2024

The Influence of By-O-Reg+ Beef, an Oregano-based Essential Oil Supplement, on Growth Performance, Carcass Traits and Meat Quality of Finishing Beef Steers

Bergin Mackenzie DeBruin
South Dakota State University, bergin1999@gmail.com

Recommended Citation

DeBruin, Bergin Mackenzie, "The Influence of By-O-Reg+ Beef, an Oregano-based Essential Oil Supplement, on Growth Performance, Carcass Traits and Meat Quality of Finishing Beef Steers" (2024). *Electronic Theses and Dissertations*. 1141.
https://openprairie.sdstate.edu/etd2/1141

THE INFLUENCE OF BY-O-REG+ BEEF, AN OREGANO-BASED ESSENTIAL OIL SUPPLEMENT, ON GROWTH PERFORMANCE, CARCASS TRAITS AND MEAT QUALITY OF FINISHING BEEF STEERS.

BY

BERGIN MACKENZIE DEBRUIN

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Animal Science

South Dakota State University

2024

THESIS ACCEPTANCE PAGE

Bergin Mackenzie DeBruin

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Amanda Blair, PhD
Advisor Date

John Jaeger, PhD
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

ACKNOWLEDGEMENTS

This degree would not be possible without my support system over the past 2 years.

First, I would like to thank my advisor, Dr. Amanda Blair. Thank you for taking me on as a student and giving me this opportunity. I want to express my sincere gratitude for answering all my random questions and gracefully guiding me throughout this process, as well as providing me with incredible opportunities to enhance my knowledge through travel and learning in all facets of life. Thank you for being willing to work with me as your student and as a judging coach. You saw the passion that I have for meat judging and coaching and never questioned my ability to handle both coaching and my life as a graduate student. Thank you for taking me into your home to me and allowing me to hang out and get to know you and your family. I can't even explain the influence that you have had on my career and will take everything that you have taught me in my professional and personal life for many years to come.

To the rest of my committee, Dr. Zach Smith, Dr. Chris Bakker, and Dr. Matthew Diersen. Thank you for agreeing to serve on my committee. I appreciate all of the time and effort that you have put in to help me succeed in all things.

To the rest of the SDSU meat science faculty, Dr. Chris Bakker, Dr. Kyle Grubbs, and Dr. Keith Underwood, thank you doesn't seem like enough. Whether it meant just answering a question, filling in for me at a meat judging practice, offering advice, or assisting me with my research project, you all were willing to go above and beyond for me, and it was appreciated. Thank you for always challenging me to think critically and outside of the box. You all have helped me become a better person, student, and researcher. Your support has been unwavering and truly appreciated.

Thank you to all the SDSU faculty and staff that have been so welcoming and made me feel like I belonged to this department. To Adam Rhody, and the meat lab employees, you all are incredible. Thank you for taking the time to help me cut product for judging practices, my research project, and help with everything in between. Your commitment to the success of the SDSU meat lab is unmatched.

To all the Meat Science graduate students that I have had the pleasure to work with: Harlee, Lydia, Clay, Sydni, Garrett, and Kylie, I have been so blessed to work with such incredible, and intelligent people. Thank you for all your help with classes, meat judging, research, and everything else. I am so grateful that I can continue to call you all dear friends of mine and can't wait to see what you all accomplish in your lives and careers, you will always have a cheerleader in me.

To all the other Animal Science graduate students that I have worked with at SDSU thank you for being great friends. I specifically would like to thank Emily Fowler for becoming one of the best friends I could have made here at SDSU. You were always there to listen to me, offer advice, and go for a tea run if we needed a break in the day. You are a true friend, and I don't know what my grad school life would have looked like without you. To Dr. Erin DeHaan, thank you for all the advice and random conversations that we've had over the past two years, you truly took me under your wing and taught me so much. Your friendship means so much to me.

I had the best opportunity to coach the 2023 SDSU Meat Judging Team, which was one of my greatest joys. Thank you to Teigen, Ty, Emmett, Jaylynn, and Cheyenne for having faith in me and allowing me to be your coach. You are some of the funniest, kindest, and smartest individuals I've ever met. I look forward to seeing the outstanding impacts you will make on the world. Looking back, watching you grow not only as meat judgers, but friends, and genuine people was the most rewarding.

To my entire family, thank you. When I started getting involved in the Meat Science Industry, I know I surprised a lot of you with what I was interested in, but you never wavered in your support and always are intrigued to know what I am up to. To Mom and Dad, I credit all of who I am because of the way you raised me to have a hard work ethic, a strong faith in God, and a positive outlook on life. You always supported me in all the passions of my life, including grad school. Mom, thank you for always teaching me to wonder and think of the science behind things. Dad, thank you for always being interested in learning more about my research and being the person that instilled a love for agriculture in me. To my grandparents, Bill and Sadie Kortemeyer, and Marlys

DeBruin, thank you for always being a phone call away and being entirely supportive throughout this whole process. I love you all very much.

To Darin, thank you for being truly one of the best things that has come from the past two years. A long-distance relationship isn’t easy, and even more difficult when in grad school, but you took it all in stride and made the best of it. Thank you for supporting me, making me smile, being my voice of reason, a shoulder to cry on, and always telling me to be confident in myself. I am truly blessed to have you in my life, I love you.

In addition to all of these acknowledgements, I would like to honor the memory of my mentor, Dr. Sherry Olsen. Without Sherry I would not be where I am today. She is the reason I found my passion in meat science. As a coach and advisor, she truly cared about all of her “kids”. I was blessed to be one of them. She taught me to do my “Dead Level Best” each and every day, and I needed to remind myself that many times throughout this degree. May her legacy never be forgotten.

Overall, I need to thank God for getting me to this point and allowing me to follow the path of higher education. He has never failed me and with him all things are possible. This journey has been nothing short of a wild ride, but a true blessing from above, and I wouldn’t have changed a moment.

TABLE OF CONTENTS

LIST OF FIGURES ix

LIST OF TABLES x

ABSTRACT xi

CHAPTER 1 LITERATURE REVIEW 1

Growth Promoting Technology in Livestock Feeding 1

Classifications of Growth Promotion Technologies 1

- Implants - Background 2
- Implants - Mode of Action 3
- Beta-adrenergic receptor agonists - Background 4
- Beta-agonist - Mode of Action 5
- Antimicrobials - Background 6
- Ionophore- Mode of Action 7

Antimicrobial Resistance Concerns 8

Livestock Industry Response to Antimicrobial Resistance 9

Technology Use in Different Beef Production Systems 10

Potential Alternatives to Antimicrobials 14

Essential Oils - Background 14

- Essential Oil - Chemical Composition 16
- Essential Oil - Antimicrobial Activity 17
- Essential Oil - Antioxidant Activity 18
- Essential Oils - Rumen Modification Activity 20

Utilization in Pork and Poultry Industry 21

Meat Quality Impacts 23

By-O-Reg in animal production 25

Summary 26

LITERATURE CITED 28

CHAPTER II: INCLUSION OF BY-O-REG+ BEEF, AN OREGANO BASED ESSENTIAL OIL FEED ADDITIVE, EFFECT ON GROWTH PERFORMANCE, HEALTH OUTCOMES, AND CARCASS CHARACTERISTICS OF YEARLING BEEF STEERS FINISHED IN AN ALL-NATURAL PROGRAM 47

ABSTRACT 47

INTRODUCTION 48

MATERIALS AND METHODS 50
Animals, Initial Processing, and Study Initiation 50
Experimental Design and Treatments 51
Diet and Intake Management...51
Feedlot Health Management..52
Cattle Management and Growth Performance Measurements 52
Carcass Characteristics..53
STATISTICAL ANALYSIS..54
RESULTS AND DISCUSSION..55
Growth Performance 55
Dietary Energetics 56
Health outcomes...57
Liver Scores and Carcass Characteristics...57
Conclusion 59
LITERATURE CITED 61
TABLES 67
Table 2.1. Ingredient and nutrient composition of finishing diet..................67
Table 2.2 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on growth performance and health outcomes of steers finished in an all-natural program ...68
Table 2.3 Influence of inclusion of By-O-Reg+ Beef on distribution of liver scores (%) 69
Table 2.4 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on carcass traits of steers finished in an all-natural program...............................70
Table 2.5 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on objective color of carcasses of steers finished in an all-natural program 71
CHAPTER III - EFFECT OF BY-O-REG+ BEEF, AN OREGANO-BASED ESSENTIAL OIL FEED ADDITIVE, ON PROXIMATE COMPOSITION AND MEAT QUALITY CHARACTERISTICS OF YEARLING BEEF STEERS FINISHED IN AN ALL-NATURAL PROGRAM. 72
ABSTRACT 72
INTRODUCTION 73
MATERIALS AND METHODS 75
    Strip Loin Collection 75
    Strip Loin Fabrication, Purge Loss, and pH 76

Proximate Analysis 76
Warner- Bratzler Shear Force and Cook Loss 78
Objective Color and Subjective Color Panel 79
Lipid Oxidation.. 80
Statistical Analysis.. 81
RESULTS AND DISCUSSION 81
Purge Loss and pH.. 81
Proximate Composition 83
Warner-Bratzler Shear Force and Cook Loss 83
Objective Steak Color and Subjective Color Panel 84
Lipid Oxidation.. 86
CONCLUSION.. 87
LITERATURE CITED 88
FIGURES.. 95
Figure 3.1 Representative image of simulated retail display...................................... 95
Figure 3.2 Representation of beef color scores utilized to for subjective color panel .. 96
TABLES 97
Table 3.1 Least square means for the effect of By-O-Reg+ Beef on pH, purge loss, and proximate composition of strip loins from steers finished in an all-natural program 97
Table 3.2 Least square means for the effect of aging day on WBSF and cook loss values of steaks.. 98
Table 3.3 Least square means for effect display day on objective color (L*, a*, b*) values, subjective color, subjective discoloration, and TBARS values of steaks over a simulated retail display 99

LIST OF FIGURES

Figure 3.1 Representative image of simulated retail display......................................95
Figure 3.2 Representation of beef color scores utilized to for subjective color panel..96

LIST OF TABLES

Table 2.1. Ingredient and nutrient composition of finishing diet 67

Table 2.2 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on growth performance and health outcomes of steers finished in an all-natural program ... 68

Table 2.3 Influence of inclusion of By-O-Reg+ Beef on distribution of liver scores (%) 69

Table 2.4 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on carcass traits of steers finished in an all-natural program.. 70

Table 2.5 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on objective color of carcasses of steers finished in an all-natural program 71

Table 3.1 Least square means for the effect of By-O-Reg+ Beef on pH, purge loss, and proximate composition of strip loins from steers finished in an all-natural program....... 97

Table 3.2 Least square means for the effect of aging day on WBSF and cook loss values of steaks 98

Table 3.3 Least square means for effect display day on objective color (L*, a*, b*) values, subjective color, subjective discoloration, and TBARS values of steaks over a simulated retail display 99

ABSTRACT

THE INFLUENCE OF BY-O-REG+ BEEF, AN OREGANO ESSENTIAL OIL SUPPLEMENT, ON GROWTH PERFORMANCE, CARCASS TRAITS AND MEAT QUALITY OF FINISHING BEEF STEERS.

BERGIN DEBRUIN

2024

The objective of this thesis was to determine the influence of an oregano-based essential oil (OEO; By-O-Reg+ Beef), on growth performance, carcass traits, and meat quality of beef steers finished in an all-natural program. Yearling steers were allotted to 16 pens (n = 8 pens per treatment, 8 steers per pen). Treatments included: 1) control (CON) fed no oregano-based essential oil and 2) group fed 4 g/steer daily of By-O-Reg+ Beef. Steers were harvested on day 149 of the feeding period. Standard carcass data was collected and instrumental color (L^*, a^*, b^*) was recorded. Strip loins were collected from a subsample of carcasses (n = 62; 4 animals closest to mean live body weight per pen). Purge loss and pH were recorded and strip loins were fabricated into steaks. One steak was utilized for proximate composition analysis. Four steaks were assigned to age for 4, 7, 14 or 21 days for Warner-Bratzler Shear Force (WBSF) analysis. One steak was overwrapped with oxygen permeable film and placed in simulated retail display for 10 days for evaluation of objective color (L^*, a^*, b^*), subjective color, and discoloration. Three steaks were aged for 4, 7, or 10 days for analysis of lipid oxidation. Treatment did not influence ($P > 0.05$) growth performance, carcass traits, liver score, initial color, purge loss, or proximate composition. Striploins from the OEO treatment had increased pH values ($P = 0.0279$) compared to CON. No treatment by day interaction ($P > 0.05$) was observed for WBSF, objective color, subjective color score, or discoloration during the retail display period. However, WBSF decreased ($P < 0.0001$) over the aging period. Further, L^* values increased ($P < 0.001$) from day 0 to day 10, while a^* values and b^* values decreased ($P < 0.0001$) during the display period. Similarly, subjective color scores increased ($P < 0.0001$) indicating samples appeared darker as display dates increased. Lipid oxidation also increased ($P < 0.0001$) over the display period. These data indicate that the inclusion of an oregano-based essential oil has limited impact on the growth performance, carcass traits and meat quality of steers.

CHAPTER 1 LITERATURE REVIEW

Growth Promoting Technology in Livestock Feeding

The agriculture industry is facing the challenge of provide sufficient high quality protein to meet the growing global demand (Henchion et al., 2017). Based on a report commissioned by the Food and Agriculture Organization (FAO), it is estimated that by the year 2050 animal protein consumption will be twice as much as it was in 2010 (McLeod, 2011). This is partially due to global population growth as well as increases in income and a growing middle class in developing countries. These trends are also driving a transition into a more urbanized population, reducing the amount of available land and resources for production agriculture (Giyarsih et al., 2023). In the United States beef industry, one strategy for meeting the growing demand for protein is improving growth performance, specifically gain efficiency. Much of the research in this area has focused on the effects of various categories of feed technologies and dietary additives on growth efficiency to support the production of more beef with less resource utilization (Stackhouse-Lawson et al., 2013). However, it is also critical to understand the influence of any new technology or additive on the composition and quality of the final product. This will allow the beef industry to meet the demand for more protein but also ensure that the quality of beef is not compromised, which could influence demand.

Classifications of Growth Promotion Technologies

Three classes of growth promoting technologies are commonly used in the beef feeding industry: implants, beta-adrenergic agonists, and ionophores/antimicrobials (Broocks et al., 2017). Each of these technologies target different functional systems of the animal, but with a similar goal of increasing the pounds of beef produced while decreasing the resources needed to produce them.

Implants - Background

Implants are a growth promoting technology with a long history of use within the U.S. beef industry. The first commercial implant was introduced in 1957 and since that time implants have been utilized extensively in multiple segments of the beef industry. Implants consist of small pellets that are administered subcutaneously in the ear. The pellets contain either natural or synthetic anabolic compounds that can produce physiological responses similar to natural hormones (Reuter et al., 2017). This response is largely influenced by the dosage or ‘potency’ of the active compound, which is generally classified as low, medium, or high potency (Reuter et al., 2017; Smith & Johnson, 2020). Active compounds in steroidal implants are based on three categories of hormones: androgens (male hormones), estrogens (female hormones), or progestins (pregnancy hormones; Smith & Johnson, 2016). Upon administration, anabolic compounds are slowly released for a time period that can range from 60 to over 200 days. This effective period, also known as the payout period, can be influenced by the formulation of the implant. It can also be modified by the addition of a delayed release coating, which slows the release of the active compounds from the coated pellets (Smith et al., 2019; Smith & Johnson, 2020). Upon release into the animal’s bloodstream, they are converted to the biologically active form of the hormone and can bind to steroid binding globulins and albumin for delivery to their target tissues such as muscle and adipose (Johnson & Beckett, 2014).

Implant formulations for the U.S. beef industry commonly utilize active compounds such as estradiol (natural or synthetic), progesterone, testosterone, or trenbolone acetate. These compounds can be utilized individually, or they can be used in combinations of one or more hormones (Smith & Johnson, 2020). Implant implementation strategies and selection can differ depending on the producer's goals and the specific production phase the animal is experiencing. These strategies ensure the formulation and potency of the implant matches with energy intake and growth potential of the animal (Reuter et al., 2017; FDA, 2023). For instance, low and moderate potency implants that may only include estrogen, or estrogen plus progesterone are generally utilized for suckling calves and stocker calves (Reinhardt & Thomson, 2016; Reuter et al., 2017; Smith & Johnson 2020). In general, high potency implants are reserved for cattle during the finishing phase and these tend to consist of trenbolone acetate alone or in combination with estrogen (Smith & Johnson, 2020). With implants, combining more than one hormone can produce a synergistic response compared with implants containing one active compound.

Implants - Mode of Action

Implants function to enhance growth performance and conversion of feedstuffs into lean tissue (Reinhardt, 2007; Reinhardt & Thomson, 2016). Specifically, androgenic hormone activity results in muscle tissue accretion by stimulating protein synthesis, resulting in leaner, heavier carcasses (Smith & Johnson, 2020). This occurs at the cellular level when a ligand binds to a hormone receptor (Smith & Johnson, 2020). This results in a gene transcription response, stimulating output of growth hormone (GH) from the hypothalamus and insulin like growth factor 1 (IGF-1) from the liver. Localized muscle

IGF-1 production has also been reported to stimulate differentiation and proliferation of muscle satellite cells (Florini et al., 1991; Johnson et al., 1996). In myogenic differentiation, IGF-1 is stimulated in satellite cells to support the increase muscle tissue hypertrophy (Florini et al., 1991). In addition, utilization of steroidal hormones has been shown to decrease protein degradation. Specifically, trenbolone acetate (TBA) has been shown to decrease circulation cortisol, which is a hormone that results in increased protein degradation (Hayden et al., 1992). Increase of circulating levels of GH and IGF-1, and decreased circulation of cortisol results in increased muscle accretion (Hayden et al., 1992).

Beta-adrenergic receptor agonists - Background

Beta-adrenergic receptor agonists have been researched for livestock production since the late 1970s (Dilger et al., 2021). Beta-adrenergic receptor agonists, commonly referred to as beta-agonists, are feed additives that are utilized in livestock production to enhance growth through alteration of body composition (Dilger, 2015; Johnson et al., 2014). Beta-agonists function as repartitioning agents, increasing lean meat yield through redirection of nutrients away from adipose tissue deposition and towards lean muscle accretion (Dilger et al., 2021; Smith, 2022). These feed additives have been shown to be effective for 28-42 days depending on the specific beta-agonist utilized (Dilger et al., 2021; Pfau et al., 2023). For this reason, they are generally fed to market cattle within the last month of the finishing period when cattle typically become more inefficient (Smith, 2022). There are three beta-adrenergic agonists approved by the FDA for utilization in finishing beef cattle: 1) ractopamine hydrochloride (Tradename - Optaflexx, Elanco Animal Health, Indianapolis, Indiana) 2) lubabegron (Tradename – Experior, Elanco

Animal Health, Indianapolis, Indiana) and 3) zilpaterol hydrochloride [Tradename - Zilmax, Merck Animal Health, Rahway, New Jersey (Dilger, 2015; Dilger et al., 2021; Smith, 2022)]. Lubabegron is marketed for the reduction of ammonia emissions in market animals (Smith, 2022) and Zilmax was voluntarily removed from the U.S. marketplace in August 2013 due to concerns with animal welfare. Therefore, ractopamine hydrochloride is the primary beta agonist currently fed to cattle to gain a repartitioning response (Smith, 2022).

Beta-agonist - Mode of Action

The beta-agonist mode of action is a complex process. It is understood that beta-agonists modify metabolic signals and receptors within the muscle and fat cells to direct nutrients toward lean growth (Smith, 2022). This is a result of upregulation of mRNA transcription in the muscle and decreased rates of lipid accretion (Anderson et al., 2004; Dilger et al., 2021; Smith, 2022). Inclusion of beta-agonists to the diet causes an increase of cyclic AMP concentration, which regulates cell metabolism (Johnson & Beckett, 2014; Johnson et al., 2014). The impact of beta-agonist inclusion is dependent on the type of beta receptors and their subtype classifications (Smith, 2022). These physiological based classifications are beta1, beta2, and beta3 (Johnson et al., 2014; Smith, 2022). The two main beta receptors of interest for livestock are beta1 and beta2 as they are the predominate receptors in muscle and adipose tissue (Smith, 2022). Feeding beta-agonists causes inhibition of *de novo* fatty acid synthesis, as well as stimulation of lipolysis (Johnson et al., 2014). Collectively, these responses cause a positive impact on growth performance by increasing protein synthesis which drives improvements in feed conversion, average daily gain (ADG), resulting in heavier hot carcass weights, higher dressing percentages, and improved carcass yield (Avendaño-Reyes et al., 2006; Strydom et al., 2009). However, as these compounds function by repartitioning energy from fat synthesis to lean growth, there haves been incidences of reduced marbling (Avendaño-Reyes et al., 2006; Strydom et al., 2009; Arp et al., 2014).

