Investigation of Alternatives to Ionophore/Antibiotic Management Strategies in Finishing Cattle and the Inherent Effect on Beef Quality and Shelf Life

By

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ABSTRACT

INVESTIGATION OF ALTERNATIVES TO IONOPHORE/ANTIBIOTIC MANAGEMENT STRATEGIES IN FINISHING CATTLE AND THE INHERENT EFFECT ON BEEF QUALITY AND SHELF LIFE

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With growing antimicrobial resistance concerns and new regulations limiting the use of antibiotics in livestock production, research for plant-based alternatives to antibiotics with antimicrobial effects is needed. This study investigated the effects of replacing monensin and tylosin with essential oils and(or) benzoic acid in finishing cattle diets. Sixty-eight crossbred steers were blocked by 3 initial weight categories and within each block, 1 of 5 finishing dietary treatments were randomly assigned: control (CON); monensin/tylosin (M/T); essential oil (EO); benzoic acid (BA); and a combination of EO and BA (COMBO). Monensin/tylosin supplemented steers had greater feed efficiency compared with steers fed CON, EO, and COMBO diets. However, EO and(or) BA supplemented beef finishing diets did not negatively affect most other major growth performance, carcass characteristics, meat quality, sensory traits, colour and oxidative stability of steaks and ground beef when compared with cattle fed no additives or conventionally fed cattle supplemented with M/T.

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Chapter 1: General Introduction

Raising livestock for meat production has progressed quite dramatically in recent years. Decades ago, the typical production practice for beef cattle was pasture-based farming whereas now, the norm in North America is to finish beef cattle in a more confined space called a feedlot. With careful management and advancements in nutritional regimes, feedlots allow producers to achieve similar lean muscle output within a much shorter time. This improvement in production efficiency makes it possible for operations to fulfill the increasing world demand for animal products (Addison, 1997). One of the most common and important feedlot management strategies is to use various feed additives for improved overall health and more consistent feed conversion (Perry, 1995). Feed additives are separated into two main groups: nutritive and non-nutritive (Addison, 1997). Nutritive additives consist of proteins, lipids, carbohydrates, vitamins and minerals. Non-nutritive additives consist of antibiotics, antibacterial drugs, hormones, and hormone-like chemicals (Addison, 1997). These ingredients could be given to animals within feed or water, by injection, through implant, paste, orally, topically, poured on, or in a bolus form (Sarmah et al., 2006).

Since Alexander Fleming discovered penicillin in 1928, a variety of antibiotics have since been developed and sold in markets throughout the world, revolutionizing medicine for both humans and animals (Sarmah et al., 2006). Antibiotics, or compounds possessing antibacterial activity, have even become one of the most popular pharmaceuticals approved for use in the agriculture sector (Sarmah et al., 2006). Antibiotics have frequently been used by livestock producers due to their ability to enhance animal health and increase feed efficiency (Sarmah et al., 2006; Hao et al., 2011; National Research Council, 1980). However, the extensive and sometimes incorrect application of antimicrobials for livestock can lead to the

emergence of drug resistant bacteria in the environment, thus posing a major risk to people and animals (Lerma et al., 2013; Kemper, 2008; CFIA, 2015). In addition, antimicrobial resistance has been observed to evolve, especially with foodborne pathogens. Mechanisms that cause antibiotic resistance include decreased cell permeability, mutated target sites, increased drug efflux and drug inactivation (Lerma et al., 2013). Since microbial ecosystems are connected between animal production facilities, the environment, and human food chain, resistant bacteria and genes have been shown to spread to products such as meat, eggs, and milk. An even greater concern is the spread of resistant bacteria to the environment, such as soil or bodies of fresh water. Overall, microbial resistance has become a serious and concerning public health problem for people all over the world (Teuber, 1999). To prevent the possibility of antimicrobial resistance becoming a worldwide crisis, governments such as United States and Canada have increased their restrictions on antibiotic use (FDA, 2017; Government of Canada, 2018) such that many drugs can only be accessed under veterinary prescription. Thus, research focuses on other potential natural feed alternatives with matching or greater efficacy as current synthetic feed additives are necessary for beef cattle production.

An alternative approach to feeding cattle antibiotics is replacement with a combination of essential oils and organic acids. Essential oils are plant-derived compounds with known antimicrobial, anti-inflammatory, antioxidative, and coccidiostatic properties. With these properties, essential oils have reduced pathogenic bacteria and their toxicity, leading to altered rumen fermentation profiles and mirroring the mode of action of ionophores (DiLorenzo, 2011; Chitprasert and Sutaphanit, 2014; Omonijo et al., 2018). There have been some studies conducted to explore the effectiveness of essential oils fed to beef cattle on animal performance and meat quality (Meyer et al., 2009; Rivaroli et al., 2016; de Oliveira Monteschio et al., 2017;

Meschiatti et al., 2019). These studies involved cattle from different breeds, sex, age, and the essential oil blends were supplemented at varying levels. But generally, cattle performance and meat qualities were similar among animals fed no additives, monensin/tylosin, and essential oil blends. Organic acids have antimicrobial effects by suppressing fungal activity and maintaining an acidic environment in the rumen (Castillo et al., 2004). However, the use of organic acids for beef cattle production is very limited (Martin et al., 1999; Foley et al., 2009) while use of organic acids has been investigated to a greater extent in swine production (Torrallardona, Badiola, and Broz, 2007; Cheng et al., 2017). Additionally, there are several research gaps regarding the effects of alternative feed additives on beef quality of multiple beef cuts, shelf life stability, and sensory quality characteristics. Therefore, this study aims to fill these research gaps and gain a better understanding of the extent of benefits that essential oils and organic acids provide.

1.1 OBJECTIVES

1. To examine and compare the effects of supplementing finishing cattle with monensin/tylosin, essential oils, and(or) benzoic acid on growth performance, carcass characteristics, meat quality, and palatability of the *longissimus thoracis* muscle.
2. To examine and compare the effects of supplementing finishing cattle with monensin/tylosin, essential oils, and(or) benzoic acid on colour and lipid oxidative stability of *longissimus thoracis* muscle and ground beef made from *semimembranosus* muscle, based on a simulated retail display shelf life study.

1.2 HYPOTHESIS

1. Finishing cattle fed monensin/tylosin will achieve similar magnitudes of success in improving feed efficiency and animal health (evaluated based on liver abscess incidences and mortality rate) than cattle fed essential oils and(or) benzoic acid.

2. Finishing diet will not affect other performance, carcass, and meat quality attributes.
3. Colour and oxidative stability among beef products will be improved with the use of essential oils and(or) benzoic acid in cattle finishing feed.

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Chapter 2: Literature Review

2.1 IONOPHORES

Ionophores are a group of compounds capable of altering rumen fermentation patterns through ion-channeling or ion-binding mechanisms in rumen microorganisms. These feed additives can form lipid soluble and dynamically reversible complexes with specific ions, allowing ions to be transported across cellular membranes and concentration gradients (Novilla, 2011). Ionophores have a hydrophilic interior pocket that allows for the binding of cations. On the other hand, the outside surface is hydrophobic such that the bound ion within the complex can move across the biological membrane, who is also hydrophobic (Freedman, 2012). Ionophores can be divided into three subclasses based on their method of ion transportation: 1) neutral ionophores, 2) channel-forming quasi-ionophores and 3) carboxylic ionophores (Butaye et al., 2003). Charged complexes created as a result of ions binding to neutral ionophores (e.g. valinomycin) are quite toxic to bacterial cells since they can disturb membranes and action potentials (Novilla, 2011). These compounds do not possess strong antibacterial activity and thus are not used as antibacterials (Butaye et al., 2003). Channel-forming quasi-ionophores (e.g. gramicidin, nystatin) carry monovalent cations and anions across cell membranes through ion conduction channels (Butaye et al., 2003). Finally, the subclass of ionophores that are supplied within animal feed, are all characterized as carboxylic ionophores (also known as polyether antibiotics) (Butaye et al., 2003). These compounds can be further subcategorized into monovalent and divalent polyether antibiotics, conditional on whether they transport monovalent or divalent cations (Westley, 1982). Carboxylic ionophores are the only group of ionophores used within animal production systems, because they form zwitterionic complexes with cations.

