (at 2.2°C).

Day	0	1	2	3	4	5	6
Control[1]	5.47±0.01	5.51±0.01	5.49±0.00	5.45±0.01	5.5±0.00	5.5±0.00	5.49±0.01
PEO[2]	5.46±0.01	5.50±0.01	5.45±0.01	5.47±0.01	5.50±0.01	5.50±0.01	5.49±0.01
M+T[3]	5.48±0.01	5.51±0.01	5.49±0.01	5.48±0.01	5.5±0.01	5.54±0.01	5.51±0.01

[1]Control: no added Prosper essential oil, monensin, or tylosin.

[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.

[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.

Table 7. Thiobarbituric acid reactive substance (TBARS;mg/kg) value (± standard error) of longissimus lumborum steaks from finishing steers fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets over a 7 d simulated retail display period (2.2°C).

Day	**0**	**1**	**2**	**3**	**4**	**5**	**6**
Control[1]	0.37±0.66^{e}	0.92±0.66^{de}	1.41±0.66^{cde}	2.76±0.66^{bcd}	3.09±0.66^{bc}	4.32±0.66^{ab}	5.55±0.66^{a}
PEO[2]	0.47±0.66^{e}	1.03±0.66^{de}	1.46±0.66^{cde}	2.43±0.66^{bcd}	3.06±0.66^{bc}	3.93±0.66^{ab}	5.33±0.66^{a}
M+T[3]	0.45±0.66^{e}	0.79±0.66^{de}	1.18±0.66^{cde}	1.82±0.66^{bcd}	2.00±0.66^{bc}	2.83±0.66^{ab}	3.71±0.66^{a}

$^{a-e}$ Means without common superscripts in a row differ ($P < 0.05$)

[1]Control: no added Prosper essential oil, monensin, or tylosin.

[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.

[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.

Table 8. Thiobarbituric acid reactive substance (TBARS; mg/kg) value (± standard error) of longissimus lumborum steaks from finishing steers fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets over a 7-d simulated retail display period (2.2°C).

	Control1	PEO[2]	M+T[3]	P-value
TBARS	2.63±.025	2.53±.025	1.82±.025[b]	0.050

1Control: no added Prosper essential oil, monensin, or tylosin.

2Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.

3Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin

Table 9. Metmyoglobin reducing activity (± standard error) of longissimus lumborum steaks from finishing steers fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets over a 7-d simulated retail display period (at 2.2°C).

Day	0	1	2	3	4	5	6
Control[1]	67.10±1.57[a]	50.40±1.89[a,b]	42.80±1.99[b]	34.90±1.45[b,c]	31.60±0.94[b,c]	16.80±0.86[c,d]	14.00±0.90[d]
PEO[2]	73.80±1.61[a]	56.00±2.72[a,b]	45.40±2.62[b]	39.30±2.07[b,c]	33.60±1.20[b,c]	28.90±2.40[c,d]	14.80±1.33[d]
M+T[3]	70.80±2.23[a]	57.30±2.72[a,b]	46.70±2.75[b]	37.10±2.70[b,c]	42.30±3.15[b,c]	26.20±2.29[c,d]	18.70±2.11[d]

[a-d] Means without common superscripts in a row differ ($P < 0.05$)

[1]Control: no added Prosper essential oil, monensin, or tylosin.

[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.

[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.

Table 10. Mean (log CFU/cm^2 ± standard deviation) aerobic counts (APC) and lactic acid bacteria counts (LABC) of longissimus lumborum steaks from cattle fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets over a 7-d simulated retail display period (at 2.2°C).

	Day	0	1	**2**	**3**	**4**	**5**	**6**
Control[1]	APC	3.63±0.65^{f}	4.35±0.50^{ef}	4.46±0.55^{d,e}	5.24±0.76^{c,d}	5.34±0.69^{b,c}	6.05±0.75^{a,b}	6.44±0.73^{a}
	LABC	3.20±0.64^{e}	3.47±0.42^{e}	4.22±0.37^{c,d}	4.99±0.77^{c,b}	5.10±0.50^{b,c}	5.82±0.10^{a,b}	6.19±0.69^{a}
PEO[2]	APC	3.59±0.59^{f}	3.79±0.66^{e,f}	4.48±0.60^{d,e}	4.83±0.74^{c,d}	5.32±0.83^{b,c}	6.01 ±0.76^{a,b}	6.36±0.99^{a}
	LABC	3.07±0.46^{e}	3.22±0.50^{e}	3.83±0.44^{c,d}	4.46±0.56^{c,b}	5.09±0.73^{b,c}	5.45±0.78^{a,b}	5.86±0.88^{a}
M+T[3]	APC	3.30±0.36^{f}	4.04±0.71^{e,f}	4.10±0.50^{d,e}	4.76±0.72^{c,d}	5.51±0.59^{b,c}	5.93±0.68^{a,b}	6.42±0.64^{a}
	LABC	2.98±0.19^{e}	3.23±0.57^{e}	4.01±0.44^{c,d}	4.39±0.72^{c,b}	5.33±0.69^{b,c}	5.78±0.55^{a,b}	6.07±0.07^{a}

$^{a-f}$ Means without common superscripts in a row differ ($P < 0.05$)

[1]Control: no added Prosper essential oil, monensin, or tylosin.

[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.

[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.

Table 11. Shear force (kg/cm^2 ; ± standard error) and cook loss (g; ± standard error) of longissimus lumborum steaks from finishing steers fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets.

	Control[1]	PEO[2]	M+T[3]	P -value
Cook Loss	53.40±5.03	67.80±4.70	67.80±4.70	0.23
Shear Force	2.16±0.18	2.39±0.17	2.50±0.17	0.17

[1]Control: no added Prosper essential oil, monensin, or tylosin.
[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.
[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.

Table 12. Crude fat (%; ± standard error)) of longissimus lumborum steaks from finishing steers fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets.

	Control[1]	PEO[2]	M+T[3]	P -value
Crude Fat (%)	6.61±0.83	7.77±0.77	6.48±0.77	0.40

[1]Control: no added Prosper essential oil, monensin, or tylosin.
[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.
[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.

Table 13. Fatty acid composition (weight %) of longissimus lumborum steaks from finishing steers fed prosper essential oil (PEO), monensin (M), or tylosin (T) in their finishing diets.

Fatty Acid	Control[1]	PEO[2]	M+T[3]
C14:0	3.10	3.08	3.03
C14:1	0.28	0.32	0.28
C16:0	33.06	33.28	32.26
C16:1	3.23	3.35	3.29
C17:0	1.29	1.34	1.32
C17:1	0.78	0.80	0.79
C18:0	14.18	13.91	13.93
C18:1	39.80	39.29	40.82
C18:2	3.15	3.45	3.05
C18:3	0.22	0.23	0.21
C20:3	0.03	0.03	0.03
C20:4	0.78	0.79	0.88
C20:5	0.07	0.07	0.07
C22:6	0.04	0.03	0.04
C24:1	0.01	0.01	0.01

[1]Control: no added Prosper essential oil, monensin, or tylosin.
[2]Diet formulated to provide: Prosper essential oil at 6 g·animal-1·day-1.
[3]Diet formulated to contain: 38.5 g/ ton of monensin and 7.7 g/ton of tylosin.