Antimicrobials - Background

In beef production, antimicrobials are utilized to positively modify the ruminal microflora as well as prevent pathogenic infection. Alteration of the microflora results in changes of the volatile fatty acid (VFA) proportions produced in the rumen. There are two classifications of antimicrobials utilized for production: ionophores and non-ionophore antimicrobials. The main difference between these antimicrobial groups is that ionophores are not utilized in human medicine. In comparison to non-ionophore antimicrobials, ionophores are fed at low dosages, and are most often fed once the rumen is functioning (Thompson et al., 2016).

Antimicrobials can be administered for three primary purposes: 1) prophylaxis 2) metaphylaxis, and 3) growth promotion (McEwen & Fedorka-Cray, 2002; Al-Dobaib & Mousa, 2009). Prophylactic administration of antimicrobials is intended as a preventative measure, such as inclusion to a group of newly weaned calves (Al-Dobaib & Mousa, 2009). Metaphylactic administration is utilized to treat an entire population exhibiting evidence of disease, to treat sick animals and prevent further spread of disease (Dewell et al., 2022). The FDA considers prophylaxis and metaphylaxis as therapeutic usage (McEwen & Fedorka-Cray, 2002), growth promotion refers to the use of antimicrobials administered to livestock to promote growth and enhance feed efficiency. Ionophores are classified as non-therapeutic antibiotics and are added to diets to increase digestive efficiency, ultimately increasing body weight. Ionophore are not bacteriocidal. They are bacteriostatic meaning that they do not kill bacteria, they simply inhibit their ability to reproduce and grow (Felix, 2017). Common ionophores used in cattle production are monensin, lasolacid, and laidlomycin propionate. The majority of ionophores originate from the *Streptomyces* family (Marques & Cooke, 2021). They function on the cellular level to move sodium, potassium, and calcium across membranes (Marques & Cooke, 2021). In addition, they alter the rumen microflora resulting in decreased acetate and methane production and increased propionate production, which improves growth performance of ruminants.

Ionophore- Mode of Action

Ionophores are lipophilic molecules that adhere to bacterial and protozoal membranes in the gastrointestinal tract and transport ions (sodium, potassium, or calcium) across bacterial cell membranes (Russel & Strobel, 1989; Mercer, 2022). This results in disruption of normal bacterial cell metabolism and inhibits the functionality of mainly gram-positive bacteria (Felix, 2017; Schären et al., 2017; Mercer, 2022). It is hypothesized that reducing the number of gram-positive bacteria gives a competitive advantage to gram-negative bacteria within the rumen environment. Gram negative bacteria are the bacteria that favor propionate production (Mercer, 2022). Cattle performance is dependent on the health on the ruminal microbiota and production Volatile Fatty Acids (VFA). Seventy-five to eighty-five percent of energy in a ruminant's diet is converted into short chain fatty acids (Huntington, 1997). The primary short chain fatty acids in the rumen are acetate, propionate, and butyrate, and their proportions are influenced by the animal's diet (Marques & Cooke, 2021). Of these short chain fatty acids, propionate is the most efficiently utilized VFA within the rumen and contributes to gluconeogenesis (Young, 1977). Gluconeogenesis is crucial in cattle as it provides most of the required glucose for ruminants. Glucose is a vital energy source for normal metabolic pathways for survival and growth (Weekes, 1991). Modification of rumen bacteria environment that supports increased propionate producing bacteria results in improved feed efficiency, dry matter intake, and ADG of beef cattle (Marques & Cooke, 2021).

Antimicrobial Resistance Concerns

The impact of antimicrobials and antibiotics on animal health and performance has led to increased use globally. Between 2010 and 2030, antimicrobial usage is expected to increase by approximately 67%, with a majority of this increase occurring in developing countries where production scale is increasing (Van Boeckel et al., 2015). However, the growing utilization of antimicrobials across the world has also become a concern relative to long-term efficacy of antibiotics for multiple species (McEwen & Fedorka-Cray, 2002; Samtiya et al., 2022).

With utilization of antimicrobials in both humans and animals, there is concern of antimicrobial resistance (AMR; Samtiya et al., 2022). The Center for Disease Control and Prevention (CDC) states that AMR occurs when microorganisms, such as bacteria and fungi, develop the ability to build a defense against the drugs that were designed to kill them (CDC, 2022). The development of AMR occurs naturally through genetic changes in bacteria and other pathogens within an animal's body (CDC, 2022; Samtiya et al., 2022; WHO, 2023). However, this phenomena may be enhanced through overuse of antimicrobials with intention to treat, prevent, or control infections in humans, animals, or plants (McEwen & Fedorka-Cray, 2002). Increasing pathogenic resistance to these antimicrobials leads to reduction of their impact (CDC, 2022). The threat of AMR has placed pressure on industries that utilize these medications – specifically the healthcare, veterinary, and agriculture industries – to become more considerate and stringent on their use (CDC, 2022).

Livestock Industry Response to Antimicrobial Resistance

Within the livestock industry, the response to AMR has led to increased legislation and regulation of antimicrobials. The European Union (EU) was one of the first groups to use legislation to address AMR. This process began in 1997, when the EU banned use of avoparcin, an antibiotic that was commonly used as a growth promoter. This was followed by a ban of virginiamycin, bacitracin, spiramycin, and tylosin in 1999 (Acar et al., 2000), followed by a ban on the use of all antibiotic feed additives in 2006 (Maron et al., 2013). In contrast, the United States began the process of restricting antimicrobials in 2012 when the FDA issued Guidance for Industry #209 'The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals (FDA, 2021). This was issued to establish the FDA's position that antimicrobials should not be utilized for improving growth performance responses, such as rate of gain or feed efficiency, but restricted to uses considered necessary for animal health. Furthermore, this guidance recommended that the use of these antimicrobials for treatment, prevention, and control should require veterinary oversight. On January 1, 2017, this policy went into effect and the Veterinary Feed Directive (VFD) was established. Under the VFD order, new guidelines for antimicrobial use were put into place that required producers to have a valid veterinary-client-patient relationship prior to obtaining a valid VFD. In addition to this requirement, all medically important antimicrobials were removed from over the counter availability and now require approval by a veterinarian (Dewell et al., 2022).

Technology Use in Different Beef Production Systems

In response to concerns regarding AMR and use of growth promoting technologies, producers in the beef cattle industry have worked to adapt their production and management systems. The industry has also responded by creating multiple programs that differentiate beef products based on the production system used to raise the animals. The majority of cattle in the United States are managed using a conventional production system, which allows producers to utilize all approved growth promoting technologies (i.e. steroidal implants, ionophores, and beta-adrenergic agonists; Smith et al., 2020). In addition to these growth promoting technologies, conventional production also allows for antimicrobials to be applied for therapeutic, prophylactic, and metaphylactic uses as long as regulations and proper withdrawal times are followed (Smith et al., 2020).

In addition to conventional cattle production, programs have been created in response to consumer demands for no added hormones or antibiotics in beef animal production. These programs allow beef producers to serve niche areas of the market if they comply with requirements for a specific program. The non-hormone treated cattle (NHTC) program was established by the USDA- Agricultural Marketing Service and requires that cattle are produced without added hormones throughout their entire life span (Smith et al., 2020). Implementation of a NHTC program also allows for greater traceability throughout the supply chain, which is a current consumer trend. Beef produced in an NHTC system is also eligible for trade into the EU. Producers that raise NHTC cattle for EU shipment must be approved and certified by USDA- FSIS, as well as listed with the FSIS PartnerShare website (USDA-AMS, 2021). Cattle listed in NHTC programs can receive other approved technologies such as antimicrobials and beta-agonists (Smith et al., 2020). When considering adoption of an NHTC program, producers have to assess the costs versus benefits of cattle raised and marketed without added hormones. Costs are associated with enrollment into the program, but there are also costs associated with removing the growth promoting effects of hormone implants (Webb, 2018; Webb et al., 2020; Kirkpatrick et al., 2023). Kirkpatrick et al. (2023) reported that non-implanted steers had a decreased ADG when compared to steers implanted with Revalor-XS, a trenbolone acetate and estradiol implant (1.30kg vs 1.42 kg, respectively). Webb et al. (2020) reported that the total cost of gain for NHTC treatments was increased compared to implanted cattle. When NHTC product moves forward through the beef supply chain costs are also added for testing and to maintain traceability, with additional costs for export. The majority of these additional production and marketing costs fall to the producer (Beckman et al., 2021), thus premiums are necessary to encourage producers to utilize this type of production system and fill the demand for NHTC product. In the current market, NHTC cattle are receiving a $19 to $24 dollar premium per hundredweight, according to the National Weekly Direct Slaughter Cattle - Premium and Discount report (USDA-AMS, 2024). While NHTC programs have been shown to increase production costs when compared to conventional cattle production, Webb et al. (2023) reported improved marbling scores and statistically more tender steaks from NHTC cattle, which could also provide consumer benefits.

All-natural programs are more restrictive on technology use compared with the NHTC program (Smith et al., 2020). Both NHTC and all-natural programs require third party audits throughout all segments of production to ensure full compliance with program requirements. While all-natural production programs are no-longer verified by USDA-AMS (USDA-AMS, 2015), most of the marketing programs follow the “Never Ever 3” (NE3) specifications established by USDA-AMS to support label claims on beef products raised without specific technologies or feed ingredients (Smith et al., 2020). These specifications state that the animal has never received any exogenous hormones, antibiotics (injectable or direct fed), or been fed animal by-products (USDA-AMS, 2015). In these types of systems, preventative management such as vaccines are still allowable (Stovall & McCaffery, 2005). Economic risk can be an enhanced concern when feeding cattle in all-natural production system due to increased cost of production and return on investment (Stovall & McCaffery, 2005) . This is partially attributed to the lack of growth promoting technologies being utilized. Use of these technologies is reported to improve feedlot performance and carcass yield (Cooprider et al., 2011; Capper, 2012; Maxwell et al., 2015; Webb et al., 2020). Webb et al. (2020) reported that in comparison to naturally produced steers, steers that received implants displayed improvement in dry matter intake and gain to feed ratio. Maxwell et al. (2015), compared the production of conventional cattle production to all-natural production and reported that conventionally fed cattle gained weight 32.8% faster and were 26.7% more efficient than all-natural steers. Hot carcass weights of conventional cattle were approximately 46 kg heavier, and they had larger loin muscle areas, suggesting increased cutability compared with all-natural cattle (Maxwell et al., 2015). Maxwell et al. 2015 reported no differences between the two groups for liver abscesses. However in other similar studies, (Maxwell et al., 2014; Smith et al., 2024) liver abscess prevalence was increased in cattle fed all-naturally, as ionophores such as tylosin or monensin are not allowed. The increase in liver abscesses in all-natural programs was suggested to be a potential factor that resulted in a decrease of feedlot and carcass performance, as ionophore inclusion such has been shown to enhance ADG and feed efficiency by decreasing liver abscesses (Brown et al., 1975). Loss of these technologies can translate into decreases in carcass performance (i.e. lighter hot carcass weight and smaller loin muscle area), resulting in a reduction in total carcass value.

However, producers that are able to fulfill the requirements for NE3 are eligible for premiums associated with natural programs (Stovall & McCaffery, 2005). According to the National Weekly Direct Slaughter Cattle - Premiums and Discounts Report for the week of June 17, 2024, cattle marketed in all-natural programs are receiving premiums of approximately $24-50 per hundredweight (USDA-AMS, 2024) . This premium is often translated down the supply chain resulting in higher priced natural beef products at retail. With the potential for premiums, interest in producing cattle for the natural market has grown. However, the constraint of fully realizing growth potential limits adoption and has resulted in increased interest in the search for alternative products that have potential to increase efficiency and health in cattle managed in programs that restrict the use of growth promoting technologies. A large risk of feeding all-natural cattle is animal health as animals natural treatments may not be sufficient to stop illnesses and animals that require treatment with antibiotics are no longer eligible for all-natural programs (Stovall & McCaffery, 2005; Smith et al., 2024). Most often, preventative health management is key in natural programs, however, there are points where health concerns reach a point where antibiotic intervention is necessary (Stovall & McCaffery, 2005). Antibiotic use in natural programs results in a loss considered as a “salvage value” (Stovall & McCaffery, 2005). Salvage value in this scenario is defined as purchasing cattle with a natural premium and then selling them on the commodity cash market because they were treated with antibiotics (Stovall & McCaffery, 2005). The culmination of decreased feedlot and carcass performance measures results in increased costs of gain and risk for loss of profits for cattle being fed in an all-natural program (Maxwell et al., 2015). With concern for antimicrobial resistance in the forefront, these natural programs are an important part of the market, however, the loss of efficiency and increased cost of gain is an issue that must be addressed. Natural alternatives to conventional growth promoting technologies have the potential to support the production of natural or NHTC beef without losing significant performance, however research of these alternatives is limited in beef cattle.

Potential Alternatives to Antimicrobials

As a result of the updated regulations and concern with utilization of antimicrobials in the livestock industry, natural additives have been investigated for their potential to deliver a similar function as antimicrobials and ionophores to enhance growth performance and animal health. These products include different direct fed microbials that act in a similar function such as yeast products, organic acids, probiotics, and phytogenic/essential oil additives. Specifically, there has been an increase in the availability and variety of essential oil products entering the market for multiple species.

Essential Oils - Background

The use of essential oils is not a new concept. It has been documented that these plant-derived compounds have been used by many cultures for centuries, dating back to in the ancient Egyptians who utilized plant compounds for medicinal purposes (Elshafie

& Camele, 2017). As civilizations advanced with new scientific discoveries, the use of essential oils has been expanded to include cosmetics, aromatherapy, and pharmaceutical purposes (Bakkali et al., 2008). Today, there are almost 3,000 different essential oils that have been discovered. Of these, only about 300 of them are commercially available and commonly used in the pharmaceutical, agriculture, sanitation, and cosmetics industries. Essential oils are generally recognized as safe (GRAS) by the FDA.

By definition, essential oils are blends of secondary metabolites derived from plants. These secondary metabolites are lipophilic by nature and consist of aromatic and volatile fractions obtained from plant material including flowers, roots, bark, leaves, seeds, peel, fruits, wood, and whole plants (Rios, 2016; Elshafie & Camele, 2017). In nature, these oils function to protect plants from bacteria, viruses, fungi, insects, herbivores, and omnivores (Bakkali et al., 2008). Many of these secondary metabolites maintain their biological function when extracted from the plant (Stevanović et al., 2018). Essential oils are collected using three main processes: steam distillation, solvent extraction, or hydro-distillation. Steam distillation is the most commonly utilized process however, the extraction process is dependent on the plant material that is being extracted (Tongnuanchan & Benjakul, 2014). Extraction method is one of the key factors influencing the quality of an essential oil, as improper extraction procedures can result in damage or altered action of the resulting oils (Tongnuanchan & Benjakul, 2014). The volatile nature of essential oils also impacts their bioactive properties (Asabahani et al., 2015; Ni et al., 2021). It has been reported that some of the volatile compounds are lost and/or oxidized at high temperatures and unstable pH environments (Asbahani et al., 2015; Nehme et al., 2021; Ni et al., 2021). Thus, use as a potential feed additive could be challenging given the environment of the digestive tract in livestock species (Nehme et al., 2021). Encapsulation technology has recently been investigated as an option for avoiding oxidation and preserving the bioactive function of essential oils and may help to overcome the hostile digestive tract environment (Asbahani et al., 2015; Nehme et al., 2021). The encapsulation process involves coating sensitive materials with a protective layer (Asbahani et al., 2015; Nehme et al., 2021). The parameters of encapsulation are dependent on targeted particle size, physiochemical characteristics, intended application of the encapsulated oils, intended release system of capsule, the production capacity, and cost (Sagiri et al., 2016; Nehme et al., 2021). Encapsulation allows for greater protection and preservation of biological activity for digestion as well as long-term storage (Nehme et al., 2021).

Essential Oil - Chemical Composition

Many factors influence the chemical composition of essential oils such as harvest season, genetics, agricultural practices, plant age, and the environment in which the plant grew (Nehme et al., 2021). Most essential oils are comprised of two main active chemical groups: terpenoids and phenylpropanoids (Benchaar et al., 2008; Calsamiglia et al., 2007; Castillejos et al., 2007). Terpenoids are characterized by a five carbon chemical structure that is also known as an isoprene unit (Calsamiglia et al., 2007). They are further classified by the number of isoprene units in the structure; for example, a monoterpenoid has one isoprene unit and a sesquiterpenoid has three isoprene units (Calsamiglia et al., 2007; Benchaar et al., 2008). Phenylpropanoids are less common than terpenoids but are classified by an aromatic ring attached to three carbons (Calsamiglia et al., 2007). Variation in these chemical structures generate different compounds with unique characteristics and functions. The modes of action of essential oils are impacted by composition; however, there has been documentation that synergistic (additive) effects can occur between components within an essential oil (Gavaric et al., 2015; Stevanović et al., 2018). For example, oregano essential oils (OEO) are composed of a group of compounds including carvacrol, thymol, beta-citronellol, and 1,8-cineole (Leyva-López et al., 2017). The predominant compounds in oregano, carvacrol and thymol, exert a synergistic impact on the antioxidant and anti-inflammatory properties as free radical scavengers and hydrogen atom donors, due to their phenolic structure, maximizing their additive effects (Gavaric et al., 2015; Leyva-López et al., 2017). Essential oils have been reported to possess antimicrobial, anti-inflammatory, antioxidant, digestive, antiviral, and cytotoxic properties (Castillejos et al., 2007; Benchaar et al., 2008; Leyva-López et al., 2017; Simitzis, 2017; Stevanović et al., 2018), and have therefore been investigated as potential alternatives to traditional growth promotants (Simitzis, 2017). There has been increased research into the use of essential oils on livestock health and performance.

Essential Oil - Antimicrobial Activity

Essential oils have a similar function to ionophores in that they both manipulate gram-positive and gram-negative bacteria (Benchaar et al., 2008). However, a combination of essential oil characteristics and the structure of gram positive bacteria make these bacteria more susceptible to the antibacterial properties of essential oils (Chouhan et al., 2017). This function is primarily attributed to the activity of the terpenoid and phenolic compounds of essential oils and their functional groups (Guimarães et al., 2019). The majority of terpenoids and phenolic compounds act as antimicrobial agents through interaction with the cell membrane of bacterial cells

(Chouhan et al., 2017). As essential oils are lipophilic and hydrophobic, they can invade the lipid bilayer and cause conformational changes in the bacterial cell membrane (Seow et al., 2014; Nehme et al., 2021). With disruption of this membrane, the ion gradient of the bacterial cells is negatively impacted, and glucose uptake is reduced resulting in slowed bacterial growth or cell death (Nazarro et al., 2013; Nehme et al., 2021). Studies have shown that common antimicrobial components of essential oils are thymol, carvacrol, p-cymene, y-terpinene, 1,8-cineole, and camphor (Nehme et al., 2021). It is hypothesized that the large number of varying antimicrobial components creates an additive effect among the components in an essential oil (Gavaric et al., 2015; Nehme et al., 2021). This is beneficial for an antimicrobial, as the different components can target different areas within a bacterial cell.

Essential Oil - Antioxidant Activity

In biological systems, oxidants are reactive species made up of ions such as oxygen and nitrogen produced through various metabolic processes in cells (Ali et al., 2020). They are well known to induce oxidative damage on lipids, proteins, and DNA (Ali et al., 2020). There are two classifications of these oxidants, either free radical species (ROS) or non-radical (Ali et al., 2020). Antioxidants are the counterpart to prevent oxidation from occuring (Jiang & Xiong, 2016; Ali et al., 2020). Antioxidants are either natural endogenous or synthetic exogenous molecules that slow or inhibit the oxidation process from occurring (Jiang & Xiong, 2016; Ali et al., 2020). Lipid oxidation in living organism is a natural process that occurs when the oxidant and antioxidant balance favors oxidant production and creates reactive oxygen species such as hydrogen peroxide, superoxide anions, hydroxyls, peroxyls and alkoxyl radicals (Leyva-López et al., 2017; Simitzis, 2017). These products are formed through interaction with oxygen. Oxygen readily reacts with unsaturated fatty acids, which makes them more susceptible to oxidation (Gunstone, 1984; Nehme et al., 2021). As the number of double bonds increases the chance for oxidation to occur also increases, which is influenced by animal species (Dinh et al., 2021). The fatty acid concentration of beef is approximately 50% unsaturated fatty acids (Dinh et al., 2021). The oxidative components that are produced adversely affect lipids, pigments, proteins, carbohydrates, ant the overall quality of animal products by losing nutritive value and limiting shelf-life of these products (Simitzis, 2017).