This neutral exchange of cations across membranes is better accepted by intact organisms compared to the charged complexes formed by neutral ionophores (Novilla, 2011).

Carboxylic ionophore polyether antibiotics are generally products of a *Streptomyces spp.* strain. They are the most widely administered antimicrobial agent for animals in the history of veterinary medicine (Dowling, 2013). Since the mid-l970s, ionophores have been used to alter rumen fermentation and promote weight gain primarily by reducing methane production, decreasing the amount of protein fermenting into ammonia, and increasing the propionic: acetate acid production ratio (Russell and Strobel, 1989). Additionally, ionophores can prevent coccidiosis and have been reported to be active against parasites (e.g. *Eimeria*, *Plasmodium)*, gram-positive bacteria, and mycoplasmas (Russell and Strobel, 1989; Augustine et al., 1987; Gumila et al., 1996; Shumard and Callender, 1968). Furthermore, ionophores can limit the occurrence of digestive disorders such as bloat from legume pastures by reducing the viscosity of rumen fluid caused by entrapped gas, and rumen acidosis by inhibiting lactate-producing rumen bacteria (McGuffey et al. 2001). Other uses include decreasing methane production in the rumen, degradation of ruminal proteins, preventing atypical bovine pulmonary emphysema, lowering serum concentrations of potassium, magnesium and phosphorus, and raising concentrations of glucose and volatile fatty acids (Dowling, 2013).

Ionophores are effective against specific ruminal gram-positive bacteria, protozoa, and fungi. Ionophores are lipophilic in nature which enables them to penetrate through bacterial cell membranes. They can modify the ion movement by binding to ions, shielding ionic charges and acting as selective mobile carriers (Pressman, 1976; Russell and Strobel, 1989). Ionophores that are fed to cattle (e.g. monensin, lasalocid) work as antiporters that bind with protons or metal ions such as sodium and potassium and move them freely across the microbial cell membrane in

opposite directions (Russell and Houlihan, 2003). Due to the changes in ion influx and efflux, the Na^+/K^+ pump and H^+ ATPase system of the bacterial cells experiences a dramatic increase in activity, in the hopes of preserving ion balance and intracellular pH. This means that the cells are consuming energy solely for basic cell purposes and there is no energy left to allocate to growth and development functions. With depleted ATP, the bacteria cells are unable to grow and reproduce, which ultimately leads to premature cellular death (Bergen and Bates, 1984).

Annual ionophore sales in U.S. in 2003 exceeded $100 million, which in turn provided benefits to the U.S. beef cattle industry for up to $1 billion per year (Russell and Houlihan, 2003). The frequent and preventative use of antimicrobials, including ionophores to achieve greater growth in food animals have become a public health concern due to the possibility of antibiotic resistance. However, ionophores are categorized by Canadian Food Inspection Agency (CFIA) as a low importance antimicrobial in human medicine (Government of Canada, 2009). This is because the mode of action of ionophores is unlike other antibiotic classes; they are not used in human medicine and they do not influence fecal shedding of possible pathogens in production facilities. In addition, ionophores resistance was found to be highly complex and specific and thus unlikely to promote the development of antibiotic resistance to important human drugs (Russell and Houlihan, 2003; Callaway et al., 2003; Lefebvre et al., 2006). Therefore, ionophores are still considered to be safe for use in animal feeds.

2.2 MONENSIN

Monensin is a carboxylic ionophore first marketed in the United States as a coccidiostat for chickens in 1971. Monensin was later granted approval in December 1975 by the FDA with growth promotion and(or) increased feed efficiency in beef cattle as its main functions (Novilla,

2011). It is the most popular ionophore used in animal production system and is used extensively in North America, Latin America, Australia, and New Zealand (Russell and Houlihan, 2003). Monensin ($C_{36}H_{62}O_{11}$; molecular weight of 670.87) is produced by *Streptomyces cinnamonensis* or monensic acid (Agtarap et al., 1967). Ionization of ionophores is pH-dependent. Specifically, if the pH of the environment is lower than the pKa of the antiporter, then the ionophore (e.g. monensin) can better penetrate the cell membrane and facilitate ion movements. Monensin has a pKa of 7.95; therefore, it works effectively in an acidic environment such as the rumen (Russell and Houlihan, 2003).

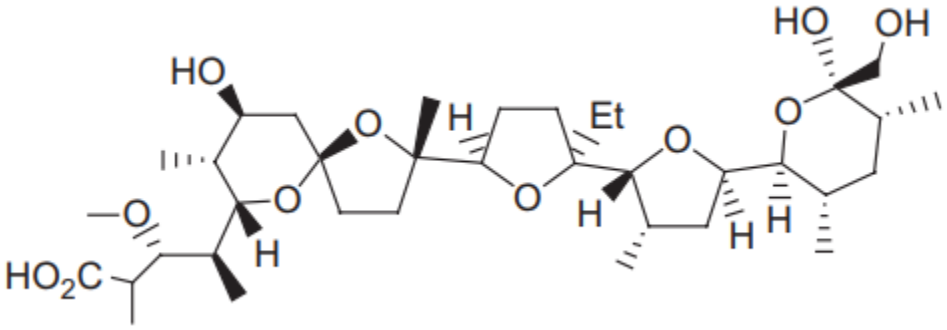


Figure 1.1. Structure of monensin, adapted from Novilla (2011).

Bacteria residing in the rumen typically have high intracellular potassium and low intracellular sodium concentrations while the ruminal environment maintains high sodium and low potassium concentrations (Chow and Russell, 1992). To acquire nutrients and form a proton motive force, ruminal bacteria uses K^+ and Na^+ ion gradients. When inserted in the phospholipid membrane, monensin as an antiporter, transporting intracellular potassium ions out and bringing in protons, or exchanges sodium in the rumen with protons within the bacteria (Russell, 1987). Since the potassium gradient is greater than the sodium gradient, protons accumulate within the bacteria. Because of this, ATP pumps are needed by the bacteria to either pump out protons or re-establish gradients by removing Na^+ and taking in K^+. This means there are less ATP pools

within the bacteria for growth, which eventually results in cellular death (Callaway et al., 2003). Monensin is a good inhibitor of gram-positive bacteria, which positively impacts energy and nitrogen metabolism efficiencies, as well as lowering risks for development of bloat and lactic acidosis (Schelling, 1984). Additionally, monensin modifies the ratio of ruminal volatile fatty acids through an increase in propionic acid and a decrease in butyric and acetic acids. This increase in propionic acid leads to increased glucose production in the animal. With a greater supply of glucose, animals can produce more energy and subsequently improve their weight gain, from their feed (Richardson et al., 1976; Prange et al., 1978).