Antioxidant activity of essential oils is provided by terpenoid groups that have a phenol chemical group attached (Nehme et al., 2021). Some examples of these components are carvacrol, thymol, and eugenol (Nehme et al., 2021). They function as antioxidants by donating hydrogen atoms to free radicals within the organism and transforming free radicals into more stable products, therefore reducing the oxidation capacity of ROS (Amorati et al., 2013; Barreras et al., 2013; Simitzis, 2017; Nehme et al., 2021). However, inclusion rate of dietary essential oils may impact antioxidant response, but results are inconsistent. In some studies, dosage has not influenced performance responses, while other studies have reported that at high levels essential oils can have a negative, cytotoxic impact on cells and cause acceleration of oxidative processes (Benchaar et. al., 2006; Pukrop et al., 2019; Dorleku et al., 2021). In addition, it has been reported that in some ruminant systems addition of essential oils increases the polyunsaturated fatty acid percentages within the body increasing the overall susceptibility to oxidation (Smeti et., 2018).

Essential Oils - Rumen Modification Activity

Rumen modifiers are feed additives that have been commonly utilized in beef cattle production to enhance growth efficiency (Marques & Cooke, 2021). These products work to improve growth performance and efficiency by altering rumen fermentation and metabolic processes. Ionophores are the most common class of rumen modifiers, however as previously stated, there has been increased legislation regulating the use of antimicrobials in the diets of livestock. It has been reported that essential oils can also modify the fermentation processes in the rumen and they do not pose a regulatory concern (Calsamiglia et al 2007; Castillejos et al., 2007; Nehme et al., 2021). It is hypothesized that within a ruminant animal, essential oils work similarly to ionophores in that they target gram-positive bacteria and alter the lipid membrane of these bacteria (Calsamiglia et al., 2007). By altering the proportions of rumen bacteria, the rumen environment can be modified to potentially improve VFA proportions, specifically propionate, with dosages of essential oil compounds (eugenol, guiaiacol, limonene, thymol, and vanillin) at 5,000 mg per L of rumen fluid, resulting in a more efficient ruminant (Castillejos et al., 2006; Dorantes-Iturbide et al., 2022). Early research into utilization of essential oils in rumen modification has been primarily *in vitro* studies (Benchaar et al., 2008). These studies have revealed that inclusion of essential oils to rumen fluid results in an increase in pH (Cardozo et al., 2005; Calsamiglia et al., 2007; Castillejos et al., 2007; Nehme et al., 2021). Inclusion of essential oils has been reported to enhance feed efficiency and nutrient utilization in ruminants (Calsamiglia et al., 2007); however, the mechanism of action is not fully understood. Determining the mechanism of action is complicated due to differences in dosage, source, and type of essential oil fed

(Calsamiglia et al., 2007; Castillejos et al., 2007; Benchaar et al., 2008). While there is variation among studies between specific essential oil fed, the most common oils referenced in trials are: thymol (found in oregano and thyme), carvacrol, (found in oregano), eugenol (found in cloves), cinnemaldehyde (found in cinnamon), and anethol (found in anise). Results of *in vitro* trials have also shown that inclusion of essential oils causes a decrease in acetate, as well as a potential to increase propionate and butyrate concentrations (Calsamiglia et al., 2007). However, there is less research investigating the rumen modification capacity of essential oils *in vivo*.

Utilization in Pork and Poultry Industry

As indicated, the application of essential oils in beef diets is limited by the rumen environment. Thus, these compounds have been much more heavily researched and utilized in the pork and poultry industries. As monogastrics, the opportunity to influence performance outcomes through feed additives is more straightforward. However, research from pork and poultry can inform possible studies and utilization of these compounds in the beef industry.

The poultry industry has utilized essential oils to aid in nutrient digestibility and absorption, which has been reported to improved feed intake and feed conversion (Brenes & Roura, 2010). In poultry, dietary essential oils have three main modes of action that are reported to improve performance and decrease morbidity. Yang et al. (2018) reported that feeding an essential oil and organic acid supplement (composed of a blend of sorbic acid, fumaric acid, and thymol essential oil) positively impacted the digestive function of broiler birds and it was determined that the essential oil supplemented diet increased villi height and crypt depth of the ileum and jejunum during the finishing phase (Yang et al., 2018). This study also investigated the effect of essential oil supplementation on digestive enzymes and determined supplementation increased activity of lipase, trypsin, and chymotrypsin in the intestinal tract, which are related to improved digestibility (Yang et al., 2018). Additional modes of action include roles as antioxidants and antimicrobials. Essential oils have been shown to function as free radical scavengers in living birds and provide an antioxidant defense in the animals (Brenes & Roura, 2010). In a study conducted by Zhang et al. (2021), when broilers were fed an oregano essential oil ADG was increased and feed conversion ratio decreased. In addition to these improvements in growth performance, the oxidative stress parameters of these birds also improved with the addition of oregano essential oil (Zhang et al., 2021). Essential oils have also been investigated for their antimicrobial activity in the poultry industry. This effect has been influenced through the lipophilic properties of essential oils, which allows for invasion of bacterial cell membranes and results in bacterial cell death within the gastrointestinal tract. A study by Mathlouthi et al., (2012) was completed to characterize the impact essential oils exert on *in vitro* antimicrobial activities of three essential oils (oregano, rosemary, or a commercial blend of essential oils) in poultry systems. Results from this study reported that both rosemary and oregano essential oil inclusion resulted in antibacterial activity against *Salmonella indiana*, and *Listeria innocua*. However, the oregano essential oil also demonstrated antimicrobial activity against pathogenic bacteria, as it was effective against *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis.*

Dietary inclusion of essential oils in swine diets has been investigated across the entire life cycle. Studies have shown that inclusion of dietary essential oil blends can improve growth performance of weaned piglets, resulting in increased ADG and final body weights when compared to a control diet (Zhang et al., 2015; Yang et al., 2018; Guillou et al., 2024). In addition to improved growth performance in weaned piglets, there has been research in swine indicating that essential oils improve nutrient digestibility of finishing pigs (Maenner et al., 2011; Zeng et al., 2015). Similar to results in poultry, this outcome is hypothesized to be the result of increased digestive enzyme secretion, as well as improvement in favorable gut microflora leading to improvements in growth performance measures. Improved ADG as well as average daily feed intake have been well documented in swine supplemented with essential oils (Janz et al., 2007; Zhang et al., 2015; Huang et al., 2022).

Meat Quality Impacts

As some of the properties of essential oils are biologically beneficial, the application of these compounds in animal diets to improve the resultant meat quality has been of interest. Utilization of essential oils as natural antioxidants has resulted in decreased oxidation in meat products from lamb, poultry, swine, and beef (Janz et al., 2007; de Oliveira Monteschio et al., 2017; Azevedo et al., 2021; Muñoz-Cuautle et al., 2022). Essential oils can be added to meat products through two different approaches. The first being the direct addition of essential oils to meat products as a natural alternative to commonly utilized synthetic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT; Rodriguez-Garcia et al., 2016). The second approach is the residual influence of essential oils fed to livestock, which have the potential for residual effects on the meat that is obtained from these animals (Simitzis, 2017). For example, meat from poultry is highly susceptible to oxidation due to the inherent fatty acid composition. Dietary inclusion of a natural antioxidant has been reported to improve antioxidant capacity of the meat (He et al., 2023). Simitzis et al., (2008) revealed that meat from lambs fed an oregano essential oil had decreased oxidation (lower thiobarbituric acid reactive substances values) compared to the control group indicating a favorable effect on oxidative stability during refrigerated storage. While there is limited research reporting the effects of dietary essential oil inclusion on beef, one study completed by de Oliveira Monteschio et al., (2017), demonstrated that inclusion of a blend of essential oils (either a combination of clove and rosemary or a blend eugenol, thymol, and vanillin) fed to feedlot finished heifers resulted in reduced lipid oxidation of vacuum packaged steaks on d 7 and 14 of retail storage display when compared to a control treatment group (de Oliveira Monteschio et al., 2017). In addition, the steaks from heifers supplemented with essential oils maintained a redder color during the storage period as evidenced by a steady $a*$ value (de Oliveira Monteschio et al., 2017). This result is supported by several other studies in beef reporting that feeding essential oils slows lipid oxidation (Rivaroli et al., 2016; de Oliveira Monteschio et al., 2017; Zhang et al., 2021; Ruiz-Hernández et al., 2023). Rivaroli et al. (2016) fed an essential oil blend of oregano, garlic, lemon, rosemary, thyme, eucalyptus, and sweet orange at an inclusion rate of 0, 3.5, or 7 g per animal daily and reported a decrease in lipid oxidation at 1 d and 14 ds postmortem when the essential oil blend was fed at a rate of 3.5 g per d. However, within the same study, inclusion at 7 g per animal daily caused a prooxidant activity (increasing oxidative stress), indicating the importance of determining the correct rate of inclusion to maximize beneficial outcomes and minimize negative results. He et al., (2023) reported that inclusion of an oregano essential oil at 0, 130, or 260 mg in beef diets daily improved total antioxidant activity by increasing enzymatic activity (i.e. increase in superoxide dismutase, glutathione peroxidase, and catalase content) and decreased malondialdehyde content. Overall, He et al., (2023) concluded that inclusion of a dietary essential oil supplement improved nutritional quality by decreasing saturated fatty acid levels (C16:0 and C18:0) and improved meat quality by maintaining water holding capacity and preventing lipid oxidation. Improvement in these characteristics is critical for maintaining quality throughout the supply chain. Generally, oxidation of meat products impacts sensory characteristics such as meat color, texture, odor, and flavor, which all affect consumer acceptability.

Of these traits, meat color is the primary factor driving initial purchase decision and case life. Consumers relate bright cherry red color to desirable beef color (Suman et al., 2014). If beef in a meat retail display deviates from the expected bright cherry red color, it may be subjected to a discount or more likely discarded (Ramanathan et al., 2022). In a study conducted by Ramanathan et al. (2022) results indicated that approximately 2.55% of total beef is discarded due to discoloration. This results in approximately $3.73 billion dollars lost annually due to discoloration. This study also noted that the pounds of beef discarded represents approximately 780,000 animals wasted (Ramanathan et al., 2022). New technologies focused on improving sustainability and reducing food waste have the potential to support the increase of beef protein to feed the growing world population. Direct-fed antioxidants, such as essential oils, have the potential to function in this role.

By-O-Reg in animal production

While essential oils have been used for centuries for human medical purposes, there has been recent interest in the application of these products as alternatives in cattle production systems. However, the inherent differences in plant source, composition, and activity creates variation in animal response among different compounds and the challenge of maintaining biological activity through the rumen environment limits use in cattle. Further, while the biologic properties of essential oils point to their potential antioxidant and antimicrobial activity, our understanding of the resultant effect on carcass and meat quality traits in beef cattle is limited. By-O-Reg+ Beef is a commercially available product that includes an encapsulated oregano essential oil product intended to be directly fed to beef cattle. Multiple studies have been conducted investigating the impact of By-O-Reg (a companion product to By-O-Reg+ Beef), in the pork industry. Inclusion of By-O-Reg in nursery pigs resulted in an increased villus height as well as improved ADG without antibiotics (Thomas et al., 2015). In addition, inclusion of By-O-Reg reduced inflammatory and humoral immune reaction of pigs resulting in improved intestinal development (Park et al., 2016). The inclusion of By-O-Reg to growing/finishing pigs also shows impacts on economically important traits. Dietary inclusion resulted in improved feed efficiency of growing and finishing pigs when fed independent of antibiotic growth promoters, as well as reduced systemic oxidative stress as evaluated by malondialdehyde and protein carbonyls within serum samples (Park et al., 2016). However, studies investigating the effects of By-O-Reg+ Beef on economically important outcomes such as growth performance, animal health, and shelf life are lacking.

Summary

Growth promotants in the beef industry have been utilized to improve growth efficiency and produce more beef with less resources. However, in recent years there have been limitations placed on usage of these technologies for various reasons. Therefore, the search for alternative products that can replace the performance and economic benefits of growth promotants have been investigated. Essential oils are a potential alternative as they have been reported to improve gain efficiency as well as improve meat quality attributes when fed to livestock. However, the role and efficacy of specific essential oils in beef cattle diets remains unclear. A challenge that is faced in the ruminant animal is protection of the biological properties of the oil past the rumen; however, encapsulation technology may protect these compounds through the rumen. By-O-Reg+ Beef is an encapsulated oregano-based essential oil, but its ability to influence growth efficiency, health, carcass characteristics, and meat quality attributes of cattle fed in an all-natural program is unclear. Therefore, the objective of this thesis is to determine the impact of dietary inclusion of By-O-Reg+ Beef, an encapsulated oregano-based essential oil, on growth performance, carcass characteristics, and meat quality of yearling beef steers finished in an all-natural production program.

LITERATURE CITED

Acar, J., Casewell, M., Freeman, J., Friis, C., & Goossens, H. (2000). Avoparcin and virginiamycin as animal growth promoters: a plea for science in decision-making. *Clinical Microbiology and Infection*, *6*(9), 477-482. https://doi.org/https://doi.org/10.1046/j.1469-0691.2000.00128.x

Al-Dobaib, S., & Mousa, H. (2009). Benefits and risks of growth promoters in animal production. *J Food Agric Environ*, *7*(2), 202-208.

Ali, S. S., Ahsan, H., Zia, M. K., Siddiqui, T., & Khan, F. H. (2020). Understanding oxidants and antioxidants: Classical team with new players. *Journal of food biochemistry*, *44*(3), e13145. https://doi.org/https://doi.org/10.1111/jfbc.13145

Amorati, R., Foti, M. C., & Valgimigli, L. (2013). Antioxidant activity of essential oils. *Journal of agricultural and food chemistry*, *61*(46), 10835-10847. https://doi.org/https://doi.org/10.1021/jf403496k

Anderson, D. B., Moody, D. E., & Hancock, D. L. (2004). Beta adrenergic agonists. *Encycl. of Anim. Sci*, 104-108.

Arp, T. S., Howard, S. T., Woerner, D. R., Scanga, J. A., McKenna, D. R., Kolath, W. H., Chapman, P. L., Tatum, J. D., & Belk, K. E. (2014). Effects of dietary ractopamine hydrochloride and zilpaterol hydrochloride supplementation on performance, carcass traits, and carcass cutability in beef steers1. *Journal of Animal Science*, *92*(2), 836-843. https://doi.org/10.2527/jas.2013-7122

Asbahani, A. E., Miladi, K., Badri, W., Sala, M., Addi, E. H. A., Casabianca, H., Mousadik, A. E., Hartmann, D., Jilale, A., Renaud, F. N. R., & Elaissari, A. (2015). Essential oils: From extraction to encapsulation. *International Journal of*

Pharmaceutics, *483*(1), 220-243. https://doi.org/https://doi.org/10.1016/j.ijpharm.2014.12.069

Avendaño-Reyes, L., Torres-Rodríguez, V., Meraz-Murillo, F. J., Pérez-Linares, C., Figueroa-Saavedra, F., & Robinson, P. H. (2006). Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers1,2. *Journal of Animal Science*, *84*(12), 3259-3265. https://doi.org/10.2527/jas.2006-173

Azevedo, I. L., Nogueira, W. C. L., de Almeida, A. C., Guedes, L. L. M., Vieira, C. R., Santos, S. H. S., Carvalho, C. M. C., da Fonseca, F. S. A., de Souza, R. M., & de Souza, C. N. (2021). Antioxidant activity and chemical composition of meat from broilers fed diets containing different essential oils. *Veterinary World*, *14*(6), 1638. https://doi.org/10.14202/vetworld.2021.1638-1643

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and chemical toxicology*, *46*(2), 446-475. https://doi.org/https://doi.org/10.1016/j.fct.2007.09.106

Barreras, A., Castro-Pérez, B., López-Soto, M., Torrentera, N., Montaño, M., Estrada-Angulo, A., Ríos, F., Dávila-Ramos, H., Plascencia, A., & Zinn, R. (2013). Influence of ionophore supplementation on growth performance, dietary energetics and carcass characteristics in finishing cattle during period of heat stress. *Asian-Australasian journal of animal sciences*, *26*(11), 1553. https://doi.org/10.5713/ajas.2013.13216

Beckman, J., Burfisher, M., Mitchell, L., & Arita, S. (2021). Hidden obstacles to trade: The case of the EU's Ban on beef hormones. *Journal of Policy Modeling*, *43*(6), 1332-1343. https://doi.org/https://doi.org/10.1016/j.jpolmod.2020.10.005

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G., Colombatto, D., McAllister, T. A., & Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology*, *145*(1-4), 209-228. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2007.04.014

Benchaar, C., Duynisveld, J. L., & Charmley, E. (2006). Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. *Canadian Journal of Animal Science*, *86*(1), 91-96. https://doi.org/10.4141/a05-027

Brenes, A., & Roura, E. (2010). Essential oils in poultry nutrition: Main effects and modes of action. *Animal Feed Science and Technology*, *158*(1-2), 1-14. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2010.03.007

Broocks, A., Rolf, M., & Place, S. (2017). *Growth promotants reduce beef's environmental impact*.

Brown, H., Bing, R., Grueter, H., McAskill, J., Cooley, C., & Rathmacher, R. (1975). Tylosin and chlortetracycline for the prevention of liver abscesses, improved weight gains and feed efficiency in feedlot cattle. *Journal of Animal Science*, *40*(2), 207-213. https://doi.org/https://doi.org/10.2527/jas1975.402207x

Calsamiglia, S., Busquet, M., Cardozo, P., Castillejos, L., & Ferret, A. (2007). Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of* *dairy science*, *90*(6), 2580-2595. https://doi.org/https://doi.org/10.3168/jds.2006-644

Capper, J. L. (2012). Is the grass always greener? Comparing the environmental impact of conventional, natural and grass-fed beef production systems. *Animals*, *2*(2), 127-143. https://doi.org/https://doi.org/10.3390/ani2020127

Cardozo, P., Calsamiglia, S., Ferret, A., & Kamel, C. (2005). Screening for the effects of natural plant extracts at different pH on in vitro rumen microbial fermentation of a high-concentrate diet for beef cattle. *Journal of Animal Science*, *83*(11), 2572-2579. https://doi.org/https://doi.org/10.2527/2005.83112572x

Castillejos, L., Calsamiglia, S., & Ferret, A. (2006). Effect of Essential Oil Active Compounds on Rumen Microbial Fermentation and Nutrient Flow in In Vitro Systems. *Journal of dairy science*, *89*(7), 2649-2658. https://doi.org/https://doi.org/10.3168/jds.S0022-0302(06)72341-4

Castillejos, L., Calsamiglia, S., Ferret, A., & Losa, R. (2007). Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology*, *132*(3-4), 186-201. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2006.03.023

CDC. (2022, October 5, 2022). *How Antimicrobial Resistance Happens* https://www.cdc.gov/drugresistance/about/how-resistance-happens.html#:~:text=Antimicrobial%20resistance%20happens%20when%20germs,is%20a%20naturally%20occurring%20process.

Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. *Medicines*, *4*(3), 58. https://www.mdpi.com/2305-6320/4/3/58

Cooprider, K., Mitloehner, F., Famula, T., Kebreab, E., Zhao, Y., & Van Eenennaam, A. (2011). Feedlot efficiency implications on greenhouse gas emissions and sustainability. *Journal of Animal Science*, *89*(8), 2643-2656. https://doi.org/https://doi.org/10.2527/jas.2010-3539

de Oliveira Monteschio, J., de Souza, K. A., Vital, A. C. P., Guerrero, A., Valero, M. V., Kempinski, E. M. B. C., Barcelos, V. C., Nascimento, K. F., & do Prado, I. N. (2017). Clove and rosemary essential oils and encapsuled active principles (eugenol, thymol and vanillin blend) on meat quality of feedlot-finished heifers. *Meat Science*, *130*, 50-57. https://doi.org/https://doi.org/10.1016/j.meatsci.2017.04.002

Dewell, G. A., Rademacher, C. J., & Sato, Y. (2022). Review of regulations and indications for the use of in-feed antimicrobials for production animals. *Journal of the American Veterinary Medical Association*, *260*(S3), S129-S132. https://doi.org/10.2460/javma.22.07.0300

Dilger, A. (2015). Beta-agonists: what are they and why do we use them in livestock production. *Fact Sheet, American Meat Science Association, Kearney, MO*.