2.2.1 EFFECTS OF MONENSIN ON RUMEN FERMENTATION

Ionophores, including monensin are compounds that are capable of manipulating rumen fermentation to improve the production efficiency of ruminants (Bergen and Bates, 1984). There are several methods in which this is achieved. First, volatile fatty acids (VFA) are produced through rumen fermentation, and they serve as the main energy supply for ruminants. The three major VFA are acetate, propionate, and butyrate (Richardson et al., 1976). Research has shown that the acetate: propionate ratio in the rumen fermentation profiles of monensin-treated ruminants, is more ruminal propionate produced than acetate (Thornton and Owens, 1981). This is beneficial for feed efficiency as feed conversion to propionate is more efficient than the other two VFA due to having a higher enthalpy. This would mean more left-over and available feed energy supplies for the animal (Richardson et al., 1976). Monensin can also inhibit methane production by 30-50% by limiting the growth of carbohydrate fermenting bacteria that produces H_2. This is another method that enables monensin to decrease the acetate to propionate ratio, although the reduction is more significant with a forage diet since the acetate to propionate ratio is much higher (up to 4:1) (Russell, 2002). Ruminal ammonia concentrations were reduced when

monensin was supplied *in vivo*. With less ammonia, there is less protein breakdown and deamination and thus more protein is spared and are available for use (Russell and Strobel, 1989; Russell, 2002). In addition, monensin have been reported to be effective in limiting the growth of lactate producers without compromising the lactate utilizers (Dennis et al., 1981a,b). Ruminal pH can decrease dramatically after cattle are fed a high grain diet. A rumen with too low of a pH is an important cause for acidosis. By lowering lactic acid production, ruminal pH can be increased, leading to reduced frequency and severity of lactic acidosis (Nagaraja et al., 1982). There has also been evidence of a greater propionate production using lactate via the acrylate pathway (Bergen and Bates, 1984). Finally, monensin can influence ruminal fermentation patterns by inhibiting protozoa populations and depressing rumen fluid viscosity which are both linked to feedlot bloat, as well as limiting the growth of undesired ruminal microbes that are associated with bloat development (Bergen and Bates, 1984).

2.2.2 EFFECTS OF IONOPHORES (MONENSIN) ON RUMEN MICROORGANISMS

The rumen microbiota is critical for influencing the digestion efficiency of ruminants and it can be manipulated for better performance via dietary intervention (e.g. monensin) (Ogunade et al., 2018). Ionophores are not capable of inhibiting all rumen microorganisms. Often times, the effectiveness of monensin is strongly related to the bacteria cell wall structure, meaning that gram-positive bacteria are more sensitive than gram-negative bacteria (Russell and Houlihan, 2003). However, the presence or absence of an outer membrane on the targeted bacterium is not the sole determinant of monensin susceptibility as studies have shown decreases in both taxa when monensin was introduced (Chen and Wolin, 1979; Kim, Eastridge, and Yu, 2013; Ogunade et al., 2018). Cattle rumen microbiota consist mainly of the phyla Bacteroidetes, Firmicutes, Proteobacteria, and Euryarchaeota. Several studies found that when steers were fed monensin,

gram-negative Bacteroidetes had higher relative abundance than gram-positive Firmicutes, which agrees with the intended purpose of monensin (Kim, Eastridge, and Yu, 2013; Thomas et al., 2017; Ogunade et al., 2018). The growth and activities of gram-negative bacteria *Prevotella* spp., *Hallella seregens*, *Parabacteroides distasonis*, *Selenomonas* spp., and *Propionispira raffinosivorans* increased when steers were administered monensin (Ogunade et al., 2018). A lower population of gram-positive bacteria means lower production of acetate, hydrogen, and lactate in the rumen, and higher production levels of propionate. This allows cattle to achieve a better energy status (Russell and Houlihan, 2003). Ogunade et al. (2018) also found decreased numbers of *Methanobacterium*, which can lead to a potential decrease in ruminal methane production due to supplying fewer primary substrates for methanogens for methanogenesis. Moreover, through decreased abundance of the *Peptostreptococcus* and *Clostridium* bacteria species, monensin can minimize amino acid degradation and ammonia accumulation in the rumen (Ogunade et al., 2018).

Following bacteria, protozoa are the next most abundant microorganisms in the rumen, accounting for as much as 50% of the total microbial genetic material (Clemmons, Voy, and Myer, 2019). *In vitro* studies have demonstrated that monensin is effective against protozoa (Hino, 1981; Hino and Russell, 1987, Shen et al., 2017). However, monensin did not attain the same level of success when applied in *in vivo* studies for cattle and sheep (Dinius, Simpson, and Marsh, 1976; Leng et al., 1984).

Anaerobic fungi comprise approximately 2% of the rumen microbiome. Fungi are only important when ruminants are fed high-forage diets because they are more capable of penetrating and breaking down the tough plant walls of fibrous forage materials than other microbes. However, ruminal fungi do not survive well in high-grain diets and are very sensitive to pH,

which is influenced by the digestion of carbohydrates in grains (Clemmons, Voy, and Myer, 2019). *In vitro* studies have found ruminal fungi can be inhibited by monensin, but results were inconsistent *in vivo* (Stewart, Duncan, and Joblin, 1987; Elliott et al., 1987; Russell and Strobel, 1989, Grenet et al., 1989).

2.2.3 EFFECT OF MONENSIN ON CATTLE PERFORMANCE

Monensin improves feed efficiency by reducing dry matter intake (DMI) and improving average daily gain of growing and finishing cattle (Duffield et al., 2012). Cattle fed 300 mg of monensin/head/day had an enhanced feed efficiency of 6% with 5 to 14% lower feed intake (depending on the amount of corn silage received in diet) compared to non-monensin fed cattle (Gill et al., 1976). In a study conducted by Mowat et al. (1977), monensin increased rate of weight gain and feed efficiency for finishing cattle on diets consisting of alfalfa silage, or alfalfa silage and high moisture shelled corn. Furthermore, Stock et al. (1995) found that monensin decreased DMI by 1.4% and improved feed efficiency by 4.2% compared with control fed cattle. In a study conducted by Benchaar et al. (2005), steers and heifers supplemented with monensin at 33 mg/kg of dry matter (DM) consumed 10% less DM than steers and heifers that were not supplemented with monensin. However, there are other studies (Burrin et al., 1988; Stock et al., 1990; Zinn and Borques, 1993; Depenbusch et al.,2008) that did not demonstrate significant positive effects for DMI and feed efficiency from feeding monensin. The range of efficacies with monensin is not well understood, and there are factors beyond sex, weight classification, usage of growth promotants, dietary metabolizable energy concentration, metabolizable energy intake, monensin consumption rate and dietary monensin concentration that could explain the variations in growth performance when monensin is fed to feedlot cattle (Goodrich et al., 1984).

2.2.4 IONOPHORE (MONENSIN) RESISTANCE

There has been an increase in bacteria resistance to antibiotics due to its wide spread usage and application in the agriculture sector. As a result, antimicrobial feed additives applied in animal production and thus present as residues in final products are causing growing concerns due to their potential negative impacts on human health. The European Union and some other countries have even banned the usage of antimicrobial growth promotors, including monensin salt in animal feed since 2006 (Stevanovic et al., 2008). However, based on several research articles, use of ionophores such as monensin in animal feed do not present an appreciable risk for consumers of animal products.

Most ionophores are produced by bacteria from the *Streptomyces* genus, and through a variety of mechanisms, these organisms are self-resistant to the antibiotics that they help create. Mechanisms include alterations to the antibiotic, alterations to the cellular target, induction of a substitute cellular component that is insensitive to the antibiotic, and removal of the antibiotic (Linton et al., 1994). However, the presence of *Streptomyces* spp. bacteria in the rumen is rare, considering that actinobacteria, the phylum that *Streptomyces* belongs to, only represent maximum 3% of the total rumen microflora (Šuľák et al., 2012). Ionophore functions can be impaired by aerobic bacteria and mammalian enzymes, although those pathways require oxygen and hence this is not a concern for anaerobic environments such as the rumen (Russell and Houlihan, 2003). Not all ruminal bacteria have been observed to be inhibited by ionophores in both *in vitro* and *in vivo* experiments. Often, monensin-resistant ruminal bacteria possess more membrane-bound fumarate reductase (a proton-translocating enzyme) activity, which can possibly counteract the ion flux caused by the ionophores (Bergen and Bates, 1984). Bacteria sensitivity and resistance to ionophores are more correlated to the type of cell envelope a specific

bacterial species has. Specifically, gram-positive are often more sensitive to monensin than gram-negative ruminal bacteria due to a thicker cell wall and peptidoglycan layer. *Prevotella* strains of bacteria (gram-negative) start off as ionophore-sensitive, then become resistant after an adaptation period (Russell and Houlihan, 2003). These ionophore adapted bacteria cells bind less ionophores possibly through reduced porin size in the cell membrane or modified outer membrane characteristics (Callaway et al., 2003). gram-positive bacteria are more sensitive to monensin especially upon initial exposure; however, these bacteria can also increase their monensin-resistance over time. This is true for ruminal bacteria such as *Streptococcus bovis, Clostridium aminophilum, Selenomonas ruminantium, and Megasphaera elsdenii*. It is not clear as to how resistance is gained, although the changes are thought to be due to phenotypic selection rather than a mutation, an acquisition, or rise in foreign and novel genes (Callaway et al., 2003; Russell and Houlihan, 2003).

Although some ruminal bacteria can become resistant to monensin *in vitro*, monensin is still highly effective for its various purposes, most notably increasing feed efficiency. Positive effects of monensin have been present when it was supplemented in animal feed for up to 400 days and rarely has ionophore-resistant isolates been found. Additionally, there is a lack of evidence present for the spread of ionophore resistance between ruminal bacteria; there is no concrete indication that ionophore resistance in cattle rumen bacteria is increasing. Monensin residues have been found in the eggs and livers of some animals, (e.g. hens, quails, pheasants, cattle, and sheep). However, the estimated consumer exposure to monensin is only 0.6% of the acceptable daily intake limit from eggs and 7.3% from liver (Dorne et al., 2013). All in all, *in vivo* resistance to monensin most often does not translate or equate to a resistance in an actual rumen. Since ionophores are not used for humans, are complex in nature, and have different

modes of actions than many other therapeutic antibiotics, usage of ionophores such as monensin in cattle and other animal feeds is unlikely to cause cross-antibiotic resistance from animals to human (Callaway et al., 2003; Russell and Houlihan, 2003). Thus, the CFIA has classified monensin and other ionophores as non-medically important antimicrobials. This means that feed manufacturers and retailers do not require the authority of a veterinarian to sell these products, and buyers do not need a veterinarian prescription to purchase these products (CFIA, 2018a).

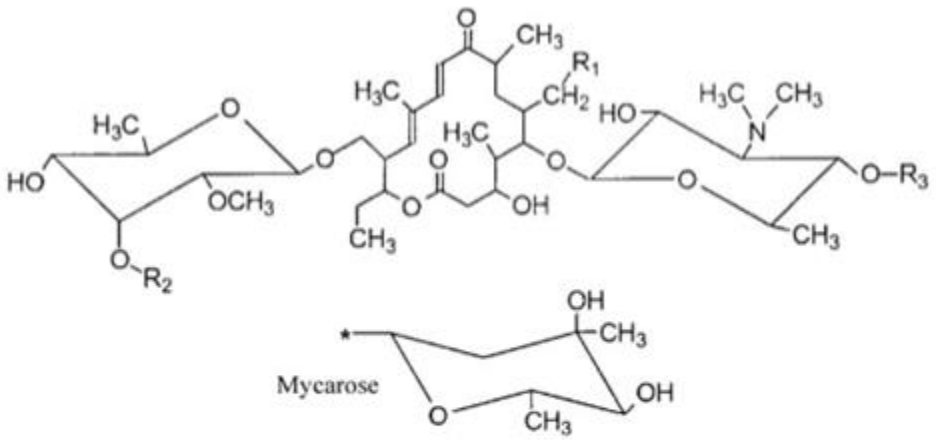
2.2.5 MONENSIN INCLUSION RATE

Toxicity, determined by haematology, blood chemistry, and necropsy measures could not be detected when giving monensin to cattle at up to 110 mg/kg in the diet or to calves at up to 120 mg/kg in the diet (Dorne et al., 2013). According to Elanco (2019a), the major manufacturer of monensin under the label Rumensin®, monensin is suggested to be supplied to feedlot cattle at between 5 – 40 mg/kg in the diet. Monensin supplemented at 5 – 40 mg/kg (90% dry matter basis) of complete feed can lead to improvements in feed efficiency, while monensin provided at 10 – 40 mg/kg (90% dry matter basis) of feed is adequate for the control and prevention of coccidiosis. CFIA (2019) recommends monensin be supplied between 33 – 48 mg/kg of the complete feed. Although, there may not be additional enhancements in feed efficiency beyond 33 mg/kg of the total feed.

2.3 TYLOSIN PHOSPHATE

Tylosin phosphate ($C_{46}H_{77}NO_{17} \cdot H_3PO_4$) is a macrolide antibiotic isolated from *Streptomyces fradiae*. It is made of a 16-membered substituted lactone ring, an amino sugar (mycaminose), and two neutral sugars (mycinose and mycarose). As seen in Figure 1.2, tylosin is composed of a mixture of four macrolides: Tylosin A, Tylosin B (desmycosin), Tylosin C

(microcin), and Tylosin D (relomycin). Around 80-90% of the compound is Tylosin A, but all components contribute to the efficacy of the antibiotic (Sarmah et al., 2006). Tylosin phosphate is approved and used for treatment of various diseases in cattle, pigs, goats, poultry, dogs, and cats (Papich, 2016). Protein synthesis by the bacteria is inhibited by the binding of tylosin to the 50S ribosomal subunits, preventing microbial transpeptidation and translocation processes. The lack of peptide bond formation leads to incomplete polypeptide chains (Giguere, 2013). In cattle, tylosin can treat bovine respiratory disease, prevent liver abscesses, and foot rot caused by various bacteria (Papich, 2016). Generally, macrolides are bacteriostatic agents but can be bactericidal against a low inoculum of bacteria when applied at high concentrations (Giguere, 2013).



	Tylosin A	Tylosin B	Tylosin C	Tylosin D
R_1	-CHO	-CHO	-CHO	$-CH_2OH$
R_2	$-CH_3$	$-CH_3$	-H	$-CH_3$
R_3	-Mycarose	-H	-Mycarose	-Mycarose

Figure 1.2. Structure of tylosin, adapted from Sarmah et al. (2006).

2.3.1 TYLOSIN AND LIVER ABSCESSES

The most important use of tylosin is as a preventative treatment and control for liver abscesses in feedlot cattle. Liver abscesses are pus-filled and composed of necrotic centers consisting of degenerating hepatocytes and leukocytes (Lechtenberg et al., 1988). Abscesses can have different capsule layer thicknesses and diameters ranging from a pinpoint to 15 cm (Nagaraja and Chengappa, 1998). They were first identified in 1940 and can occur in all types and ages of cattle species. Incidence rate in commercial feedlots range from 12-32%. Past research has found liver abscesses to be unfavourable against feed intake, weight gain, feed efficiency, and dressing percentage in feedlot cattle. Thus, they are a big economic liability for everyone involved, from producers to beef consumers (Smith, 1940; Brink et al., 1990; Nagaraja and Chengappa, 1998). The prevalence of liver abscesses is mainly connected to the type of finishing diet, specifically high concentrate diets comprising of grains that are "rapidly and extensively fermented". Diets high in corn, barley and wheat cause more instability in ruminal pH and intake than other feed grains, which can eventually lead to liver abscesses (Nagaraja and Chengappa, 1998). Besides diets, days of feeding, cattle type, breed, gender, geographic location, and season could also be related factors to abscess formation (Reinhardt and Hubbert, 2015). Abscessed livers are condemned and not sold, making them an economic liability for the beef industry. Liver abscesses are visually evaluated at slaughter by trained professionals and are scored based on their number and size. A score of 0 means a healthy liver with no abscesses; A- means 1-2 small abscesses or scars under 2.54 cm in diameter; A means 2-4 small abscesses; A+ = 1 or more large abscesses and/or a liver adhered to the gastrointestinal tract, diaphragm or both (Elanco, 2018).