Dilger, A. C., Johnson, B. J., Brent, P., & Ellis, R. L. (2021). Comparison of beta-ligands used in cattle production: structures, safety, and biological effects. *Journal of Animal Science*, *99*(8). https://doi.org/10.1093/jas/skab094

Dinh, T., Schilling (2021). Fatty Acid Composition of Meat Animals as Flavor Precursors. *Meat and Muscle Biology*, *5(1)*(34), 1-16. https://doi.org/https://doi.org/10.22175/mmb.12251

Dorantes-Iturbide, G., Orzuna-Orzuna, J. F., Lara-Bueno, A., Mendoza-Martínez, G. D., Miranda-Romero, L. A., & Lee-Rangel, H. A. (2022). Essential oils as a dietary additive for small ruminants: A meta-analysis on performance, rumen parameters, serum metabolites, and product quality. *Veterinary Sciences*, *9*(9), 475. https://doi.org/https://doi.org/10.3390/vetsci9090475

Elshafie, H. S., & Camele, I. (2017). An overview of the biological effects of some mediterranean essential oils on human health. *BioMed research international*, *2017*. https://doi.org/ https://doi.org/10.1155/2017/9268468

FDA. (2023). *FDA Provides a List of the Currently-Approved and Currently-Marketed Implants Available by Beef Cattle Target Animal Class Which Clarifies the Approved Conditions of Use Regarding Use in a Reimplantation Program*. Retrieved April 4 from

FDA, U. (2021). CVM GFI# 209: the judicious use of medically important antimicrobial drugs in food-producing animals. 2019. In.

Felix, T. L. (2017). Ionophores: A Technology to Improve Cattle Efficicency. https://extension.psu.edu/ionophores-a-technology-to-improve-cattle-efficiency

Florini, J. R., Ewton, D. Z., & Roof, S. L. (1991). Insulin-Like Growth Factor-I Stimulates Terminal Myogenic Differentiation by Induction of Myogenin Gene Expression. *Molecular Endocrinology*, *5*(5), 718-724. https://doi.org/10.1210/mend-5-5-718

Gavaric, N., Mozina, S. S., Kladar, N., & Bozin, B. (2015). Chemical Profile, Antioxidant and Antibacterial Activity of Thyme and Oregano Essential Oils, Thymol and Carvacrol and Their Possible Synergism. *Journal of Essential Oil Bearing Plants*, *18*(4), 1013-1021. https://doi.org/10.1080/0972060X.2014.971069

Giyarsih, S. R., Armansyah, A., Zaelany, A. A., Latifa, A., Setiawan, B., Saputra, D., Haqi, M., Fathurohman, A., & Lamijo, L. (2023). Interrelation of Urban Farming and Urbanization: An Alternative Solution to Urban Food and Environmental Problems Due to Urbanization in Indonesia. *Frontiers in Built Environment*, *9*, 1192130. https://doi.org/https://doi.org/10.3389/fbuil.2023.1192130

Guillou, D., Brillouet, D., Bryte, J., & Lemoine, N. (2024). 194 Effects of combining plant and yeast extracts with essential oils or protected betaine on performance and health of weaned piglets. *Journal of Animal Science*, *102*(Supplement_2), 141-142. https://doi.org/10.1093/jas/skae102.157

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M., & Scherer, R. (2019). Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules*, *24*(13), 2471. https://www.mdpi.com/1420-3049/24/13/2471

Gunstone, F. D. (1984). Reaction of oxygen and unsaturated fatty acids. *Journal of the American Oil Chemists' Society*, *61*(2), 441-447. https://doi.org/10.1007/BF02678811

Hayden, J. M., Bergen, W. G., & Merkel, R. A. (1992). Skeletal muscle protein metabolism and serum growth hormone, insulin, and cortisol concentrations in growing steers implanted with estradiol-17β, trenbolone acetate, or estradiol-17β plus trenbolone acetate2. *Journal of Animal Science*, *70*(7), 2109-2119. https://doi.org/10.2527/1992.7072109x

He, P., Lei, Y., Zhang, K., Zhang, R., Bai, Y., Li, Z., Jia, L., Shi, J., Cheng, Q., Ma, Y., Zhang, X., Liu, L., & Lei, Z. (2023). Dietary oregano essential oil supplementation alters meat quality, oxidative stability, and fatty acid profiles of beef cattle. *Meat Science*, *205*, 109317. https://doi.org/https://doi.org/10.1016/j.meatsci.2023.109317

Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., & Tiwari, B. (2017). Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. *Foods*, *6*(7), 53. https://doi.org/https://doi.org/10.3390/foods6070053

Huang, C., Chen, D., Tian, G., He, J., Zheng, P., Yu, J., Mao, X., Huang, Z., Yan, H., & Wang, Q. (2022). Effects of dietary plant essential oil supplementation on growth performance, nutrient digestibility and meat quality in finishing pigs. *Journal of Animal Physiology and Animal Nutrition*, *106*(6), 1246-1257. https://doi.org/https://doi.org/10.1111/jpn.13673

Huntington, G. B. (1997). Starch utilization by ruminants: from basics to the bunk. *Journal of Animal Science*, *75*(3), 852-867. https://doi.org/https://doi.org/10.2527/1997.753852x

Janz, J. A. M., Morel, P. C. H., Wilkinson, B. H. P., & Purchas, R. W. (2007). Preliminary investigation of the effects of low-level dietary inclusion of fragrant essential oils and oleoresins on pig performance and pork quality. *Meat Science*, *75*(2), 350-355. https://doi.org/https://doi.org/10.1016/j.meatsci.2006.06.027

Jiang, J., & Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, *120*, 107-117. https://doi.org/https://doi.org/10.1016/j.meatsci.2016.04.005

Johnson, B., & Beckett, J. (2014). Application of growth enhancing compounds in modern beef production executive summary. *Reference Paper. Champaign (IL): American Meat Science Association*, 1-15.

Johnson, B. J., Hathaway, M. R., Anderson, P. T., Meiske, J. C., & Dton, W. R. (1996). Stimulation of circulating insulin-like growth factor I (IGF-I) and insulin-like growth factor binding proteins (IGFBP) due to administration of a combined trenbolone acetate and estradiol implant in feedlot cattle. *Journal of Animal Science*, *74*(2), 372-379. https://doi.org/10.2527/1996.742372x

Johnson, B. J., Smith, S. B., & Chung, K. Y. (2014). Historical overview of the effect of β-adrenergic agonists on beef cattle production. *Asian-Australasian journal of animal sciences*, *27*(5), 757. https://doi.org/ 10.5713/ajas.2012.12524

Kirkpatrick, T. J., Wesley, K. R., Pillmore, S. L., Cooper, K. B., Francis, F. L., Tennant, T. C., Nichols, W. T., Hutcheson, J. P., Walter, L.-A., & Lawrence, T. E. (2023). Effects of ds on feed and growth implant administration on live growth performance, live and carcass biometric measures, and carcass grading outcomes. *Applied Animal Science*, *39*(5), 330-348. https://doi.org/https://doi.org/10.15232/aas.2023-02395

Leyva-López, N., Gutiérrez-Grijalva, E. P., Vazquez-Olivo, G., & Heredia, J. B. (2017). Essential oils of oregano: Biological activity beyond their antimicrobial properties. *Molecules*, *22*(6), 989. https://doi.org/https://doi.org/10.3390/molecules22060989

Maenner, K., Vahjen, W., & Simon, O. (2011). Studies on the effects of essential-oil-based feed additives on performance, ileal nutrient digestibility, and selected bacterial groups in the gastrointestinal tract of piglets1. *Journal of Animal Science*, *89*(7), 2106-2112. https://doi.org/10.2527/jas.2010-2950

Maron, D. F., Smith, T. J., & Nachman, K. E. (2013). Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. *Globalization and health*, *9*, 1-11. https://doi.org/https://doi.org/10.1186/1744-8603-9-48

Marques, R. d. S., & Cooke, R. F. (2021). Effects of ionophores on ruminal function of beef cattle. *Animals*, *11*(10), 2871.

Mathlouthi, N., Bouzaienne, T., Oueslati, I., Recoquillay, F., Hamdi, M., Urdaci, M., & Bergaoui, R. (2012). Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: In vitro antimicrobial activities and effects on growth performance1. *Journal of Animal Science*, *90*(3), 813-823. https://doi.org/10.2527/jas.2010-3646

Maxwell, C. L., Bernhard, B. C., O'Neill, C. F., Wilson, B. K., Hixon, C. G., Haviland, C. L., Grimes, A. N., Calvo-Lorenzo, M. S., VanOverbeke, D. L., Mafi, G. G., Richards, C. J., Step, D. L., Holland, B. P., & Krehbiel, C. R. (2015). The effects of technology use in feedlot production systems on feedlot performance and carcass characteristics1. *Journal of Animal Science*, *93*(3), 1340-1349. https://doi.org/10.2527/jas.2014-8127

Maxwell, C. L., Krehbiel, C. R., Wilson, B. K., Johnson, B. T., Bernhard, B. C., O'Neill, C. F., VanOverbeke, D. L., Mafi, G. G., Step, D. L., & Richards, C. J. (2014). Effects of beef production system on animal performance and carcass characteristics1. *Journal of Animal Science*, *92*(12), 5727-5738. https://doi.org/10.2527/jas.2014-7639

McEwen, S. A., & Fedorka-Cray, P. J. (2002). Antimicrobial Use and Resistance in Animals. *Clinical Infectious Diseases*, *34*(Supplement_3), S93-S106. https://doi.org/10.1086/340246

McLeod, A. (2011). *World livestock 2011-livestock in food security*. Food and agriculture organization of the united nations (FAO).

Mercer, M. A. (2022). *Ionophores Use in Animals*. Retrieved April 9 from https://www.merckvetmanual.com/pharmacology/antibacterial-agents/ionophores-use-in-animals

Muñoz-Cuautle, A., Ortega-Cerrilla, M. E., Herrera-Haro, J. G., Nava-Cuellar, C., Gutiérrez-Olvera, C., Ramírez-Bribiesca, J. E., & Zetina-Córdoba, P. (2022). Effect of Oregano (Lippia graveolens) Essential Oil as a Phytogenic Feed Additive on Productive Performance, Ruminal Fermentation, and Antioxidant Activity in Lamb Meat. *Agriculture*, *12*(7), 973. https://www.mdpi.com/2077-0472/12/7/973

Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of Essential Oils on Pathogenic Bacteria. *Pharmaceuticals*, *6*(12), 1451-1474. https://www.mdpi.com/1424-8247/6/12/1451

Nehme, R., Andrés, S., Pereira, R. B., Ben Jemaa, M., Bouhallab, S., Ceciliani, F., López, S., Rahali, F. Z., Ksouri, R., & Pereira, D. M. (2021). Essential oils in livestock: From health to food quality. *Antioxidants*, *10*(2), 330. https://doi.org/https://doi.org/10.3390/antiox10020330

Ni, Z.-J., Wang, X., Shen, Y., Thakur, K., Han, J., Zhang, J.-G., Hu, F., & Wei, Z.-J. (2021). Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils. *Trends in Food Science & Technology*, *110*, 78-89. https://doi.org/https://doi.org/10.1016/j.tifs.2021.01.070

Park, I., Lee, J. K., Wang, J., & Kim, S. W. (2016). 0948 Effects of dietary supplementation of phytobiotics on intestinal health and growth performance of nursery pigs. *Journal of Animal Science*, *94*(suppl_5), 456-456. https://doi.org/10.2527/jam2016-0948

Park, I., Parnsen, W., & Kim, S. W. (2016). 271 Effects of dietary supplementation of phytobiotics on growth performance and health status of growing-finishing pigs. *Journal of Animal Science*, *94*(suppl_2), 127-128. https://doi.org/10.2527/msasas2016-271

Pfau, A. P., Shepherd, E. A., Martin, M. G., Ascolese, S., Mason, K. M., Egert-McLean, A. M., Voy, B. H., & Myer, P. R. (2023). Beta-Adrenergic Agonists, Dietary Protein, and Rumen Bacterial Community Interactions in Beef Cattle: A Review. *Veterinary Sciences*, *10*(9), 579. https://doi.org/https://doi.org/10.3390/vetsci10090579

Ramanathan, R., Lambert, L. H., Nair, M. N., Morgan, B., Feuz, R., Mafi, G., & Pfeiffer, M. (2022). Economic loss, amount of beef discarded, natural resources wastage, and environmental impact due to beef discoloration. *Meat and Muscle Biology*, *6*(1). https://doi.org/https://doi.org/10.22175/mmb.13218

Reinhardt, C. (2007). Growth-promotant implants: Managing the tools. *Veterinary Clinics of North America: Food Animal Practice*, *23*(2), 309-319. https://doi.org/https://doi.org/10.1016/j.cvfa.2007.03.004

Reinhardt, C. D., & Thomson, D. U. (2016). Growth promotant implants in suckling calves and stocker cattle: Mode of action, performance response, and practical recommendations. *The Bovine Practitioner*, 40-46. https://doi.org/https://doi.org/10.21423/bovine-vol50no1p40-46

Reuter, R., Mourer, G., Lalman, D., & Richards, C. (2017). *Implants and their use in beef cattle production*.

Ríos, J.-L. (2016). Essential oils: What they are and how the terms are used and defined. In *Essential oils in food preservation, flavor and safety* (pp. 3-10). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-416641-7.00001-8

Rivaroli, D. C., Guerrero, A., Velandia Valero, M., Zawadzki, F., Eiras, C. E., Campo, M. D. M., Sañudo, C., Mendes Jorge, A., & Nunes do Prado, I. (2016). Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Sci*, *121*, 278-284. https://doi.org/10.1016/j.meatsci.2016.06.017

Rodriguez-Garcia, I., Silva-Espinoza, B. A., Ortega-Ramirez, L. A., Leyva, J. M., Siddiqui, M. W., Cruz-Valenzuela, M. R., Gonzalez-Aguilar, G. A., & Ayala-Zavala, J. F. (2016). Oregano Essential Oil as an Antimicrobial and Antioxidant Additive in Food Products. *Critical Reviews in Food Science and Nutrition*, *56*(10), 1717-1727. https://doi.org/10.1080/10408398.2013.800832

Ruiz-Hernández, K., Sosa-Morales, M. E., Cerón-García, A., & Gómez-Salazar, J. A. (2023). Physical, chemical and sensory changes in meat and meat products induced by the addition of essential oils: A concise review. *Food Reviews International*, *39*(4), 2027-2056. https://doi.org/https://doi.org/10.1080/87559129.2021.1939369

Russell, J. B., & Strobel, H. (1989). Effect of ionophores on ruminal fermentation. *Applied and Environmental Microbiology*, *55*(1), 1-6. https://doi.org/Doi: 0099-2240/89/010001-06$02.00/0

Sagiri, S. S., Anis, A., & Pal, K. (2016). Review on Encapsulation of Vegetable Oils: Strategies, Preparation Methods, and Applications. *Polymer-Plastics Technology and Engineering*, *55*(3), 291-311. https://doi.org/10.1080/03602559.2015.1050521

Samtiya, M., Matthews, K. R., Dhewa, T., & Puniya, A. K. (2022). Antimicrobial Resistance in the Food Chain: Trends, Mechanisms, Pathways, and Possible Regulation Strategies. *Foods*, *11*(19), 2966. https://www.mdpi.com/2304-8158/11/19/2966

Schären, M., Drong, C., Kiri, K., Riede, S., Gardener, M., Meyer, U., Hummel, J., Urich, T., Breves, G., & Dänicke, S. (2017). Differential effects of monensin and a blend of essential oils on rumen microbiota composition of transition dairy cows. *Journal of dairy science*, *100*(4), 2765-2783. https://doi.org/https://doi.org/10.3168/jds.2016-11994

Seow, Y. X., Yeo, C. R., Chung, H. L., & Yuk, H.-G. (2014). Plant Essential Oils as Active Antimicrobial Agents. *Critical Reviews in Food Science and Nutrition*, *54*(5), 625-644. https://doi.org/10.1080/10408398.2011.599504

Simitzis, P. E. (2017). Enrichment of animal diets with essential oils—a great perspective on improving animal performance and quality characteristics of the derived products. *Medicines*, *4*(2), 35. https://doi.org/https://doi.org/10.3390/medicines4020035

Simitzis, P. E., Deligeorgis, S. G., Bizelis, J. A., Dardamani, A., Theodosiou, I., & Fegeros, K. (2008). Effect of dietary oregano oil supplementation on lamb meat characteristics. *Meat Science*, *79*(2), 217-223. https://doi.org/https://doi.org/10.1016/j.meatsci.2007.09.005

Smeti, S., Hajji, H., Mekki, I., Mahouachi, M., & Atti, N. (2018). Effects of dose and administration form of rosemary essential oils on meat quality and fatty acid profile of lamb. *Small Ruminant Research*, *158*, 62-68. https://doi.org/https://doi.org/10.1016/j.smallrumres.2017.10.007

Smith, J., Darambazar, E., Penner, G. B., Erickson, N., Larson, K., McKinnon, J., Damiran, D., & Lardner, H. A. (2024). Effects of conventional and natural management systems on backgrounding and finishing performance of beef steers. *Applied Animal Science*, *40*(2), 150-166. https://doi.org/10.15232/aas.2023-02425

Smith, Z. K. (2022). Use of Beta-Adrenergic Receptor Agonists in Animals https://www.merckvetmanual.com/pharmacology/growth-promotants-and-production-enhancers/use-of-beta-adrenergic-receptor-agonists-in-animals#:~:text=The%20major%20use%20of%20beta,by%207%25%E2%80%9320%25.

Smith, Z. K., Anderson, P. T., & Johnson, B. J. (2020). Finishing cattle in all-natural and conventional production systems. https://doi.org/https://doi.org/10.4236/ojas.2020.102013

Smith, Z. K., Holland, B. P., Word, A. B., Crawford, G. I., Nichols, W. N., Nuttelman, B. L., Streeter, M. N., Hutcheson, J. P., & Johnson, B. J. (2019). Effects of a single initial and delayed release implant on arrival compared with a non-coated initial implant and a non-coated terminal implant in heifers fed across various ds on feed. *Translational Animal Science*, *3*(4), 1182-1193. https://doi.org/10.1093/tas/txz127

Smith, Z. K., & Johnson, B. J. (2020). Mechanisms of steroidal implants to improve beef cattle growth: a review. *Journal of Applied Animal Research*, *48*(1), 133-141. https://doi.org/https://doi.org/10.1080/09712119.2020.1751642

Stackhouse-Lawson, K. R., Calvo, M. S., Place, S. E., Armitage, T. L., Pan, Y., Zhao, Y., & Mitloehner, F. M. (2013). Growth promoting technologies reduce greenhouse gas, alcohol, and ammonia emissions from feedlot cattle. *Journal of Animal Science*, *91*(11), 5438-5447. https://doi.org/10.2527/jas.2011-4885

Stevanović, Z. D., Bošnjak-Neumüller, J., Pajić-Lijaković, I., Raj, J., & Vasiljević, M. (2018). Essential oils as feed additives—Future perspectives. *Molecules*, *23*(7), 1717. https://doi.org/https://doi.org/10.3390/molecules23071717

Stovall, T., & McCaffery, J. (2005). Natural beef in the feedlot: Risk & return to feeder calf premiums. Range Beef Cow Symposium, Strydom, P., Frylinck, L., Montgomery, J., & Smith, M. (2009). The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. *Meat Science*, *81*(3), 557-564. https://doi.org/https://doi.org/10.1016/j.meatsci.2008.10.011

Suman, S. P., Hunt, M. C., Nair, M. N., & Rentfrow, G. (2014). Improving beef color stability: Practical strategies and underlying mechanisms. *Meat Science*, *98*(3), 490-504. https://doi.org/https://doi.org/10.1016/j.meatsci.2014.06.032

Thomas, L., Woodworth, J., Goodband, R., DeRouchey, J., Tokach, M., & Dritz, S. (2015). Determining the Impact of By-O-Reg+ in Diets with or without a Feed Grade Antibiotic on Growth Performance of Nursery Pigs. *Kansas Agricultural Experiment Station Research Reports*, *1*(7), 35. https://doi.org/https://doi.org/10.4148/2378-5977.1140

Thompson, A. J., Smith, Z. K. F., Corbin, M. J., Harper, L. B., & Johnson, B. J. (2016). Ionophore strategy affects growth performance and carcass characteristics in feedlot steers1. *Journal of Animal Science*, *94*(12), 5341-5349. https://doi.org/10.2527/jas.2016-0841

Tongnuanchan, P., & Benjakul, S. (2014). Essential oils: extraction, bioactivities, and their uses for food preservation. *Journal of food science*, *79*(7), R1231-R1249.

USDA-AMS. (2015, November 13,2015). *NE3 Marketing Program*. Retrieved April 5 from https://www.ams.usda.gov/content/ne3-marketing-program

USDA-AMS. (2021). Food Safety and Inspection Service's Program for Certifying Non-Hormone Treated Beef to the European Union. In.

USDA-AMS. (2024). *National Weekly Direct Slaughter Cattle-Premiums and Discounts*.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., Teillant, A., & Laxminarayan, R. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences*, *112*(18), 5649-5654. https://doi.org/https://doi.org/10.1073/pnas.1503141112

Webb, M. J. (2018). *Influence of production system on animal performance, carcass characteristics, meat quality, environmental impacts, production economics, and consumer preference for beef*. South Dakota State University.

Webb, M. J., Block, J. J., Harty, A. A., Salverson, R. R., Daly, R. F., Jaeger, J. R., Underwood, K. R., Funston, R. N., Pendell, D. P., Rotz, C. A., Olson, K. C., & Blair, A. D. (2020). Cattle and carcass performance, and life cycle assessment of production systems utilizing additive combinations of growth promotant technologies. *Translational Animal Science*, *4*(4). https://doi.org/10.1093/tas/txaa216

Weekes, T. (1991). Hormonal control of glucose metabolism. In *Physiological aspects of digestion and metabolism in ruminants* (pp. 183-200). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-702290-1.50016-3

WHO. (2023, November 21, 2023). *Antimicrobial Resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance#:~:text=AMR%20is%20a%20natural%20process,in%20humans%2C%20animals%20and%20plants.

Yang, C., Zhang, L., Cao, G., Feng, J., Yue, M., Xu, Y., Dai, B., Han, Q., & Guo, X. (2018). Effects of dietary supplementation with essential oils and organic acids on the growth performance, immune system, fecal volatile fatty acids, and microflora community in weaned piglets. *Journal of Animal Science*, *97*(1), 133-143. https://doi.org/10.1093/jas/sky426

Yang, X., Xin, H., Yang, C., & Yang, X. (2018). Impact of essential oils and organic acids on the growth performance, digestive functions and immunity of broiler chickens. *Animal Nutrition*, *4*(4), 388-393. https://doi.org/https://doi.org/10.1016/j.aninu.2018.04.005

Young, J. (1977). Gluconeogenesis in cattle: significance and methodology. *Journal of dairy science*, *60*(1), 1-15.

Zeng, Xu, X., Zhang, Q., Li, P., Zhao, P., Li, Q., Liu, J., & Piao, X. (2015). Effects of essential oil supplementation of a low-energy diet on performance, intestinal morphology and microflora, immune properties and antioxidant activities in weaned pigs. *Animal Science Journal*, *86*(3), 279-285. https://doi.org/https://doi.org/10.1111/asj.12277

Zeng, Zhang, S., Wang, H., & Piao, X. (2015). Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. *Journal of animal science and biotechnology*, *6*, 1-10. https://doi.org/https://doi.org/10.1186/s40104-015-0004-5

Zhang, L. Y., Peng, Q. Y., Liu, Y. R., Ma, Q. G., Zhang, J. Y., Guo, Y. P., Xue, Z., & Zhao, L. H. (2021). Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. *Poultry Science*, *100*(7), 101163. https://doi.org/https://doi.org/10.1016/j.psj.2021.101163

CHAPTER II: INCLUSION OF BY-O-REG+ BEEF, AN OREGANO BASED ESSENTIAL OIL FEED ADDITIVE, EFFECT ON GROWTH PERFORMANCE, HEALTH OUTCOMES, AND CARCASS CHARACTERISTICS OF YEARLING BEEF STEERS FINISHED IN AN ALL-NATURAL PROGRAM.

ABSTRACT

The objective of this study was to determine the influence of an oregano-based essential oil on growth performance, health outcomes, and carcass characteristics of yearling beef steers finished in an all-natural program. Single-sourced yearling steers [n = 128; initial body weight (BW) = 335 ± 7.98 kg] were allotted to 16 pens (n = 8 pens/treatment with 8 steers/pen). Steers were blocked by initial BW grouping in a randomized complete block design (n = 8 blocks total). Treatment groups consisted of 1) control group fed no oregano-based essential oil product (CON) and 2) a group fed 4 g/steer daily of By-O-Reg+ Beef (Advanced Ag Products, Canton, SD; OEO). Steers were individually weighed on d 0 (arrival), d 38, d 66, d 108, and d 149 for growth performance measures. Steers were transitioned from a 70% concentrate diet to a diet that contained 90% concentrate over the first 14 d and remained on the finishing diet until harvest on d 149. The finishing diet provided 1.37 Mcal/kg of NEg. Steers were evaluated daily for indications of disease or visible digestive disorders by a trained technician blinded to the treatments. Health outcomes were not influenced by dietary treatment ($P \geq 0.18$). Inclusion of OEO did not influence ($P \geq 0.73$) carcass-adjusted final BW, dry matter intake (DMI), average daily gain (ADG), gain:feed, observed dietary NEm or NEg, observed-to-expected dietary net energy for maintenance (NEm) or net

energy for gain (NEg). No differences ($P \geq 0.30$) were observed between treatment groups for carcass traits or the distribution of USDA Yield and Quality Grades ($P = 0.94$ and $P = 0.79$ respectively). Collectively, growth performance, health, and carcass traits of steers finished in an all-natural program in this experiment were not influenced by dietary inclusion of an oregano-based essential oil feed additive.

INTRODUCTION

Antibiotics and ionophores have been shown to improve animal health and increase feed efficiency within the beef industry (Bergen & Bates, 1984; Russell & Strobel, 1989; Stackhouse-Lawson et al., 2013; Thompson et al., 2016; Gadberry et al., 2022; Mercer, 2022). However, in recent years government agencies have enforced more stringent regulations on the use of antibiotics in livestock diets such as the veterinary feed directive (Sneeringer, 2015; FDA, 2017). These regulations, as well as the growth of niche markets offering premiums for decreased utilization of growth promoting technologies have caused producers to consider the benefits and limitations of different production systems and seek options for maintaining growth efficiency in programs that limit use of growth promoting technologies.

The introduction of programs such as ‘non-hormone treated cattle’ (NHTC) and ‘all-natural’ have specific requirements that limit the utilization of growth promoting technologies. For example, all-natural cattle production often follows the Never-Ever 3 requirements, which include no utilization of animal by-products, no antibiotics, and no growth promoting hormones at any point of the animals life (USDA-AMS, 2015). Feeding cattle in these types of programs pose challenges for producers working to maintain animal health and efficiency, while capturing premiums that are associated with these niche marketing programs. Natural alternatives have been investigated for their potential to deliver similar functions as antibiotics and ionophores to enhance growth performance and animal health. These natural alternatives consist of different categories such as probiotics, yeast products, enzymes, and essential oils (Beck & Biggs, 2022). Essential oils are secondary plant metabolites that have been shown to exhibit antimicrobial, antioxidant, cytotoxic, anti-inflammatory activity, and have grown in popularity due to interest in natural programs (Calsamiglia et al., 2007; Benchaar et al., 2008; Simitzis et al., 2017; Beck &Biggs, 2022). However, differences in plant source, composition, volatility, and activity of essential oils create variation in products and subsequent animal response (Ashabani et al., 2015; Nehme et al., 2021; Ni et al., 2021). The volatile nature of essential oils impacts their bioactive properties. It has been reported that some volatile compounds are oxidized at high temperatures and unstable pH conditions, making them a challenge to feed to ruminants (Asbahani et al., 2015; Ni et al., 2021). Thus, research is needed to evaluate the efficacy of these compounds on health and growth performance of beef cattle given the challenging environment of the rumen (Asbahani et al., 2015; Nehme et al., 2021; Ni et al., 2021). Encapsulation technology has been investigated as an option to avoid oxidation and aid in preservation of the bioactive function of essential oils to overcome the hostile digestive tract (Asbahani et al., 2015; Nehme et al., 2021). By-O-Reg+ Beef is an encapsulated oregano-based essential oil product approved for beef cattle. We hypothesized that encapsulation of the active components of oregano (mainly carvacrol and thymol), would allow these active compounds to by-pass the rumen and improve growth performance, health outcomes, and carcass traits of steers in an all-natural feeding system. Therefore, the objective of this study was to determine the influence of a primarily oregano-based encapsulated essential oil product on growth performance, health outcomes and carcass characteristics of yearling beef steers finished in an all-natural program in which administering conventional antibiotics and growth promoting hormones is not permitted.

MATERIALS AND METHODS

Animals, Initial Processing, and Study Initiation

All experimental procedures were approved by South Dakota State University Institutional Animal Care and Use Committee (Approval Number: 2202-005E). Single-sourced predominately Angus yearling steers (n = 128) with an initial body weight (BW) of 335 ± 7.9 kg were used in this 149-d feedlot finishing phase experiment. The steers were procured from a central South Dakota auction facility and were transported to the Southeast Research Facility in Beresford, South Dakota (~250km) to finish. Pens were open dirt lot pens that provided 54.4m^2 of pen space per steer. Approximately 10 d after arrival at the Southeast Research Farm steers were administered an individual ID tag, vaccinated against respiratory pathogens [infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD) types 1 and 2, parainfluenza-3 (PI3), and bovine respiratory syncytial virus (BRSV); Bovi-Shield Gold 5; Zoetis, Parsippany, NJ] and clostridial species (Bovilis Vision 7 with Spur, Merck Animal Health Rahway, NJ) and administered pour-on moxidectin (Cydectin, Bayer Healthcare LLC, Shawnee Mission, KS). Steers were initially weighed and allotted to 16 pens (n = 8 pens per treatment; 8 steers per pen). Steers were blocked by pen location and were grouped in a randomized complete block design (RCBD), with a total of 8 blocks (n = 16 pens). The BW on d 0 (study initiation) was used as the initial BW [initial shrunk (4%) BW= 335 ± 7.97kg]. The study period was initiated on 17 February 2023 and terminated on 16 July 2023.

Experimental Design and Treatments

This study used 16 pens (n = 8 pens/ treatment: n = 8 steers/pen) of cattle finished in an all-natural production system [no exogenous hormones, antibiotics (injectable or fed), or animal by-products]. Each pen was randomly assigned to 1 of 2 dietary treatment groups: 1) the control diet group that were not fed By-O-Reg+ Beef, an oregano-based essential oil product (CON) or 2) a diet that contained 4g per steer daily of By-O-Reg+ Beef (Advanced Ag Products, Canton, SD; OEO) in a ground corn carrier mixed with the diets.

Diet and Intake Management

Steers were fed once daily in the morning using a slick bunk style management system. The diets throughout the step-up periods are presented in Table 2.1. Steers were transitioned from a 70% concentrate diet to a diet that contained 90% concentrate over 14 d and remained on the finishing diet until study termination on d 149. Diets included dry-rolled corn, suspended supplement, modified distillers grains, grass hay, and sorghum silage. Composition of this supplement is listed in Table 2.1. Ingredient samples were obtained monthly and stored in a freezer at -20˚C until nutrient analyses were completed. Dry matter was determined (method no. 935.29; AOAC, 2012). Each ingredient was analyzed for N (method no. 968.06; AOAC, 2016; Rapid Max N Exceed; Elementar; Mt. Laurel, NJ) and ash (method no. 941.05; AOAC, 2012). The OEO treatment group varied from the CON group with inclusion of 4 grams of By-O-Reg+ Beef per steer daily mixed with a ground corn carrier.

Feedlot Health Management

Steers were evaluated daily for indication of disease or visible digestive disorders by a trained technician blinded to treatments. Due to the nature of this study, if cattle required treatment for digestive upset (bloating), non-antibiotic intervention was administered first. However, if antibiotic treatment was deemed necessary for any reason, the affected animal(s) was removed from the study. Growth performance data was reported on a deads and removals excluded basis. However, these were included in the evaluation of health outcomes.

Cattle Management and Growth Performance Measurements

Steers were individually weighed on d 0 (study initiation), d 38, d 99, d 108, and d 149, which also served as study termination. Body weights were measured before morning feedings with a 4% pencil shrink applied BW measurements. Cumulative growth performance was reported on both live and carcass-adjusted basis. The carcass-adjusted final BW was calculated from hot carcass weight (HCW) divided by 0.625. Average daily gain (ADG) was determined as the difference between final body weight and initial shrunk BW divided by ds on feed (149 d). Efficiency of weight gain [gain:feed (G:F)] was calculated by dividing ADG by daily dry matter intake (DMI), which was tabulated at weekly intervals.

Carcass-adjusted growth performance was used to calculate performance-based dietary net energy (NE) to determine dietary NE utilization. Net energy on a performance basis was calculated from daily energy gain (EG; Mcal/d) using the following equation :

$$EG = ADG^{1.097} \times 0.0557BW^{0.75},$$

where BW is metabolic shrunk BW (kg) calculated as shrunk BW × (478/AFBW). Where AFBW is the adjusted final body weight at 28% body fat.

Maintenance Energy (EM; Mcal/d) was calculated by the equation:

$$EM = 0.077 \times BW^{0.75}.$$

Dietary net energy for gain was calculated from net energy for maintenance using the equation (DMI = ME/0.877 NEm - 0.41), and can be resolved for estimation of dietary NEm through the quadratic formula

$$x = \frac{-b - \sqrt{b^2 - 4ac}}{2c}$$

Where x = NEm, Mcal/kg, a = -0.41EM, b = 0.811EM+0.41DMI+ EG, c = -0.877DMI

Dietary net energy for gain (NEg; Mcal/d) was determined from NEm using the following equation : NEg = 0.877NEm – 0.41.

Carcass Characteristics

Steers were weighed off trial on d 149, when visually appraised to meet a common compositional endpoint (~1.27 cm of fat at the 12th rib). Steers were randomly assigned to one of three trucks and shipped to a commercial packing facility in Omaha, Nebraska (~240km). Steers were harvested the following d. At time of harvest, liver scores and hot carcass weight (HCW) were recorded. Liver scores were determined by a trained technician and classified according to the Elanco Liver Scoring System: Normal (no abscesses), A- (1 or 2 small abscesses or abscess scars), A (2 to 4 well organized abscesses less than 2.5 cm diameter), or A+ (1 or more large active abscesses greater than 2.5 cm diameter with inflammation of surrounding tissue). After approximately 24 hr of chilling, carcasses were ribbed between the 12th and 13th rib and video image data was obtained for ribeye area (REA), rib fat (RF), marbling scores and USDA Quality and Yield grades. Initial lean color (*L*, a*, b**) was obtained from a sub-sample of carcasses (n = 32 per treatment; four steers per pen closest to the pen average BW at d 108) using a handheld colorimeter (Model CR-31, Minolta Corp. Ramsey, NJ, US; 50 mm diameter measuring space; C illuminant with a 2˚observer) following an approximately 20-minute bloom period.

STATISTICAL ANALYSIS

Growth performance, health data, and carcass characteristics were analyzed as a Randomized Complete Block Design (RCBD) using the GLIMMIX procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with pen as experimental unit. The model included a fixed effect of dietary treatment and random effect of block (pen location). Health outcomes were evaluated on an individual animal basis using PROC GLIMMIX procedure of SAS assuming a multinomial distribution. Least Squares Means were generated using the LSMEANS statement of SAS. An α of 0.05 or less determined significance and an α of 0.06 to 0.10 was considered a tendency.

RESULTS AND DISCUSSION

Growth Performance

Growth performance responses are shown in Table 2.2. There were no differences between treatments for final BW (*P* = 0.55), ADG (*P* = 0.52), DMI (*P* = 0.97), G:F (*P* = 0.22), or gain efficiency (*P* = 0.83) indicating that inclusion of By-O-Reg+ Beef did not hinder or improve growth performance measures for these cattle fed in an all-natural system. Dorleku et al. (2021) reported similar results for BW, ADG, DMI and G:F when evaluating the replacement of monensin and tylosin in beef finishing diets with two commercial blends of essential oils. Benchaar et al. (2006) also reported that DMI, ADG, and G:F were not affected by increasing doses of an essential oil product (including thymol, eugenol, vanillin and limonene; fed at 2 or 4g) when compared to a control treatment with no added essential oils, antibiotics or antimicrobials. However, in the initial growth performance trial, a quadratic effect was detected for feed efficiency being highest at 2 g of essential oil. More recently, Angus-Simmental crossbred steers (n = 72) were finished on a diet including a commercial blend of essential oils (DSM Nutritional Products, Parsippany, NJ; included at a rate of 1 g per head daily) and compared to a treatment group that received 90 mg per d of tylosin or a control group containing no antibiotics or added essential oils (Pukrop et al., 2019). Similar to the current study, final BW, ADG, DMI, and gain: feed did not differ among treatments, indicating no impact of essential oils or tylosin on growth performance attributes (Pukrop et al., 2019). Overall, results from this study align with previous findings that essential oil products can be utilized as a feed additive with little effect on the growth performance of feedlot cattle.

Dietary Energetics

Dietary energetics are shown in Table 2.2. Overall, treatment did not influence (*P* ≥ 0.86) any measures of applied energetics including NEm, NEg, observed to expected NEg, NEm, or DMI. It is important to note that the observed to expected net energy values were 0.95, indicating that the expected net energy values were higher than how the cattle performed. However, observed to expected DMI values were 1.05 indicating that the DMI was slightly higher than the expected value based on the diet formulation. These reports are similar to Estrada-Angulo et al. (2021), where finishing lambs fed a blend of essential oils containing thymol, eugenol, limonene, and vanillin compounds maintained all dietary energetic values compared to control lambs fed a high energy finishing diet containing no essential oil additive. There is limited research on the effects of essential oils on the dietary energetics of finishing cattle.

Many studies investigating the inclusion of essential oils in beef diets have evaluated inclusion at different dosages. The literature broadly indicates that the effects of essential oils are dependent upon many factors including essential oil type, source, dosage, and the mixture/synergism of the essential oils if blended. Differing dosages have resulted in contradictory findings between studies (Calsamiglia et al., 2007; Benchaar et al., 2008; Nehme et al., 2021). These contradictory findings may be caused by differences in composition between specific products or the production phase in which the product is being tested. However, additional work has revealed that low doses of essential oils (up to 4 g per animal daily) may improve subsequent meat quality characteristics (color, antioxidant activity, and/or lipid oxidation; Castillejos et al., 2008; Nehme et al., 2021). In contrast, high doses may have detrimental impacts on health and meat quality outcomes (Nehme et al., 2021) . In the current study, 4 grams per head daily were fed to yearling cattle, which is within the range of what has previously been determined to improve meat quality characteristics, but not high enough to be detrimental to health.

Health outcomes

Health outcomes did not differ ($P \geq 0.44$) between the control and OEO treatment groups in this study (Table 2.2). The portion of steers that were documented to have a digestive disorder and treated non medically once, twice or three times (1x , 2x, or 3x) did not differ between treatment groups. However, numerically the OEO treatment group did have a numerical increase in digestive disorders that were treated one or more times when compared to the control. Similarly, while there was a numerical increase in the number of OEO animals removed from the trial (treated with an antibiotic treatment for illness or died), there was no significant difference ($P \geq 0.18$) between groups for removals. Smith et al., (2024) compared natural cattle production to conventional production and reported that cattle produced in a natural system had increased occurrence of digestive disorders. There is an increased potential for cattle fed under natural production practices to be subjected to digestive issues, as common mitigation strategies, such as ionophores, are not used.

Liver Scores and Carcass Characteristics

Liver scores are reported in Table 2.3. There were no differences in the distribution of liver scores between treatments ($P = 0.11$). However, a numerically higher percentage of carcasses displayed liver abscesses in categories (A-, A, A+) in the OEO group compared to the CON. In a similar comparison, Pukrop et al., (2019) observed no difference in the proportion of normal liver scores between the control group and essential oil treatment group, however, a tendency was detected with essential oil treatment group displaying more liver scores in the category ‘A’ (2 to 4 well organized abscesses less than 1 in. diameter). Combined with the results from the current study this outcome might indicate additional research is warranted to determine the impact of essential oils on prevalence and severity of liver abscess.