Although presence of *Actinomyces pyogenes*, *Bacteroides* spp., *Clostridium* spp., *Peptostreptococcus* spp., *Staphylococcus* spp., and *Streptococcus* spp. have been isolated from liver abscesses, various culture studies examining the bacterial flora of bovine liver abscesses have concluded *Fusobacterium necrophorum* as the main etiologic agent (Simon and Stovell, 1971; Berg and Scanlan, 1982; Scanlan and Hathcock, 1983). Isolated in 71-95% of liver abscesses, *F. necrophorum* is a gram-negative, anaerobic, nonmotile bacterium that naturally occurs in both animal and human gastrointestinal tracts.

In a high-grain diet, more lactate is available in the rumen for *F. necrophorum* cells to use as an energy substrate, and ferment into acetate, butyrate, and some propionate (Lechtenberg et al., 1988). Thus, there could be more than 10 times more *F. necrophorum* in the rumen of a steer on a grain dominant diet versus a roughage-based diet (Tan, Nagaraja, and Chengappa, 1994). A rapid change to a high energy finishing diet is often correlated with an acid-induced rumenitis. This condition along with the possibility of being penetrated by foreign objects in the feed causes damage to the ruminal wall, allowing it to be susceptible to a *F. necrophorum* attack and colonization. Afterwards, the bacterium can shed bacterial emboli into the portal vein that eventually gets filtered to the liver via portal blood, causing infection and liver abscess development (Nagaraja and Chengappa, 1998). Penetration and damage to the ruminal epithelium is also aided by key virulence factors including leukotoxins, endotoxins, haemolysins, haemagglutinins, and adhesins (Tan, Nagaraja, and Chengappa, 1996). However, the liver is an oxygen-rich environment whereas *F. necrophorum* is an anaerobe, meaning there may be other factors influencing the growth of abscesses (Nagaraja and Chengappa, 1998). Instead of being a primary causative agent, *F. necrophorum* could be just an opportunistic bacterium that is

"passing by" and taking advantage of a vulnerable environment caused by liver abscesses (Rothman and Greenland, 2005; Tjalsma et al., 2012).

Out of the antimicrobials allowed for use in animal feeds, tylosin is generally the most effective in preventing liver abscesses, while bacitracin is the least effective (Nagaraja et al., 1999). The mechanism of action for tylosin is due to its inhibitory effect on *F. necrophorum* mostly in the rumen since the antibiotic is absorbed in the gut; microbial inhibition could also occur in the liver if tylosin could travel there (Gingerich, Baggot, and Kowalski, 1977). Tylosin, a macrolide, is mostly effective against gram-positive bacteria, but *F. necrophorum,* a gram-negative bacterium is also sensitive to tylosin (Lechtenberg, Nagaraja, and Chengappa, 1998). The incidence of liver abscesses decreased from 29% in control cattle not fed an antibiotic to 10% in feedlot cattle fed tylosin at 11 mg/kg of feed, including a large reduction in A+ abscesses from 20% to only 1.1% (Heinemann, Hanks, and Young, 1978). Crossbred steers fed 75 mg of tylosin per day had fewer liver abscesses compared to steers that were not fed tylosin (Pendlum, Boling, and Bradley, 1978). Similarly, there were fewer live abscesses in crossbred yearling heifers on a steam-flaked corn finishing diet supplemented with 90 mg of tylosin per day as compared to heifers that were only consuming corn or corn accompanied with monensin (Depenbusch et al., 2008). Furthermore, feedlot cattle fed 11 mg/kg of feed of tylosin had 18% less liver abscesses and improved average daily gain than cattle there not fed an antibiotic (Potter et al., 1985). Available literature has indicated the effectiveness of tylosin at reducing 40-70% of liver abscesses (Nagaraja and Chengappa, 1998). A complete suppression of liver abscesses has not been observed, and reasons for this could be due to 1) inadequate concentrations of tylosin were given and absorbed by the rumen; 2) reduction of *F. necrophorum* may have allowed the growth of other opportunistic bacteria that could damage the ruminal wall and eventually reach

the liver; 3) development of antibiotic resistant bacteria due to consumption of tylosin (Nagaraja et al., 1999).

2.3.2 TYLOSIN RESISTANCE

Antimicrobial resistance (AMR) is defined as the natural phenomenon of bacteria and fungi acquiring the ability to resist and defeat the drugs that were intended to kill them, following exposure. Resistant microbes that are not killed by the antimicrobial agents are able to keep multiplying and cause more harm to its host (Séveno et al., 2002; CDC, 2018). AMR can be intrinsic or acquired. Naturally occurring mechanisms of resistance are generally due to cells being inaccessible or lacking the required target or receptor sites for the antimicrobial agent to attach to (Schwarz, Cloeckaert, and Roberts, 2006). Acquired resistance mechanisms include reduced cell permeability, drug target site mutation, improved efflux pump expression, drug enzymatic inactivation, and horizontal gene transfer of resistance determinants via mobile genetic elements such as plasmids, transposons, integrons, and bacteriophages (Alekshun and Levy, 2007; Lerma et al., 2013). Intrinsic resistance is a bacterial genus- or species-specific property while acquired resistance is strain-specific (Schwarz, Cloeckaert, and Roberts, 2006).

Tylosin is part of the macrolide-lincosamide-streptogramin B (MLS_B) superfamily of antibiotics and is strictly used in food animals. As a member of this superfamily, tylosin can cross-select for resistance to all other MLS_B drugs, including ones used to treat human infections (e.g. clarithromycin, clindamycin, azithromycin) (Chen et al., 2008). As of May 2019, there are 107 genes identified that give resistance to macrolides and influence drug resistance patterns (Roberts, 2019; Leclercq, 2002). Bacteria can acquire resistance to macrolides using 3 methods: efflux of the antibiotic, inactivation of the antibiotic, and most importantly, alteration of the

target-site by methylation or mutation such that the antibiotic cannot bind to its ribosomal target (Leclercq, 2002).

Ribosomal RNA methylation is the most common and studied mechanism of macrolide resistance (Leclercq, 2002; Roberts et al., 1999). The majority of methylases are encoded by *erm* (erythromycin ribosome methylation) genes, who are one of the most commonly developed resistance genes in bacteria and antimicrobial genes of MLS_B (Chen et al., 2007). To date, there have been 41 classes of *erm* genes found that can confer resistance to macrolides (Roberts, 2019). *erm* gene codes work by adding one or two methyl groups to an adenine residue located at the A2058 position (in *E. coli*) of a 23S rRNA, which is also a component of the large 50S ribosomal subunit. Consequently, the binding site is modified and binding to tylosin is no longer possible (Leclercq, 2002; Roberts et al., 1999). Due to overlapping binding sites in 23 rRNA, there is cross-resistance to all other drugs within the MLS_B superfamily, as previously mentioned (Leclercq, 2002).

Antibiotic resistance genes code for efflux proteins to drive the antibiotic out of the cell or cellular membrane. This allows a low intracellular antibiotic concentration and prevents ribosomes from binding the antibiotic (Roberts et al., 1999). In gram-negative bacteria, efflux pumps mostly belong to the resistance/nodulation/division family with 12 membrane-spanning regions, while in gram-positive bacteria, efflux proteins mostly belong to the ATP-binding-cassette transporter superfamily or the major facilitator superfamily (Leclercq, 2002). Drug inactivation only confers resistance to antibiotics with similar structures. Different genes that inactivate enzymes consist of 4 esterases, 2 lyases, 16 transferases, and 15 phosphotransferases (Leclercq, 2002; Roberts, 2019).

In a study by Chen et al. (2008), fecal samples from beef cattle fed tylosin at 11 mg/kg of feed were found to have increased *erm* and *tet* genes who confer resistance to MLS_B and tetracyclines, respectively. Zaheer et al. (2013) administered tylosin therapeutically and subtherapeutically to beef cattle and found a larger population of antimicrobial resistant *Enterococcus* spp. within the intestinal tract. However, those species are not commonly associated with human nosocomial infections. Beukers et al. (2015) reported an increase in tylosin resistant enterococci population in the fecal samples of feedlot steers, but resistant populations decreased after a 28-day tylosin withdrawal period. Prevalence of tylosin resistant enterococci collected from cattle fecal samples also increased as reported by Amachawadi et al. (2015). Surprisingly, there has not been an observed increase in resistance in *F. necrophorum* or *T. pyogenes* (Amachawadi et al., 2017).

2.3.3 INCLUSION RATE

Tylosin is commonly used in the diets of livestock. 71% of feedlot cattle in United States have received tylosin, making it the second most popular feed additive after ionophores (USDA, 2013). Tylosin however, is not an antibiotic for human use but its structure is similar to another macrolide antibiotic, erythromycin, which is being used for both animals and humans (Mellon et al., 2001; Chen et al., 2008). Uses for macrolides in humans include treating infections caused by *Streptococcus pneumoniae, S. pyogenes, Staphylococcus aureus,* and sometimes pathogens such as *Legionella pneumophila, Chlamydophila pneumoniae,* and *Mycoplasma pneumoniae* (Wierzbowski et al., 2006). Concerns for increased prevalence of resistant bacteria in animals and cross-resistance to human bacteria have led to a ban of tylosin use in the European Union since July 1, 1999 (Teuber, 1999). The Government of Canada has placed macrolides in Category II, meaning that it is an antimicrobial drug of high importance due to its uses against various infections (Government of

Canada, 2009). As previously mentioned, new CFIA regulations require medically important antimicrobials such as tylosin to only be bought and used in feeds or sold with a veterinary prescription obtained by the end-user (CFIA, 2018a).

For beef producers that have a veterinary prescription, Elanco, the manufacturer of Tylan® Premix (commercial name of tylosin phosphate), recommends its inclusion level to be at 8-10 mg/kg of feed at 90% dry matter basis. This would allow the "reduction of incidence of liver abscesses in beef cattle associated with *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*" (Elanco, 2019b). CFIA approves tylosin to be included in the complete diet of beef cattle at a level of 11 mg/kg (100% dry matter basis), to reduce the incidence of liver abscesses. Tylosin should also be used carefully and for the shortest amount of time necessary after achieving the desired clinical effect to minimize the development of antimicrobial resistance (CFIA, 2018b).

2.4 ESSENTIAL OILS

Essential oils (EO) are concentrated hydrophobic and aromatic oily liquids extracted from plant material. They can be obtained from components such as flowers, leaves, herbs, and roots. Processing techniques are most commonly water or steam distillation, but also through expression, fermentation, enfleurage, or extraction (Burt, 2004). Aromatic oils have been used since 4500 BC in ancient Egypt for cosmetics and ointments. The Chinese and Indians have also been recorded to use EO for medicinal purposes between 3000 and 2000 BC (Elshafie and Camele, 2017). Pharmacies started distilling EO during the 13th century and they became commonly used in Europe for medicinal purposes by the 16th century. Due to their diverse composition and activities, these secondary metabolites are incredibly important plant-derived

products; EO are currently being used in the production of various foods, perfumes, cosmetics, and pharmaceuticals (Burt, 2004; Calsamiglia et al., 2007).

Plant EO are complex, natural mixtures made up between 20-60 components that are characterized by 2 or 3 dominant components due to their abundance (Chouhan, Sharma, and Guleria, 2017). The composition of EO are dependent on plant type, plant part, environmental conditions before and after harvest, chemotypes, and method of extraction. However, they are composed of some variation of hydrocarbons, aliphatic aldehydes, alcohols, and esters (Elshafie and Camele, 2017). This in turn impacts the functionality of these oils. The two most important groups of active compounds are terpenoids and phenylpropanoids, which are made from different precursors and are produced through different metabolic pathways (Calsamiglia et al., 2007). Terpenoids are hydrocarbons made of a combination of multiple isoprene units (C_5H_8) and are named based on the number of units in their skeleton. Examples of terpenoids include thymol, carvacrol, and menthol (Chouhan, Sharma, and Guleria, 2017). Phenylpropanoids are 6 carbons aromatic ring compounds attached with a chain of 3 carbons, synthesized from the amino acid, phenylalanine through the shikimate metabolic pathway (Calsamiglia et al., 2007; Chouhan, Sharma, and Guleria, 2017). While not many EO consist of this compound, their proportions are significantly dominant in certain plants. Examples of phenylpropanoids include eugenol, vanillin, and cinnamaldehyde (Calsamiglia et al., 2007).

EO have been found to possess a wide variety of desirable properties, including anti-inflammatory, antioxidant, and most importantly, antimicrobial activities based on composition, functional group, and any synergistic interactions (Chouhan, Sharma, and Guleria, 2017). Terpenoids and phenylpropanoids are thought to be effective against bacteria mainly due to the hydrophobicity of the hydrocarbons, which allow the bioactive components to attach and interact

with the bacteria cell membrane. They are then able to accumulate and consequently, penetrate through the phospholipid bilayer of the bacteria membrane. This disrupts the structural integrity and stability of the membrane, thus compromising cell metabolism and eventually leading to cell death (Calsamiglia et al., 2007; Chouhan, Sharma, and Guleria, 2017). EO are also more effective against gram-positive versus gram-negative bacteria since the outer cell wall that gram-negative bacteria possess is rigid, complex, and hydrophilic. This makes the hydrophobic EO compounds harder to diffuse through and cause damage (Chouhan, Sharma, and Guleria, 2017).

2.4.1 THYMOL

Thymol EO ($C_{10}H_{14}O$) is a terpenoid and a phenolic molecule which is one of the major components of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) (Burt, 2004). Thymol can be extracted through a variety of methods, including steam distillation, solid-liquid extraction methods, pressurized liquid extraction through solvents, and chromatography (Garcia-Risco et al., 2011; Bermejo et al., 2014). Due to its pleasurable odor and distinctive taste, thymol has long been added as a flavoring agent for fish and meat products (El-Hack et al., 2016). Its most significant use however is for its anti-inflammatory, antioxidant, antibacterial, and antifungal activities (Soliman and Badeaa, 2002; Braga et al., 2006; Hoferl et al., 2009).

The known antimicrobial properties of thymol are believed to be due to its ability to damage the structures and functions of cytoplasmic membranes of bacterial cells. As a phenolic compound, thymol possesses an active hydroxyl (─OH) group that can interact with water and disrupt the membrane through hydrogen bridges (Chouhan, Sharma, and Guleria, 2017). As the membrane gets weakened, cellular components are lost, and electrons are delocalized, thus minimizing and possibly disintegrating the proton motive force gradient. As a result, ATP

synthesis is compromised, and the pool becomes depleted, eventually leading to cell death (Purwar, 2019). The antibacterial properties of compounds are affected by the number and location of the hydroxyl groups on the aromatic ring, with thymol exhibiting stronger activity than its isomer carvacrol due to having the hydroxyl group in the ortho position. Juven et al. (1994) also found thymol to have greater antibacterial activity at pH 5.5 versus 6.5 because the molecules are undissociated and more hydrophobic at lower pH, allowing more interaction with membrane proteins to increase cell permeability.