Carcass trait responses are displayed in Table 2.4. Treatment did not influence ($P \geq 0.30$) hot carcass weight, dressing percentage, ribeye area, rib fat thickness, marbling score, or yield grade. Estimated body fat (EBF) and adjusted final body weight (AFBW) did not differ ($P \geq 0.47$) between treatment groups. There were also no differences ($P = 0.79$) in USDA Quality Grade distribution between treatments, which follows the marbling score outcomes of both groups. Overall, the Quality Grade data indicates that majority of the cattle across both treatment groups were Upper 2/3rd Choice or above. In addition, there was no difference in distribution of USDA Yield Grades ($P = 0.94$), with most of the cattle assigned to Yield Grade 2 and 3 categories. Pukrop et al. (2019), observed similar results for carcass traits of cattle fed 1 gram per steer daily of an proprietary essential oil blend. In addition, a study by Wang et al. (2020) compared five dietary treatments provided to cattle in the last 98 d of the finishing period: 1) control 2) monensin/tylosin (monensin at 33mg/kg on DM basis, and tylosin supplement at 11mg/kg on DM basis; 3) essential oils [Victus Liv (DSM Nutritional Products, Parsippany NJ)] diet (supplemented at 1.0 g per steer daily); 4) benzoic acid supplemented at 0.5% on DM basis or 5) combination diet of essential oils and 0.5% benzoic acid and also reported no differences in carcass characteristics. However, Wang et al. (2020) did observe a tendency for improved marbling scores in carcasses from the essential oil groups compared to the control. This is similar to the numerically increased proportion of Prime carcasses in the OEO carcasses of the current study. When compared with other studies investigating the carcass characteristics of cattle managed in natural programs , these cattle performed similarly in regard to REA and marbling score, achieving a mean quality grade of 'Average Choice' and indicating that inclusion of OEO did not negatively impact marbling development.

Initial objective lean color values ($L*$, $a*$, and $b*$) recorded at the ribeye surface prior to fabrication did not differ ($P \geq 0.48$) between treatment groups. Wang et al. (2020) evaluated $L*$, $a*$, and $b*$ values of samples post-fabrication and reported no differences between the control diet containing no added supplement and the essential oil supplemented diet (proprietary blend of thymol eugenol, vanillin, guaiacol, and limonene; DSM Nutritional Products; Parsippany NJ). In addition, Dorleku et. al. (2021) reported no differences in instrumental color of lean from steers fed a control diet compared to treatment diet including one of two individual proprietary essential oil blends [Victus Liv (DSM Nutritional Products, Parsippany NJ) at 1 g per steer daily or Fortissa Fit 45 (Provimi Canada ULC, St-Valerien-de Milton, QC, Canada) at 4 g per steer daily].

Conclusion

We reject our hypothesis that inclusion of By-O-Reg+ Beef, an encapsulated essential oil product, would improve growth performance, health outcomes, and carcass characteristics of steers fed in an all-natural system. While there were no significant enhancements in growth performances or carcass characteristics, there were no detrimental effects with inclusion of By-O-Reg+ Beef in the diet indicating commercially available essential oil products may be viable alternatives to conventional feed additives without causing negative effects to performance and carcass traits. In general, there is a lack of consistency in the active compounds, dosage, and bioavailability of essential oils warranting further research to determine optimal dosage, location of absorption, and possible causes of oxidation within the ruminant system.

LITERATURE CITED

Asbahani, A. E., Miladi, K., Badri, W., Sala, M., Addi, E. H. A., Casabianca, H., Mousadik, A. E., Hartmann, D., Jilale, A., Renaud, F. N. R., & Elaissari, A. (2015). Essential oils: From extraction to encapsulation. *International Journal of Pharmaceutics*, *483*(1), 220-243. https://doi.org/https://doi.org/10.1016/j.ijpharm.2014.12.069

Beck, P., & Biggs, R. (2022). Feed Additives for Beef Cattle Production [Fact Sheet]. *Oklahoma Cooperative Extension Service Fact Sheets*. Retrieved 1/9/2024, from https://extension.okstate.edu/fact-sheets/feed-additives-for-beef-cattle-production.html

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G., Colombatto, D., McAllister, T. A., & Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology*, *145*(1-4), 209-228. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2007.04.014

Benchaar, C., Duynisveld, J. L., & Charmley, E. (2006). Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. *Canadian Journal of Animal Science*, *86*(1), 91-96. https://doi.org/10.4141/a05-027

Bergen, W. G., & Bates, D. B. (1984). Ionophores: their effect on production efficiency and mode of action. *Journal of Animal Science*, *58*(6), 1465-1483. https://doi.org/https://doi.org/10.2527/jas1984.5861465x

Calsamiglia, S., Busquet, M., Cardozo, P., Castillejos, L., & Ferret, A. (2007). Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of* *dairy science*, *90*(6), 2580-2595. https://doi.org/https://doi.org/10.3168/jds.2006-644

Castillejos, L., Calsamiglia, S., Martín-Tereso, J., & Ter Wijlen, H. (2008). In vitro evaluation of effects of ten essential oils at three doses on ruminal fermentation of high concentrate feedlot-type diets. *Animal Feed Science and Technology*, *145*(1), 259-270. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2007.05.037

Dorleku, J. B., Wang, L. M., Zhou, Z. Y., Mandell, I. B., & Bohrer, B. M. (2021). Effects of feeding two different blends of essential oils to finishing steers on growth performance, carcass characteristics, meat quality, meat composition, and shelf life. *Canadian Journal of Animal Science*, *101*(3), 507-526. https://doi.org/10.1139/cjas-2020-0075

Estrada-Angulo, A., Arteaga-Wences, Y. J., Castro-Pérez, B. I., Urías-Estrada, J. D., Gaxiola-Camacho, S., Angulo-Montoya, C., Ponce-Barraza, E., Barreras, A., Corona, L., & Zinn, R. A. (2021). Blend of Essential Oils Supplemented Alone or Combined with Exogenous Amylase Compared with Virginiamycin Supplementation on Finishing Lambs: Performance, Dietary Energetics, Carcass Traits, and Nutrient Digestion. *Animals*, *11*(8), 2390.

Veterinary Feed Directive (VFD), (2017). https://www.fda.gov/animal-veterinary/development-approval-process/veterinary-feed-directive-vfd

Gadberry, S., Lalman, D., White, F., Linneen, S., & Beck, P. (2022). Meta-analysis of the effects of monensin on growth and bloat of cattle on pasture. *Translational Animal Science*, *6*(2). https://doi.org/10.1093/tas/txac031

Guiroy, P., Tedeschi, L., Fox, D., & Hutcheson, J. (2002). The effects of implant strategy on finished body weight of beef cattle. *Journal of Animal Science*, *80*(7), 1791-1800. https://doi.org/ https://doi.org/10.2527/2002.8071791x

Maxwell, C. L., Bernhard, B. C., O'Neill, C. F., Wilson, B. K., Hixon, C. G., Haviland, C. L., Grimes, A. N., Calvo-Lorenzo, M. S., VanOverbeke, D. L., Mafi, G. G., Richards, C. J., Step, D. L., Holland, B. P., & Krehbiel, C. R. (2015). The effects of technology use in feedlot production systems on feedlot performance and carcass characteristics1. *Journal of Animal Science*, *93*(3), 1340-1349. https://doi.org/10.2527/jas.2014-8127

Maxwell, C. L., Krehbiel, C. R., Wilson, B. K., Johnson, B. T., Bernhard, B. C., O'Neill, C. F., VanOverbeke, D. L., Mafi, G. G., Step, D. L., & Richards, C. J. (2014). Effects of beef production system on animal performance and carcass characteristics1. *Journal of Animal Science*, *92*(12), 5727-5738. https://doi.org/10.2527/jas.2014-7639

Mercer, M. A. (2022). *Ionophores Use in Animals*. Retrieved April 9 from https://www.merckvetmanual.com/pharmacology/antibacterial-agents/ionophores-use-in-animals

NASEM. (2016). *Nutrient Requirements of Beef Cattle* (N. A. Press, Ed. 8th revised ed.).

Nehme, R., Andrés, S., Pereira, R. B., Ben Jemaa, M., Bouhallab, S., Ceciliani, F., López, S., Rahali, F. Z., Ksouri, R., & Pereira, D. M. (2021). Essential oils in livestock: From health to food quality. *Antioxidants*, *10*(2), 330. https://doi.org/https://doi.org/10.3390/antiox10020330

Ni, Z.-J., Wang, X., Shen, Y., Thakur, K., Han, J., Zhang, J.-G., Hu, F., & Wei, Z.-J. (2021). Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils. *Trends in Food Science & Technology*, *110*, 78-89. https://doi.org/https://doi.org/10.1016/j.tifs.2021.01.070

Pukrop, J. R., Campbell, B. T., & Schoonmaker, J. P. (2019). Effect of essential oils on performance, liver abscesses, carcass characteristics and meat quality in feedlot steers. *Animal Feed Science and Technology*, *257*, 114296. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2019.114296

Russell, J. B., & Strobel, H. (1989). Effect of ionophores on ruminal fermentation. *Applied and Environmental Microbiology*, *55*(1), 1-6. https://doi.org/Doi: 0099-2240/89/010001-06$02.00/0

Simitzis, P. E. (2017). Enrichment of animal diets with essential oils—a great perspective on improving animal performance and quality characteristics of the derived products. *Medicines*, *4*(2), 35. https://doi.org/https://doi.org/10.3390/medicines4020035

Smith, J., Darambazar, E., Penner, G. B., Erickson, N., Larson, K., McKinnon, J., Damiran, D., & Lardner, H. A. (2024). Effects of conventional and natural management systems on backgrounding and finishing performance of beef steers. *Applied Animal Science*, *40*(2), 150-166. https://doi.org/10.15232/aas.2023-02425

Sneeringer, S. (2015). REstrictions on Antibiotic Use for Production Purposes in U.S. Livestock Industries Likely to Have Small Effects on Prices and Quantities *Animal Products*. Retrieved May 7, 2024, from https://www.ers.usda.gov/amber-waves/2015/november/restrictions-on-antibiotic-use-for-production-purposes-in-u-s-livestock-industries-likely-to-have-small-effects-on-prices-and-quantities/#:~:text=In%202006%2C%20the%20European%20Union,in%20livestock%20for%20production%20purposes.

Stackhouse-Lawson, K. R., Calvo, M. S., Place, S. E., Armitage, T. L., Pan, Y., Zhao, Y., & Mitloehner, F. M. (2013). Growth promoting technologies reduce greenhouse gas, alcohol, and ammonia emissions from feedlot cattle. *Journal of Animal Science*, *91*(11), 5438-5447. https://doi.org/10.2527/jas.2011-4885

Thompson, A. J., Smith, Z. K. F., Corbin, M. J., Harper, L. B., & Johnson, B. J. (2016). Ionophore strategy affects growth performance and carcass characteristics in feedlot steers1. *Journal of Animal Science*, *94*(12), 5341-5349. https://doi.org/10.2527/jas.2016-0841

USDA-AMS. (2015, November 13,2015). *NE3 Marketing Program*. Retrieved April 5 from https://www.ams.usda.gov/content/ne3-marketing-program

Wang, L. M., Mandell, I. B., & Bohrer, B. M. (2020). Effects of feeding essential oils and benzoic acid to replace antibiotics on finishing beef cattle growth, carcass characteristics, and sensory attributes. *Applied Animal Science*, *36*(2), 145-156. https://doi.org/https://doi.org/10.15232/aas.2019-01908

Webb, M. J., Block, J. J., Harty, A. A., Salverson, R. R., Daly, R. F., Jaeger, J. R., Underwood, K. R., Funston, R. N., Pendell, D. P., Rotz, C. A., Olson, K. C., & Blair, A. D. (2020). Cattle and carcass performance, and life cycle assessment of production systems utilizing additive combinations of growth promotant technologies. *Translational Animal Science*, *4*(4). https://doi.org/10.1093/tas/txaa216

Zinn, R. A., Barreras, A., Owens, F. N., & Plascencia, A. (2008). Performance by feedlot steers and heifers: Daily gain, mature body weight, dry matter intake, and dietary energetics. *Journal of Animal Science*, *86*(10), 2680-2689. https://doi.org/10.2527/jas.2007-0561

TABLES

Table 2.1. Ingredient and nutrient composition of finishing diet

Item	d 1 to 7	d 8 to 14	d 15 to 50	d 51 to 149
Ingredient Composition				
Dry-Rolled Corn, %	51.98	61.99	73.03	69.01
Liquid Supplement[1], %	4.03	4.02	4.00	3.93
Modified Distillers Grains, %	14.29	14.25	15.02	15.18
Grass Hay, %	14.57	9.68	3.85	7.98
Sorghum Silage, %	15.13	10.05	4.10	3.90
Nutrient Composition[2]				
DM, %	61.11	65.80	72.50	73.84
CP, %	12.25	12.12	12.19	12.34
NDF, %	26.54	21.54	15.78	17.96
ADF, %	14.47	11.16	7.32	8.86
Ash, %	5.76	5.30	4.79	4.94
EE,%	3.32	3.35	3.40	3.40
NEm, Mcal/kg[3]	85.49	89.63	94.55	92.94
NEg, Mcal/kg[3]	54.43	58.52	63.38	61.75

[1] Liquid Supplement contained (DM basis): 26.92 % CP, 19.75% NPN(Non-Protein Nitrogen), 0.21% Crude Fat, 0.04% Crude Fiber, 57.97% Ash, 13.08% total sugars, 1.00% K, 16.92% Ca, 5.01% Cl, 0.34% Mg, 0.46% P, 3.36% Na, 0.55% S, 4.77ppm Co, 249.23 ppm Cu, 148.25 ppm Fe, 40.0 ppm I, 500.0 ppm Mn, 4.0 ppm Se, 2,249.23 ppm Zn, 50.0 ppm EDDI, 2.03 mg/kg F, 79,470 IU/kg Vitamin A, 552.85 IU/kg Vitamin E, 19,870 IU/kg Vitamin D. 48.66 Mcal/cwt NEm, 0.58 Mcal/kg NEg. Did not contain any additional ionophore.

[2] DM = Dry Matter; CP = Crude Protein; NDF = Neutral Detergent Fiber; ADF = Acid Detergent Fiber; NEm = net energy for maintenance; NEg = net energy for gain

[3] Calculated using values from NASEM (2016)

Table 2.2 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on growth performance and health outcomes of steers finished in an all-natural program[1]

	Treatment[2]			
Item	Control	OEO	SEM[3]	*P* – value[4]
Steers, n	64	64	-	-
Pens, n	8	8	-	-
Initial BW[5], kg	335	335	0.1	0.45
Initial to d 149				
Final BW[5], kg	572	568	4.6	0.55
ADG, kg	1.59	1.59	0.031	0.52
DMI, kg	10.22	10.21	9.176	0.97
G:F	0.156	0.154	0.0016	0.22
Gain Efficiency[6]	3.50	3.45	0.070	0.54
Cumulative Carcass Adjusted[7]				
Final BW, kg	573	572	12.7	0.87
ADG, kg	1.59	1.59	0.039	0.85
DMI, kg	10.2	10.2	0.176	0.97
G:F	0.157	0.156	0.0024	0.73
Gain Efficiency[6]	1.60	1.58	0.031	0.83
Applied energetics[8]				
NEm, Mcal/cwt	88.43	88.34	0.916	0.93
NEg, Mcal/cwt	58.96	58.88	0.803	0.92
O/E NEm	0.95	0.95	0.011	1.00
O/E NEg	0.96	0.96	0.013	0.93
O/E DMI	1.05	1.05	0.013	0.86
Health outcomes, % (n)				
Digestive 1×[9]	4.7 (3)	7.8 (5)	-	0.44
Digestive 2×[9]	0.0 (0)	3.1 (2)	-	0.97
Digestive 3×[9]	0.0 (0)	1.6 (1)	-	0.97
Removal[10]	4.7 (3)	10.9 (7)	-	0.18
Dead[11]	4.7 (3)	7.8 (5)	-	0.44

[1] All values are based on a deads and removed basis, excluding the values for health outcomes

[2] Control = No inclusion of By-O-Reg+ Beef in the diet. OEO = Inclusion of By-O-Reg+ Beef (oregano-based feed additive) at a rate of 4 grams per steer daily

[3] Standard error of the mean

[4] Probability of difference among least square means

[5] A 4% pencil shrink was applied to all BW measures to account for digestive tract fill.

[6] Calculated as ADG at the same DMI

[7] Calculated based on the following equation for Final Body Weight (Hot Carcass Weight/0.625)

[8] Applied energetics determined from carcass-adjusted growth performance; Nem = Net energy for maintenance; NEg = Net energy for gain; O/E = Observed to Expected

[9] Digestive = animal was observed to have symptoms of digestive disorder and treated non-medically one time (1x) two times (2x) or three times (3x)

[10] Steers that were removed from trial for reasons such as medically treated or died during trial period

[11] Steers that died during trial period

Table 2.3 Influence of inclusion of By-O-Reg+ Beef on distribution of liver scores (%)[1]

	Treatment		
Item	Control	OEO	*P*-value[2]
Liver scores, n	61	56	-
Normal, %	86.9	76.8	0.11
A- , %	4.9	5.4	
A,%	1.6	3.6	
A+ or greater, %	6.6	14.2	

[1] Liver abscess prevalence and severity was determined by a trained technician using the Elanco system as Normal (no abscesses), A- (1 or 2 small abscesses or abscess scars), A (2 to 4 well organized abscesses less than 1 in. diameter), or A+ (1 or more large active abscesses greater than 1 in. diameter with inflammation of surrounding tissue).

[2] Probability of difference among least square means

Table 2.4 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on carcass traits of steers finished in an all-natural program

	Treatment[1]			
Item	Control	OEO	SEM[2]	*P*-value[3]
Steers, n	61	56		
Hot carcass weight, kg	357.9	357.4	3.58	0.88
Dressing percentage, %	62.6	62.9	0.280	0.45
Ribeye area, sq. cm	76.77	77.68	1.250	0.48
Rib Fat Thickness, cm	0.84	0.89	0.030	0.43
Marbling score[4]	551	568	15.8	0.30
Yield grade	3.03	3.01	0.052	0.69
Retail yield, %	49.94	50.00	0.108	0.62
Empty body fat, %[5]	28.86	29.06	0.276	0.49
Adjusted final body weight, kg[6]	551.6	547	5.3	0.47
Quality Grade, %				
Select	3.3	3.6	-	
Low Choice	27.9	23.2	-	
Average Choice	41.0	41.0	-	0.79
High Choice	23.0	19.6	-	
Prime	4.8	12.6	-	
Yield Grade, %				
1	4.9	5.3	-	
2	59.0	58.9	-	
3	36.1	35.8	-	0.94
4	0.0	0.0		
5	0.0	0.0		

[1] Control= No inclusion of By-O-Reg+ Beef in the diet. OEO = Inclusion of By-O-Reg+ Beef (oregano-based feed additive) at a rate of 4 grams per steer daily

[2] Standard error of the mean

[3] Probability of difference among least square means

[4] Small= 400, Modest= 500, Moderate=600

[5] Calculated from Guiroy et al (2002)

[6] Final BW at 28% estimated empty body fatness calculated from Guiroy et al (2002)

Table 2.5 Influence of By-O-Reg+ Beef, an oregano-based essential oil (OEO), on objective color[1] of carcasses of steers finished in an all-natural program

	Treatment			
Item	Control	By-O-Reg	SEM[2]	*P*-value[3]
n	32	32		
*L**	43.1	43.1	0.289	0.87
*a**	25.41	25.32	0.167	0.69
*b**	10.24	10.12	0.117	0.48

[1] Objective color collected utilizing the handheld colorimeter following an approximately 20-minute bloom time, and prior to camera grading

[2] Standard Error of the Mean

[3] Probability of difference among least square means

CHAPTER III - EFFECT OF BY-O-REG+ BEEF, AN OREGANO-BASED ESSENTIAL OIL FEED ADDITIVE, ON PROXIMATE COMPOSITION AND MEAT QUALITY CHARACTERISTICS OF YEARLING BEEF STEERS FINISHED IN AN ALL-NATURAL PROGRAM.