Within the agriculture and food industries, the most significant functions that thymol exhibit appear to be for flavor and for its antimicrobial and antioxidant effects. The bactericidal activity of thymol has been well researched in both foods and broth systems. It has been reported to be effective against a wide range of pathogens such as *E. coli, S. aureus, S. typhimurium*, and *L. monocytogenes*, spoilage bacteria such as *B. thermosphacta*, and moulds such as *Penicillium* spp. (Smith-Palmer et al., 1998; Cosentino et al. 1999; Hammer et al., 1999, Lambert et al., 2001). In a study conducted by Del Nobile et al. (2007), supplementation of 750 mg of thymol in a kg of ground beef reduced the growth of coliforms and *Enterobacteriaceae*, improved product quality, and extended shelf life. Another study reported thymol capable of lowering pathogen concentrations and fermentation products in beef cattle manure slurries (Wells et al., 2015).

Various studies have reported high antioxidant activity from thymol due to its phenolic content (Wojdylo, Oszmianski, and Czemerys, 2007; Shan et al., 2005; Wang, 2003). One study reported that the use of liquid thyme extract was able to inhibit lipid oxidation of pork by 64% (Tanabe, Yoshida, and Tomita, 2002). In another study, *Carum copticum* otherwise known as ajowan and similar to caraway, was examined as an antioxidant for use in mined beef (Mahmoudzadeh et al., 2016). These researchers found that thymol was present in 36.4% of total

phenolic compounds for *Carum copticum* with strong antioxidant activities to prevent off-color and off-odor while also inhibiting lipid oxidation in minced beef.

2.4.2 EUGENOL

Eugenol ($C_{10}H_{12}O_2$) is a phenolic aromatic organic compound within the family of phenylpropanoids. It is a clear to pale yellow oily liquid that was first isolated from *Eugenia caryophyllata* (*Syzygium aromaticum*) or clove in 1929, for which it was named after. Although eugenol is most often extracted from clove buds and leaves (45-90%), this EO is also found in basil, cinnamon, nutmeg, and bay leaf species (Chouhan, Sharma, and Guleria, 2017; Marchese et al., 2017). Similar to thymol, eugenol contains a high number of phenolic compounds and is capable of increasing cytoplasmic membrane permeability, disrupting proton motive force, electron flow, pH gradient, and active transport as well as coagulating bacterial cell components (Burt, 2004). Based on these properties, eugenol has a wide range of antibacterial properties that are effective against *E. coli, S. typhimurium*, and *L. monocytogenes* (Kim et al., 1995). The antimicrobial activity is also attributed to its free hydroxyl group, which has been hypothesized to bind to the hydrophobic parts of membrane proteins, limiting enzyme action in *E. aerogenes* (Burt, 2004; Wendakoon and Sakaguchi, 1995).

In foods, eugenol has significantly inhibited the growth of *A. hydrophila* and *L. monocytogenes* in ready-to-eat beef slices and refrigerated, cooked chicken breast meat (Hao, Brackett, and Doyle, 1998a,b). Zengin and Baysal (2014) reported *S. typhimurium* and native coliforms were inhibited by clove EO (75.2% eugenol as determined by gas-chromatography mass-spectrophotometry) in ground beef samples and color was maintained throughout 9 days of storage; use of eugenol significantly reduced TBA quantities as compared to when no EO was

incorporated within the ground beef. Eugenol applied to novel coating films on beef *longissimus thoracis* steaks also significantly inhibited lipid oxidation and improved color stability (Navikaite-Snipaitiene et al., 2018).

2.4.3 LIMONENE

Limonene (p-mentha-1,8-diene) is a cyclic monoterpene and an organic compound that can be extracted from the oils of many citrus plants (e.g. orange, lemon, grapefruit), as well as herbs (e.g. mint, basil, thyme) (Elshafie and Camele, 2017; Bacanli, Basaran, and Basaran, 2018). Limonene provides flavor for the food and beverage industries, aromas for cosmetics and household products, and is used even for some pharmaceutical products, although a high concentration is required for pharmacological effects (Hirota et al., 2010; Crowell et al., 1994). This EO has two enantiomers, R and S, with the more abundant form being the R isomer (Bacanli, Basaran, and Basaran, 2018). Limonene has antibacterial effects, mainly against gram-negative bacteria, including *E. coli, Salmonella spp., C. jejuni,* and *C. coli* (Samii et al., 2016; Nannapaneni et al., 2008) because its polarity is low, making it difficult to move through and penetrate through the rigid cell membranes that gram-positive bacteria have (Samii et al., 2016; Bacanli, Basaran, and Basaran, 2018). Although it does not possess a phenolic ring, limonene has an alkenyl instead of an alkyl constituent. This extra bond increases the antibacterial activity, and this alkylation is perhaps another reason why gram-negative bacteria are more susceptible to limonene (Dorman and Deans, 2000). The bioactive properties of limonene include antioxidant, anti-inflammatory, and antifungal activities that are beneficial for human health (Bacanli, Basaran, and Basaran, 2018).

Citrus lemon essential oil contains 39.74% limonene in its chemical composition. The lemon EO has acceptable radical scavenging activity and was effective in limiting the growth of *L. monocytogenes* in minced beef. pH and TBARS were also lower in the EO treated beef, demonstrating its antioxidant ability (Hsouna et al., 2017). In a study by Samii et al. (2016), limonene decreased growth of *F. necrophorum*, the bacterium that causes liver abscesses in feedlot cattle in an in vitro study and was found to be even more effective than thymol for bacterial suppression. Limonene also lowered ruminal *F. necrophorum* concentrations in heifers when supplementing limonene in vivo, demonstrating the potential of incorporating limonene in feedlot diets instead of tylosin.

2.4.4 VANILLIN

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a phenylpropene and the main constituent of vanilla beans (Fitzgerald et al., 2004). Presently, vanillin is rarely obtained from natural sources since it is 300 times more expensive than the synthetically produced version, and there is not enough natural vanillin to keep up with global demands (Rana et al., 2013). Synthetic vanillin is identical to natural vanillin and is made from lignin, eugenol, or guaiacol (Fitzgerald et al., 2004). As a generally recognized as safe (GRAS) product, vanillin is widely used as a flavor and odor agent in a variety of foods and cosmetics, as well as an intermediate in agrochemicals and medicinal drugs (Fitzgerald et al., 2004; Rana et al., 2013). Additionally, past studies have reported strong antimicrobial activities for vanillin against yeasts and molds in fruit purees and fruit-based agar systems (Cerrutti and Alzamora, 1996; Lopez-Malo, Alzamora, and Argaiz, 1997; Matomoros-Leon, Argaiz, and Lopez-Malo, 1999). Bacteria inhibition is thought to be from vanillin detrimentally damaging the cytoplasmic membrane, and in turn causing a loss of ion gradient, pH homeostasis, and respiratory activity. However, membrane damage is not as

extensive as phenolic antimicrobials, and thus their effects are more bacteriostatic instead of bactericidal (Fitzgerald et al., 2004). In a study by Castillejos, Calsamiglia, and Ferret (2006), vanillin was supplemented in a basal diet of alfalfa hay, corn, barley, and soybean meal and fed to lactating dairy cows. Adding vanillin to the 60% forage:40% concentrate diet did not affect nor improve rumen microbial fermentation. In an in vitro study conducted by Patra and Yu (2014), vanillin reduced ruminal production of methane, ammonia, and protozoa populations with increasing dosage, but populations of *Ruminococcus flavefaciens, Prevotella bryantii, Butyrivibrio fibrisolvens, Prevotella ruminicola, Clostridium aminophilum*, and *Ruminobacter amylophilus* actually increased as more vanillin was added. Thus, efficacy of this compound was mixed in animal studies.