ABSTRACT

The objective of this study was to determine the influence of an oregano-based essential oil feed additive (By-O-Reg+ Beef) on meat quality attributes of yearling beef steers finished in an all-natural program. Yearling steers [n = 128, initial body weight (BW) = 335 ± 7.98 kg] were allotted to 16 pens (n = 8 pens per treatment with 8 steers per pen). Steers were blocked by initial BW grouping in a randomized complete block design (n = 8 blocks total). Treatments included: 1) control group fed no oregano-based essential oil (CON) and 2) group fed 4 g/steer daily of By-O-Reg+ Beef (Advanced Ag Products, Canton, SD; OEO). Steers were harvested at a commercial packing facility following a 149-d finishing period. Following chilling (~36 h) strip loins were collected from a subsample of carcasses (n = 62) and transported to the South Dakota State University Meat Laboratory for further analysis. Purge loss and pH were recorded for each strip loin prior to fabrication into 2.54-cm steaks. One steak was vacuum packaged, aged for 4 ds postmortem then frozen for evaluation of proximate composition. Four steaks were vacuum packaged and aged for 4, 7, 14 or 21 ds for Warner-Bratzler shear force (WBSF) analysis. One steak from each striploin was overwrapped with a high oxygen permeable film and placed under a simulated retail display for 10 ds for evaluation of objective color (L^*, a^*, b^*) using a handheld colorimeter. Color score and discoloration were also evaluated by a trained panel once daily for 11 ds (d 0 -10). Three additional steaks were overwrapped and placed under a simulated retail display for 4, 7, or 10 d then removed and frozen for evaluation of lipid oxidation using the thiobarbituric acid reactive substances (TBARS) assay. Strip loins from the OEO treatment group had increased pH values ($P = 0.02$) compared to CON. Treatment did not influence ($P > 0.05$) purge loss of strip loins. Proximate composition (percentage of moisture, protein, fat, and ash) of steaks was not influenced by treatment ($P > 0.05$). There was no treatment effect or treatment × aging d interaction for WBSF, however, a d effect was observed as steaks became more tender from d 4 to 21. Cook loss was not affected by treatment, aging d, or their interaction ($P > 0.05$). No treatment effect or treatment × aging d interaction was detected ($P > 0.05$) for objective color, subjective color, discoloration, or lipid oxidation; however a d effect was observed ($P < 0.001$) for these measures. From d 0 to d 10 the L^* values of steaks increased ($P < 0.001$) while a^* and b^* decreased ($P < 0.001$) over the display period. Similarly, subjective color scores increased ($P < 0.0001$) indicating samples appeared darker red and panel ratings of discoloration increased ($P < 0.0001$) over the display period. Steaks also became more oxidized ($P < 0.0001$) as TBARS values were lowest ($P > 0.05$) at d 4, highest ($P > 0.05$) at d 10, with d 7 samples intermediate and different ($P < 0.05$). Data indicate that the inclusion of an oregano-based essential oil did not have any detrimental impacts on composition or meat quality of steaks from steers fed in an all-natural program.

INTRODUCTION

As the desire for beef produced through natural cattle production has grown, producers within these markets have sought products that have the potential to return the efficiencies that are often lost with all-natural production systems (Smith et al., 2020;

Smith et al., 2024). One of the commercially available feed additives investigated as an alternative to conventional antibiotics and growth promotants are essential oils.

Essential oils are blends of secondary metabolites derived from plants. These secondary metabolites are lipophilic and hydrophobic by nature and consist of aromatic and volatile fractions obtained from plant materials including flowers, roots, bark, leaves, and whole plants. In nature, these oils function to protect plants from bacteria, viruses, fungi, insects, herbivores and omnivores (Rios, 2016; Elshafie & Camele, 2017). Many of these secondary metabolites maintain their biological function when extracted from plants (Stevanović et al., 2018). Essential oils have been reported to possess antimicrobial, antioxidant, cytotoxic, and anti-inflammatory activities (Calsamiglia et al., 2007; Benchaar et al., 2008; Amorati et al., 2013; Leyva-López et al., 2017; Guimarães et al., 2019; Nehme et al., 2021).

Essential oils have been reported to improve gain efficiency as well as improve meat quality attributes when fed to livestock. However, inherent differences in plant source, composition, and plant activity creates variation in animal response among different compounds and the challenge of maintaining biological activity through the rumen environment limits use in cattle . However, encapsulation technology may protect these compounds through the rumen (Asbahani et al., 2015; Nehme et al., 2021). While the biologic properties of essential oils point to their potential antioxidant and antimicrobial activity, our understanding of the resultant effect on meat quality traits in beef cattle is limited (Calsamiglia et al., 2007, Castillejos et al., 2007; Benchaar et al., 2008; Nehme et al., 2021). Thus, research is needed to evaluate the effects of dietary inclusion of essential oils on meat quality characteristics. We hypothesized that the antimicrobial properties of oregano-based essential oils would improve animal health resulting in increased intramuscular fat and improved tenderness of beef and that the antioxidant properties would extend shelf life. Therefore, the objective of this study was to determine the influence of By-O-Reg+ Beef, an encapsulated oregano based essential oil, on meat quality traits from yearling beef steers finished in an all-natural program.

MATERIALS AND METHODS

Strip Loin Collection

Details regarding animals, experimental design, and treatments are provided in Chapter 2. Briefly, yearling steers [n = 128, initial body weight (BW) = 335 ± 7.98 kg] were allotted to 16 pens (n = 8 pens per treatment with 8 steers per pen). Steers were blocked by initial BW grouping in a randomized complete block design (RCBD; n = 8 blocks total). Treatments included: 1) control group fed no oregano-based essential oil (CON) and 2) group fed 4 g/steer daily of By-O-Reg+ Beef (Advanced Ag Products, Canton, SD; OEO). Steers remained on the finishing diet until harvest on d 149 of the trial. Following carcass chilling (approximately 36 h postmortem) strip loins (IMPS #180; *M. longissimus lumborum*) were collected from the left side of a subsample of carcasses (n = 62; 31 per treatment). The subsample was selected based on the BW on d 108 of the finishing period (four head closest to the pen average BW). After collection strip loins were vacuum packaged and transported under refrigeration (maintained at approximately 4˚C) to the South Dakota State University (SDSU) Meat Laboratory.

Strip Loin Fabrication, Purge Loss, and pH

Strip loin samples arrived at SDSU Meat Lab at 3-d postmortem. Upon arrival, all striploins were weighed in the vacuum packaging, and then removed from bags and re-weighed to determine purge loss. When strip loins were removed from packaging, they were also trimmed of external fat. Ultimate pH was recorded at the posterior end of the strip loin with a hand-held pH meter (Thermo-Scientific Orion Star, Beverly, MA, USA, Model #A221 and Star A321 Portable pH Probe). The strip loins were then fabricated into 2.54-cm steaks for further analysis. One steak was vacuum packaged, aged 4 d and frozen for later evaluation of proximate composition. Four steaks were vacuum packaged, aged 4-, 7-, 14-, or 21-d then frozen for Warner-Bratzler Shear force (WBSF) analysis. One steak from each striploin was overwrapped with a high oxygen permeable film (15,500-16,275 $cm^3/m^2/24h$) for use in a simulated retail display and three additional steaks were overwrapped for evaluation of lipid oxidation during retail display.

Proximate Analysis

To determine proximate nutrient composition steaks collected from anterior portion of each striploin were thawed slightly, trimmed of accessory muscles, minced with a knife, submerged in liquid nitrogen, and powdered using a stainless-steel blender (Waring Products Division, Model # 51BL32, Lancaster, PA, USA). Homogenized samples were stored at -20˚C in 4.5cm × 9cm sterile plastic bags until further chemical composition analysis.

To determine protein content, duplicate powdered samples were weighed (~ 250 mg) into crucibles and were subjected to dumas combustion by a nitrogen analyzer

(Rapid Max N Exceed, Elementar, Hanau Germany, Serial #29161032). Percent protein content was determined based on the protein factor (6.25) multiplied by the percent nitrogen detected for each sample.

To determine ash content, duplicate powdered samples were weighed (~3 g) into pre-dried (100˚C for 24h) 42 mL aluminum weigh boats and placed in an oven (Precision Scientific, Winchester, VA, Cat. #51220159) at 100˚C for 24 h. Dried samples were then placed into a glass desiccator and samples were reweighed after cooling for at least 1 h and then placed into a muffle furnace (Thermo Scientific Thermolyne Furnace Benchtop Industrial Type FD1500M, Thermo Scientific, Waltman MA) at 525˚C and ashed for 24 h. Ashed samples were removed and placed into a desiccator once the furnace cooled down to approximately 150˚C. Ashed samples were cooled in the desiccator for at least 1 h then re-weighed. Proximate ash content was calculated as the difference between pre- and post ashed sample weights and expressed as percent of the pre-ashed sample weight.

Percent crude fat and moisture were determined using the ether extract method outlined by Mohrhauser et al. (2015). Powdered samples (~5 g) were weighed into dried aluminum tins (FisherBrand, Pittsburgh, PA, Cat. #08-732-101), covered with dried filter papers, (Whatman, Buckinghamshire, UK, Cat. # 1001-1055) and dried in an oven (Precision Scientific, Winchester, VA, Cat. #51220159) at 101˚C for 24 h. Dried samples were then placed in a desiccator (Scienceware, Wayne, NJ Cat. #420320000) and samples were re-weighed after cooling for at least 1 h. Proximate moisture content was calculated as the difference between pre- and post-drying sample weights and expressed as percent of the pre-drying sample weight. Dried samples were then extracted with petroleum ether in a side-arm Soxhlet extractor (ThermoFischer Scientific, Rockville, MD) for a 60-h reflux period followed by evaporation under the laboratory hood at room temperature for 4 h and subsequent drying in an oven at 101˚C for 4 h (Bruns et al., 2004). Dried, extracted samples were placed in desiccators to cool for 1 h and then re-weighed. Proximate intramuscular fat content was calculated as the difference between pre-and post-extraction sample weight and expressed as a percent of the pre-extraction sample weight.

Warner- Bratzler Shear Force and Cook Loss

Warner Bratzler Shear Force was utilized to compare objective tenderness of CON and OEO supplemented steaks. In preparation for WBSF, frozen steaks were thawed at 4˚C for approximately 24 h before cooking. All steaks were weighed prior to cooking. Steaks were cooked to an internal temperature of 71˚C on an electric clamshell grill (George Foreman 9 Serving Classic Plate Grill, Model GR2144P, Middleton, WI, USA) and internal temperature was monitored using a digital thermometer (Cooper-Atkins, Middlefield, CT, Model 41-983430-5) placed near the geometric center of each steak. Steaks were removed from the grill and targeted to reach 71˚C and then monitored to determine the peak temperature of each steak. After cooking, all steaks were cooled overnight at approximately 4˚C for approximately 16 h. Steaks were removed from refrigeration approximately 4 h prior to shearing to allow steaks to reach room temperature (approximately 25˚C). Steaks were then re-weighed to determine cook loss. Cook loss was reported as a percentage of the raw weight using the following equation [raw weight- cooked weight/raw weight] ×100. Six cores (1.27cm in diameter) were removed from each steak parallel to muscle fiber orientation. A texture analyzer

(Shimadzu Scientific Instruments Inc. Lenexa, KS, USA, Model EZ-SX) with a Warner-Bratzler attachment was used to determine peak force required to shear each core. An average shear peak force value was then reported for each steak.

Objective Color and Subjective Color Panel

Immediately after fabrication one steak per strip loin was designated for observation by a trained color panel throughout an 11-d simulated retail display (d 0 -10 post-fabrication). A meat soaker pad was placed in a styrofoam tray, steaks were placed on the pad and overwrapped with oxygen permeable polyvinyl chloride wrap (15,500-16,275 $cm^3/m^2/24h$). Trays were then arranged on tables illuminated by two 1.22 m long fluorescent lights (32 watt; 3,000 lumens; 3500K) per table (31 steaks per table) as shown in Figure 3.1. Steaks were rotated approximately every 24 h through the display area to ensure even distribution of light exposure among samples. Light intensity was measured daily to ensure intensity was maintained between 1,612.5 – 2,152 lux throughout the entirety of the retail display. Objective color measurements (L^*, a^* and b^*) were measured using a handheld colorimeter (Model CR-31, Minolta Corp. Ramsey, NJ, US; 50 mm diameter measuring space; D65 illuminant) on each d of the color panel at the same time each d. Additionally, seven trained panelists evaluated steaks daily for subjective color score and surface discoloration. Panelists rated color score on an 8-point scale (1= Bleached Red, 2 = Very Light Cherry Red, 3 = Moderately Light Cherry Red, 4 = Cherry Red, 5 = Slightly Dark Red, 6 = Moderately Dark Red, 7 = Dark Red, 8= Very Dark Red; full scale is shown in Figure 3.2), and discoloration on a 6-point scale (1= No discoloration or 0%, 2 = Slight Discoloration or 1-20%, 3= Small Discoloration or 21-40%, 4 = Modest Discoloration or 41-60%, 5 = Moderate Discoloration or 61-80 %, 6=

extreme discoloration or 81-100%) according to procedures described by the American Meat Science Association (AMSA, 2023). Following the display period, steaks from each tray were individually vacuum sealed and frozen for analysis of lipid oxidation (this served as the d 10 TBARS sample).

Lipid Oxidation

The thiobarbituric acid reactive substances (TBARS) assay was used to evaluate markers of lipid oxidation in steaks following simulated retail display. Immediately after fabrication, steaks were overwrapped with a high oxygen polyvinyl chloride and placed under a simulated retail display as described above. In addition to the d 10 steak used for the color panel, two additional steaks were also removed from the display at d 4 and 7. As steaks reached their assigned display d specified (4, 7, or 10) they were vacuum packaged and frozen for TBARS analysis. Samples were prepped as described previously. Homogenized samples were stored at -20˚C in 4.5cm × 9cm sterile plastic bags. Oxidation was determined using the TBARS protocol outlined by Leick et. al., (2010). However, instead of making a spiked sample following every 10 samples, 18 samples were randomly chosen to be utilized for percent recovery calculations to represent 10 percent of the total samples. Absorbance values of samples, blanks, and standards were obtained using a spectrophotometer (SpectraMax 190 Absorbance microplate reader; Molecular Devices San Jose, CA) at 530 nm and TBARS were expressed as mg malondialdehyde (MDA) per kg of meat.

Statistical Analysis

Purge loss, pH, and proximate composition were analyzed as a RCBD using the mixed model procedures of SAS (SAS Institute Inc., Cary, NC, USA) with pen included as the experimental unit. The model included fixed effect of dietary treatment and random effect of block (pen location). Objective tenderness (WBSF), cook loss, objective color, subjective color, and lipid oxidation were analyzed as repeated measures using the Toeplitz variance structure for subjective color and compound symmetry for all other analyses for the effects of treatment, aging or display d, and their interactions. Peak temperature was included as a covariate for WBSF and cook loss. Panelist was included as a random effect for subjective color. Least squares means were generated using the LSMEANS statement of SAS and significance was considered at an α of ≤ 0.05 and tendencies were considered at an α of > 0.05 to 0.10.

RESULTS AND DISCUSSION

Purge Loss and pH

Purge loss and pH data are shown in Table 3.1. Supplementation with OEO did not influence ($P > 0.05$) purge loss of strip loins compared to CON. In contrast, Pukrop et al. (2019) reported that inclusion of a proprietary essential oil blend (DSM Nutritional Products, Parsippany, NJ) to the diet of cattle decreased purge loss compared to samples from cattle fed tylosin or a control diet containing no essential oil or tylosin. Differences between studies may be related to the method of evaluating purge loss as Pukrop et al. (2019) evaluated the moisture loss of 14 d aged steaks that were frozen then thawed, while in the present study purge was evaluated on whole strip loins aged 3 d following removal from vacuum packaging.

Ultimate pH was increased (*P* = 0.0279) for OEO striploins compared to CON. In contrast, He et al., (2023) reported that steers fed a low inclusion (130mg/d) level, or a high inclusion (230 mg/d) level of oregano essential oils had a decreased pH at 30 min and 24 h postmortem of *longissimus thoracis* muscle compared to steers fed a control diet. de Oliveira Monteschio et al. (2017) reported that the inclusion of essential oils (rosemary, clove, or a mixture of thymol, eugenol, and vanillin) did not affect the pH of samples from Nellore heifers. In a similar study, Rivaroli et al. (2016) also reported no difference in pH of meat from young, crossbred bulls fed with or without dietary essential oils. Conflicting data among studies may be related to differences in specific essential oils fed, dosage levels, evaluation of different muscles, and timing of pH measurement.

Previous research has shown that purge loss is correlated with water holding capacity of meat and water holding capacity is correlated to pH (Huff-Lonergan & Lonergan, 2005). Thus alterations to meat pH can impact the ability of meat to retain moisture (Huff-Lonergan & Lonergan, 2005). When pH drops too quickly in a carcass, it may denature proteins, and result in poor meat quality known as pale, soft, and exudative (PSE) meat, which is a common issue in pork. In beef, the phenomenon of dark, firm, and dry (DFD) meat is characterized by a dark color and a dry/firm surface, as the water is held tightly within muscle. The condition of DFD is at an ultimate pH of 5.7-7.0 (Miller, 2007). While pH values differed statistically in the current study, strip loins from both treatments are within the normal pH range for beef (5.2 - 5.6; Page et al., 2001) and it is unlikely that the difference is biologically significant and did not approach DFD conditions. Additionally, the lack of difference in purge loss supports the suggestion that differences in pH are likely not biologically significant to the point of affecting the capacity of the strip loins to hold water.

Proximate Composition

Proximate composition data are shown in Table 3.1. There was no effect of treatment ($P > 0.05$) on moisture, fat, protein or ash content of the steaks. These results are similar to results of Rivaroli et al., (2016) indicating that dietary addition of different levels (0 g, 3.5 g, or 7 g daily) of an essential oil supplement (MixOil®, Animal Wellness Products, Oakland, Nebraska with components of oregano, garlic, lemon, rosemary, thyme, eucalyptus, and sweet orange) did not affect chemical composition of steaks from crossbred young bulls. In addition, other studies have reported that proportion of moisture and fat did not differ with inclusion of essential oil products (Wang et al., 2020; Dorleku et al., 2021).

Warner-Bratzler Shear Force and Cook Loss

WBSF and cook loss are shown in Table 3.2. There was no treatment effect or treatment × aging d interaction ($P > 0.05$) observed for WBSF or cook loss of the steaks. However, aging d did influence ($P < 0.0001$) WBSF and cook loss. Steaks aged for 4 d were the least tender ($P = 0.0440$), while steaks aged 7 d were less tender ($P = 0.0473$) than 21 d samples with 14 d intermediate and similar ($P > 0.05$) to 7 and 21 d steaks. Steaks aged for 14 ds exhibited the least percentage of cook loss overall ($P > 0.05$), while steaks aged 4, 7, or 21 d exhibited similar cook loss results ($P > 0.05$). Other studies have also reported no impact on WBSF with inclusion of dietary essential oils (Rivaroli et al., 2016; Wang et al., 2020; Dorleku et al., 2021). Additionally, the results from this study agree with previous studies that indicate an improvement in tenderness (decreased WBSF value) with aging (Huff & Parrish Jr, 1993; George-Evins et al., 2004; de Oliveira Monteschio et al., 2017; Nair et al., 2019; Foraker et al., 2020). The WBSF values of both the OEO and CON groups would be categorized as “Very Tender” using the tenderness claim standard introduced by the USDA. To meet USDA “Tender” and “Very Tender” steaks must have WBSF values of less than4.4 kg and 3.9 kg, respectively. At all aging ds, steaks from both groups were lower the “Very Tender” threshold (Yates et al., 2013). Other studies have also reported inclusion of dietary essential oils did not influence the cook loss of steaks (Rivaroli et al., 2016; Pukrop et al., 2019; Wang et al., 2020).

Objective Steak Color and Subjective Color Panel

No treatment effect or treatment × d of retail display interaction ($P > 0.05$) was observed for objective color (L^*, a^*, or b^*) of steaks; however, L^*, a^*, and b^* were influenced ($P < 0.001$) by display d (Table 3.3). From d 0 to d 10 the L^* values of steaks increased ($P = 0.0005$) while a^* ($P < 0.0001$) and b^* ($P < 0.0001$) decreased over the display period indicating that steaks became lighter and less red in color over the retail display period. Wang et al. (2020) also reported similar responses for L^*, a^*, and b^* during retail display. Consumers purchasing choices are correlated with the a^* and b^* values of steaks (Holman et al., 2016) and a^* value impacts consumers’ likeliness and willingness to purchase beef (Lybarger et al., 2023).

No treatment effect or treatment × d interaction ($P > 0.05$) was detected for color scores or discoloration by the trained panel indicating no influence of treatment on shelf life of steaks. Overall, subjective color score remained within the desired range (3 – 4; bright cherry-red) until approximately d 5 of the panel, when the color scores increased to 5 (slightly dark red) and continued to increase throughout the termination of the panel. Discoloration mimicked the results for subjective color, where scores remained within the 1 category (No discoloration) until d 5 where they reached category 2 (slight discoloration 1- 20%) and increased until d 8 when steaks reached category 3 (small discoloration 21- 40%) and remained until termination of the panel. Shelf life is determined by the point at which consumers are able to detect oxidation products or observe color changes within beef (Domínguez et al., 2019) and is often signified by the appearance of a dull brown colored pigmentation called metmyoglobin, which is associated with the deterioration of quality (Kropf, 2003). Many factors can contribute to the metmyoglobin concentration within a steak, such as the extent and type of lighting that steaks are subjected to in retail displays (Tomasevic et al., 2021). This can result in conditions that cause lipid oxidation and subsequently protein oxidation and metmyoglobin formation. There are two types of lipid oxidation that are common within retail display settings, auto oxidation and photo-oxidation (Pateiro et al., 2018; Domínguez et al., 2019). Auto oxidation is the intrinsic free radical formation and oxidation that occurs within meat. Photo oxidation is oxidation that occurs when meat is exposed to light for extensive periods of time (Pateiro et al., 2018; Domínguez et al., 2019). The exposure to intense lighting is a common in retail settings as it is appealing to consumers however, extensive exposure does enhance the oxidation process and therefore results in promotion of metmyoglobin concentration and accelerated discoloration (Domínguez et al., 2019).

Lipid Oxidation

No treatment effect or treatment × display d interaction ($P > 0.05$) was detected for TBARS (Table 3.3). However, TBARS values were influenced by display d as the amount of MDA content within the steaks increased and steaks became more oxidized ($P < 0.0001$) over time. The TBARS values were lowest ($P > 0.043$) at d 4, highest ($P < 0.0001$) at d 10, with d 7 samples intermediate and different ($P < 0.0001$). These results are in agreement with other studies reporting negligible impacts of dietary essential oils on TBARS values of beef (Pukrop et al. 2019; Wang et. al., 2020). However, Rivaroli et al. (2016) reported that inclusion of essential oils (oregano, garlic, lemon, rosemary, thyme eucalyptus and sweet orange) in the diet at 7 g per d increased lipid oxidation after 14 d of storage. This outcome was assumed to be due to the high inclusion level of the essential oil blend, because in the same study there was no impact on lipid oxidation of steaks from cattle fed 3.5 g per d. Results of Rivaroli et al. (2016) suggest investigation of increased dosage of dietary OEO may be warranted. In addition, Ornaghi et al. (2020) observed a linear reduction of MDA between d 7 and d 14 of storage when an essential oil was added to the diet of young bulls. The influence of display d observed in the present study is common among most studies as lipid oxidation normally occurs over time (Domínguez et al., 2019; Ornaghi et al., 2020). Malondialdehyde is the most distinct aldehyde produced during secondary lipid oxidation in food therefore allowing for the quantification of oxidation that has occurred at a given storage time. Lipid oxidation is commonly associated with unsaturated fatty acids and their reaction with oxygen via free radical mechanisms (Domínguez et al., 2019). Oxidation is represented by three stages: initiation, propagation and termination. As storage time increases, there is an increase in potential of radicals entering the initiation phase due to iron being released from heme proteins, which catalyzes reactions into the initiation and propagation phase of oxidation and causing damage to lipids over time (Domínguez et al., 2019). Results of this study suggest that the combination of extended exposure to light and storage time is likely the cause of the oxidation and that inclusion of OEO in the diet did not have detrimental impacts on lipid oxidation.

CONCLUSION

In summary, the dietary inclusion of an encapsulated oregano-based essential oil increased the pH of strip loin steaks from steers fed in an all-natural program. However, this did not translate to differences in other measures of meat quality. Dietary inclusion of OEO did not improve or hinder objective color or subjective color scores of steaks in a simulated retail display. In addition, inclusion of OEO did not influence purge loss, proximate composition, WBSF, or cook loss. Feeding steers OEO in the finishing diets did not result in any negative compositional or meat quality outcomes and future research into the optimal dosage of OEO in beef finishing diets may be warranted.

LITERATURE CITED

Amorati, R., Foti, M. C., & Valgimigli, L. (2013). Antioxidant activity of essential oils. *Journal of agricultural and food chemistry*, *61*(46), 10835-10847. https://doi.org/https://doi.org/10.1021/jf403496k

Asbahani, A. E., Miladi, K., Badri, W., Sala, M., Addi, E. H. A., Casabianca, H., Mousadik, A. E., Hartmann, D., Jilale, A., Renaud, F. N. R., & Elaissari, A. (2015). Essential oils: From extraction to encapsulation. *International Journal of Pharmaceutics*, *483*(1), 220-243. https://doi.org/https://doi.org/10.1016/j.ijpharm.2014.12.069

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G., Colombatto, D., McAllister, T. A., & Beauchemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology*, *145*(1-4), 209-228. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2007.04.014

Calsamiglia, S., Busquet, M., Cardozo, P., Castillejos, L., & Ferret, A. (2007). Invited review: essential oils as modifiers of rumen microbial fermentation. *Journal of dairy science*, *90*(6), 2580-2595. https://doi.org/https://doi.org/10.3168/jds.2006-644

Castillejos, L., Calsamiglia, S., Ferret, A., & Losa, R. (2007). Effects of dose and adaptation time of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology*, *132*(3-4), 186-201. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2006.03.023

de Oliveira Monteschio, J., de Souza, K. A., Vital, A. C. P., Guerrero, A., Valero, M. V., Kempinski, E. M. B. C., Barcelos, V. C., Nascimento, K. F., & do Prado, I. N.

(2017). Clove and rosemary essential oils and encapsuled active principles (eugenol, thymol and vanillin blend) on meat quality of feedlot-finished heifers. *Meat Science*, *130*, 50-57. https://doi.org/https://doi.org/10.1016/j.meatsci.2017.04.002

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants*, *8*(10), 429. https://doi.org/https://doi.org/10.3390/antiox8100429

Dorleku, J. B., Wang, L. M., Zhou, Z. Y., Mandell, I. B., & Bohrer, B. M. (2021). Effects of feeding two different blends of essential oils to finishing steers on growth performance, carcass characteristics, meat quality, meat composition, and shelf life. *Canadian Journal of Animal Science*, *101*(3), 507-526. https://doi.org/10.1139/cjas-2020-0075

Elshafie, H. S., & Camele, I. (2017). An overview of the biological effects of some mediterranean essential oils on human health. *BioMed research international*, *2017*. https://doi.org/ https://doi.org/10.1155/2017/9268468

Foraker, B. A., Gredell, D. A., Legako, J. F., Stevens, R. D., Tatum, J. D., Belk, K. E., & Woerner, D. R. (2020). Flavor, tenderness, and related chemical changes of aged beef strip loins. *Meat and Muscle Biology*, *4*(1). https://doi.org/https://doi.org/10.22175/mmb.11115

George-Evins, C. D., Unruh, J. A., Waylan, A. T., & Marsden, J. L. (2004). Influence of quality classification, aging period, blade tenderization, and endpoint cooking temperature on cooking characteristics and tenderness of beef gluteus medius steaks12. *Journal of Animal Science*, *82*(6), 1863-1867. https://doi.org/10.2527/2004.8261863x

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M. C. C., Endringer, D. C., Fronza, M., & Scherer, R. (2019). Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules*, *24*(13), 2471. https://www.mdpi.com/1420-3049/24/13/2471

He, P., Lei, Y., Zhang, K., Zhang, R., Bai, Y., Li, Z., Jia, L., Shi, J., Cheng, Q., Ma, Y., Zhang, X., Liu, L., & Lei, Z. (2023). Dietary oregano essential oil supplementation alters meat quality, oxidative stability, and fatty acid profiles of beef cattle. *Meat Science*, *205*, 109317. https://doi.org/https://doi.org/10.1016/j.meatsci.2023.109317

Holman, B. W. B., Mao, Y., Coombs, C. E. O., van de Ven, R. J., & Hopkins, D. L. (2016). Relationship between colorimetric (instrumental) evaluation and consumer-defined beef colour acceptability. *Meat Science*, *121*, 104-106. https://doi.org/https://doi.org/10.1016/j.meatsci.2016.05.002

Huff-Lonergan, E., & Lonergan, S. M. (2005). Mechanisms of water-holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Science*, *71*(1), 194-204. https://doi.org/https://doi.org/10.1016/j.meatsci.2005.04.022

Huff, E., & Parrish Jr, F. (1993). Bovine longissimus muscle tenderness as affected by postmortem aging time, animal age and sex. *Journal of food science*, *58*(4), 713-716. https://doi.org/https://doi.org/10.1111/j.1365-2621.1993.tb09341.x

Jiang, J., & Xiong, Y. L. (2016). Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: A review. *Meat Science*, *120*, 107-117. https://doi.org/https://doi.org/10.1016/j.meatsci.2016.04.005

King, D. A., Hunt, M. C., Barbut, S., Claus, J. R., Cornforth, D. P., Joseph, P., Kim, Y. H. B., Lindahl, G., Mancini, R. A., & Nair, M. N. (2023). American Meat Science Association guidelines for meat color measurement. *Meat and Muscle Biology*, *6*(4). https://doi.org/https://doi.org/10.22175/mmb.12473

Kropf, D. (2003). Enhancing meat color stability. Proc. 56th Recip. Meat Conf., Columbia, MO. Natl. Livest. Meat Board, Chicago, IL,

Leick, C. M., Puls, C. L., Ellis, M., Killefer, J., Carr, T. R., Scramlin, S. M., England, M. B., Gaines, A. M., Wolter, B. F., Carr, S. N., & McKeith, F. K. (2010). Effect of distillers dried grains with solubles and ractopamine (Paylean) on quality and shelf-life of fresh pork and bacon. *Journal of Animal Science*, *88*(8), 2751-2766. https://doi.org/10.2527/jas.2009-2472

Leyva-López, N., Gutiérrez-Grijalva, E. P., Vazquez-Olivo, G., & Heredia, J. B. (2017). Essential oils of oregano: Biological activity beyond their antimicrobial properties. *Molecules*, *22*(6), 989. https://doi.org/https://doi.org/10.3390/molecules22060989

Miller, M. (2007). Dark, firm and dry beef. *Beef facts product enhancement. Texas Tech University*, *2007*.

Mohrhauser, D. A., Taylor, A. R., Gonda, M. G., Underwood, K. R., Pritchard, R. H., Wertz-Lutz, A. E., & Blair, A. D. (2015). The influence of maternal energy status during mid-gestation on beef offspring tenderness, muscle characteristics, and gene expression. *Meat Science*, *110*, 201-211. https://doi.org/https://doi.org/10.1016/j.meatsci.2015.07.017

Nair, M. N., Canto, A. C. V. C. S., Rentfrow, G., & Suman, S. P. (2019). Muscle-specific effect of aging on beef tenderness. *LWT*, *100*, 250-252. https://doi.org/https://doi.org/10.1016/j.lwt.2018.10.038

Nehme, R., Andrés, S., Pereira, R. B., Ben Jemaa, M., Bouhallab, S., Ceciliani, F., López, S., Rahali, F. Z., Ksouri, R., & Pereira, D. M. (2021). Essential oils in livestock: From health to food quality. *Antioxidants*, *10*(2), 330. https://doi.org/https://doi.org/10.3390/antiox10020330

Ornaghi, M. G., Guerrero, A., Vital, A. C. P., de Souza, K. A., Passetti, R. A. C., Mottin, C., de Araújo Castilho, R., Sañudo, C., & do Prado, I. N. (2020). Improvements in the quality of meat from beef cattle fed natural additives. *Meat Science*, *163*, 108059. https://doi.org/https://doi.org/10.1016/j.meatsci.2020.108059

Page, J. K., Wulf, D. M., & Schwotzer, T. R. (2001). A survey of beef muscle color and pH. *Journal of Animal Science*, *79*(3), 678-687. https://doi.org/10.2527/2001.793678x

Pateiro, M., Barba, F. J., Domínguez, R., Sant'Ana, A. S., Khaneghah, A. M., Gavahian, M., Gómez, B., & Lorenzo, J. M. (2018). Essential oils as natural additives to prevent oxidation reactions in meat and meat products: A review. *Food Research International*, *113*, 156-166. https://doi.org/https://doi.org/10.1016/j.foodres.2018.07.014

Pukrop, J. R., Campbell, B. T., & Schoonmaker, J. P. (2019). Effect of essential oils on performance, liver abscesses, carcass characteristics and meat quality in feedlot steers. *Animal Feed Science and Technology*, *257*, 114296. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2019.114296

Ríos, J.-L. (2016). Essential oils: What they are and how the terms are used and defined. In *Essential oils in food preservation, flavor and safety* (pp. 3-10). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-12-416641-7.00001-8

Rivaroli, D. C., Guerrero, A., Velandia Valero, M., Zawadzki, F., Eiras, C. E., Campo, M. D. M., Sañudo, C., Mendes Jorge, A., & Nunes do Prado, I. (2016). Effect of essential oils on meat and fat qualities of crossbred young bulls finished in feedlots. *Meat Sci*, *121*, 278-284. https://doi.org/10.1016/j.meatsci.2016.06.017

Smith, J., Darambazar, E., Penner, G. B., Erickson, N., Larson, K., McKinnon, J., Damiran, D., & Lardner, H. A. (2024). Effects of conventional and natural management systems on backgrounding and finishing performance of beef steers. *Applied Animal Science*, *40*(2), 150-166. https://doi.org/10.15232/aas.2023-02425

Smith, Z. K., Anderson, P. T., & Johnson, B. J. (2020). Finishing cattle in all-natural and conventional production systems. https://doi.org/https://doi.org/10.4236/ojas.2020.102013

Stevanović, Z. D., Bošnjak-Neumüller, J., Pajić-Lijaković, I., Raj, J., & Vasiljević, M. (2018). Essential oils as feed additives—Future perspectives. *Molecules*, *23*(7), 1717. https://doi.org/https://doi.org/10.3390/molecules23071717

Tomasevic, I., Djekic, I., Font-i-Furnols, M., Terjung, N., & Lorenzo, J. M. (2021). Recent advances in meat color research. *Current Opinion in Food Science*, *41*, 81-87. https://doi.org/https://doi.org/10.1016/j.cofs.2021.02.012

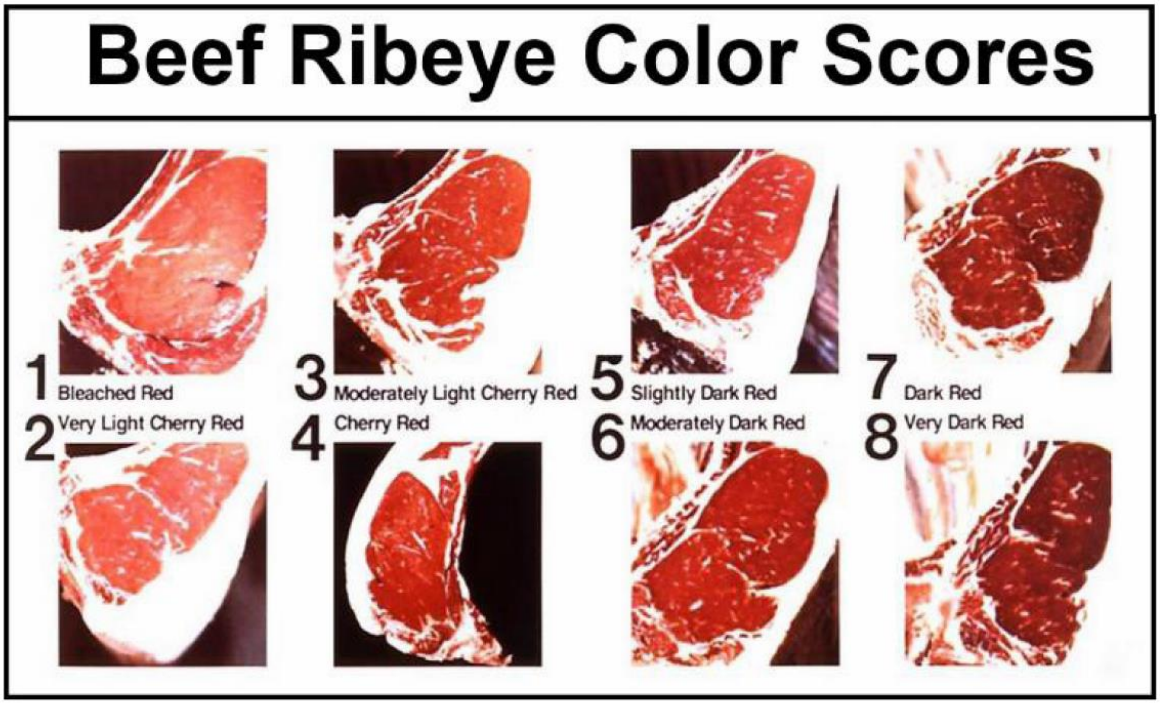
Wang, L. M., Mandell, I. B., & Bohrer, B. M. (2020). Effects of feeding essential oils and benzoic acid to replace antibiotics on finishing beef cattle growth, carcass characteristics, and sensory attributes. *Applied Animal Science*, *36*(2), 145-156. https://doi.org/https://doi.org/10.15232/aas.2019-01908

Yates, L. D., Doerscher, D. R., & David Bowden, J. (2013). Agricultural Marketing Service's Beef Tenderness Certification Program. Reciprocal Meat Conference

FIGURES

Figure 3.1 Representative image of simulated retail display

Figure 3.2 Representation of beef color scores utilized to for subjective color panel



TABLES

Table 3.1 Least square means for the effect of By-O-Reg+ Beef on pH, purge loss, and proximate composition of strip loins from steers finished in an all-natural program

	Treatment			
Item	Control	OEO	SEM[1]	*P*-value[2]
n	31	31	-	-
pH	5.63	5.67	0.012	0.02
Purge Loss[3], %	1.5	1.4	0.180	0.59
Moisture, %	70.7	70.6	0.303	0.71
Crude Fat, %	6.9	7.1	0.394	0.69
Protein,%	21.30	21.49	0.240	0.58
Ash, %	1.31	1.35	0.076	0.71

[1] Standard error of the mean

[2] Probability of difference among least square means

[3] Calculated as: [weight in bag- (weight out of bag + bag weight /weight in bag)] * 100

Table 3.2 Least square means for the effect of aging day on WBSF and cook loss values of steaks

	Day					
Item	4	7	14	21	SEM[1]	*P*-value[2]
WBSF, kg	2.48^{a}	2.30^{b}	2.22^{bc}	2.13^{c}	0.061	< 0.0001
Cook loss, %	18.29	18.17	17.47	18.64	0.387	0.16

[1] Standard error of the mean

[2] Probability of difference among least square means

[a,b,c] Means lacking common superscripts differ $P < 0.05$

Table 3.3 Least square means for effect display day on objective color ($L*$, $a*$, $b*$)[1] values, subjective color, subjective discoloration, and TBARS values of steaks over a simulated retail display

	Day												
Item	0	1	2	3	4	5	6	7	8	9	10	SEM[2]	*P*-value[3]
$L*$	47.71^{d}	48.64^{bcd}	49.36^{abc}	48.50^{cd}	49.47^{ab}	49.63^{a}	49.08^{abc}	49.01^{abc}	48.80^{abc}	48.86^{abc}	49.05^{abc}	0.347	0.0005
$a*$	20.42^{a}	20.74^{a}	20.29^{ab}	20.08^{abc}	19.55^{bc}	19.40^{c}	18.27^{d}	17.68^{d}	17.46^{de}	16.68^{ef}	16.23^{f}	0.030	<0.0001
$b*$	7.61^{a}	7.88^{a}	7.72^{a}	7.62^{a}	7.63^{a}	7.58^{a}	7.22^{b}	7.10^{b}	7.20^{b}	7.07^{b}	6.97^{b}	0.126	<0.0001
Color Score[4]	3.71^{a}	3.69^{a}	4.09^{b}	4.33^{b}	4.67^{c}	5.12^{d}	5.24^{d}	5.56^{e}	5.88^{f}	5.90^{f}	6.10^{f}	0.093	<0.0001
Discoloration Score[5]	1.0^{a}	1.12^{a}	1.17^{a}	1.36^{b}	1.78^{c}	2.08^{d}	2.46^{e}	2.85^{f}	3.28^{g}	3.21^{g}	3.65^{h}	0.166	<0.0001
TBARS (mg MDA/kg)	–	–	–	–	0.378^{a}	–	–	0.473^{b}	–	–	0.725^{c}	0.0329	<0.0001

[1] $L*$: 0 = Black, 100 = White; $a*$: Negative values = green, Positive values = red; $b*$: Negative values = blue, Positive values = yellow

[2] Standard error of the mean

[3] Probability of difference among least square means

[4] Color Score: 1 = Extremely bright cherry red, 2 = Bright cherry red, 3 = Moderately bright cherry red, 4 = Slightly bright cherry red, 5 = Slightly dark cherry red, 6 = Moderately dark red, 7 = Dark red, 8 = Extremely dark red

[5] Discoloration: 1 = No discoloration; 0%, 2 = Slight discoloration; 1-21%, 3 = Small discoloration; 21-40%, 4 = Modest discoloration; 41-60%, 5 = Moderate discoloration; 61-80%, 6 = Extreme discoloration; 81-100%

[a,b,c,d,e,f,g,h] Within a row means lacking a common superscript differ ($P < 0.05$)