2.5 ORGANIC ACIDS

Organic acids (OA) are acidic organic compounds that contain carbon atoms and are used as additives in food and feed production. They either exist within foods or are chemically manufactured, then included directly or indirectly to the targeted product. Common OA used within foods are acetic, benzoic, citric, formic, lactic, and propionic acids. OA partially dissociate in aqueous solutions, often producing weak acids and buffers in aqueous solution (Quitmann, Fan, and Czermak, 2014; Anyasi et al., 2017). OA can be grouped based on the number of carboxyl (─COOH) groups, hydroxyl groups, and carbon─carbon double bonds that make up the compound (Theron and Lues, 2011). Furthermore, OA classification depends on the type of their carbon chain, saturated or unsaturated, substituted or non-substituted, and the number of functional groups (Theron and Lues, 2011).

OA have a wide range of functions in food and beverages. As a pH regulator, OA lowers and maintains pH to a desired level such that microbial growth is minimized. As a primary antioxidant, OA such as gallic or ferulic acids have been found to directly scavenge free radicals (Aruoma et al., 1993; Graf, 1992). Others such as ascorbic and citric acids behave more like synergists or secondary antioxidants; these boost the effect of primary oxidants by chelating metal ions, lowering pH of the medium, scavenging oxygen, or converting unstable hydroperoxides (Pokorny, Yanishlieva, and Gordon, 2001; Yen, Duh, and Tsai, 2002). As a preservative, OA inhibit undesired microbial growth and slow down product deterioration by compromising bacteria DNA activities, and functions (Quitmann, Fan, and Czermak, 2014). In animal feed, OA also act as a preservative by lowering pH and targeting specific microorganisms. They also produce positive gut health by inhibiting growth of pathogenic bacteria, and improve growth performance effects by increasing feed intake, average daily gain, feed conversion ratio for livestock animals such as swine. This may be attributed to the antimicrobial properties of OA, which reduce microbial populations, aid protein digestion, and enhance mineral absorption and retention in animals (Papatsiros, Cristodoulopoulos, and Filippopoulos, 2012; Suryanarayana, Suresh, and Rajasekhar, 2012; Quitmann, Fan, and Czermak, 2014).

2.5.1 BENZOIC ACID

Benzoic acid ($C_7H_6O_2$), a colorless crystalline solid, is an aromatic monocarboxylic acid. It is one of the oldest and most popular preservatives with uses in food, beverages, and household items. Although it is naturally occurring in many fruits such as cranberries and plums, benzoic acid is manufactured industrially, mainly through the partial oxidation of toluene using oxygen. The antimicrobial properties of benzoic acid are thought to be connected to its ability to

disturb cellular membranes, dissociate within the cell to release toxic anions, hinder metabolic functions, and lower cytoplasmic pH to reduce homeostasis (Anyasi et al., 2017). Benzoic acid is effective against yeasts and molds, including ones in the *Aspergillus* and *Penicillium* genera, and against various bacteria, including *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus*, and *Pseudomonas aeruginosa*, especially at pH levels between 2.5 and 4.5 (Olmo, Calzada, and Nunez, 2017).

Benzoic acid incorporated into animal feeds have been investigated previously. A positive effect on body weight gain and feed efficiency as well as lower *E. coli* numbers and better gut health were observed in piglets fed on diets that were supplemented with 0.5% benzoic acid (Papatsiros et al., 2011). Other research has reported possible improvements in growth performance, intestinal antioxidant capacity, and microbial populations in sows and piglets with dietary inclusion of benzoic acid (Kluge et al., 2010; Diao et al., 2016; Torrallardona, Badiola, and Broz, 2007). Moreover, a synergistic antibacterial effect was found between benzoic acid and essential oils when fed to turkeys and weaned piglets (Giannenas et al., 2014; Diao et al., 2015). When benzoic acid was supplemented in chicken diets at 1, 2, and 2.5 g/kg of the total diet, weight gain was not different than the chickens that were not fed benzoic acid (Józefiak et al., 2007; Józefiak et al., 2010; Olukosi and Dono, 2014). However, at higher inclusion levels of 5 and 7.5 g/kg of benzoic acid, weight gain and feed conversion ratio were negatively affected (Józefiak et al., 2007). This less desirable result suggests there could be mechanism variances with benzoic acid between different animal species.

2.6 CONCLUSION

Ionophores and antibiotics possess many useful functions which makes them valuable to the livestock production industry. Due to growing concerns of antimicrobial resistance that may threaten human health, stricter regulations have been implemented by both the FDA and CFIA to limit the usage of antibiotics in livestock production. In addition, there is growing consumer interest in foods that are free of synthetic additives. Thus, there is an increased need for the beef industry to find alternative feed additives with similar or improved functions found with use of ionophores and antibiotics. Potential alternatives from natural sources worth exploring are essential oils and organic acids, since their modes of actions are similar to antimicrobials. Comprehensive studies that examine the impact of alternative feed additives fed to finishing steers are extremely limited. Therefore, the following two research chapters will focus on the effects of finishing diets supplemented with monensin/tylosin, essential oils, benzoic acid, or a combination of essential oils and benzoic acid to finishing cattle on animal growth performance, carcass characteristics, meat quality, sensory evaluation, and shelf life stability during simulated retail display.

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Chapter 3

Running head: Cattle fed essential oils and/or benzoic acid

The effects of feeding essential oils and(or) benzoic acid for replacing antibiotics on finishing cattle growth performance, beef carcass characteristics, and beef sensory attributes

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For this research chapter, the primary author (Lydia Wang) planned & conducted experiments, collected & analyzed data, and prepared the manuscript draft.

ABSTRACT

Objective: This study examined the effects of feeding essential oils and(or) benzoic acid, as a replacement for replacing monensin/tylosin, on finishing cattle growth performance, carcass characteristics, meat quality, and sensory properties.

Materials and Methods: Crossbred steers (N = 68; BW = 539 ± 36 kg) were placed into three blocks based on initial weight. Within each block, one of five dietary treatments were randomly assigned: (1) control (CON) diet (no supplement); (2) monensin/tylosin (M/T) diet (monensin supplemented at 33 mg/kg on DM basis; tylosin supplemented at 11 mg/kg on DM basis); (3) essential oils (EO) diet (supplemented at 1.0 g/steer/day); (4) benzoic acid (BA) diet (supplemented at 0.5% on DM basis); and (5) combination (COMBO) diet (essential oils supplemented at 1.0 g/steer/day and benzoic acid supplemented at 0.5% on DM basis). Steers were fed their designated diets for the last 98 days of the finishing period using an Insentec feeding system.

Results and Discussion: Key findings were that gain to feed ratio differed ($P = 0.05$) with M/T steers having greater feed efficiency compared with CON, EO, and COMBO steers. Dietary treatment affected ($P \leq 0.05$) quality grade along with tenderness, chewiness, juiciness, and beef flavor attributes for steaks as determined by trained panelists. Specifically, EO cattle had greater quality grade than CON, M/T, and COMBO treatments, and CON and COMBO steaks were tougher, chewier, and less juicy as compared to steaks from the other dietary treatments. Overall, results from this study suggest that growth performance was similar for steers supplemented with essential oils and(or) benzoic acid versus CON steers, and beef quality from alternative treatments to M/T was not compromised.

Key words: antibiotic alternatives, beef quality, feed additives, feed supplements

1. INTRODUCTION

Monensin, an ionophore is used to improve nitrogen and energy utilization, control coccidiosis, and decrease risk of certain digestive diseases such as ruminal acidosis in feedlot cattle (Goodrich et al., 1984; McGuffey et al., 2001; Lemos et al., 2012; Vyas et al., 2018). Tylosin is a macrolidic antibiotic used strictly in veterinary medicine and is used to improve weight gain and prevent liver abscesses in feedlot cattle finished on high-concentrate diets (Giguère, 2013). Despite the many functions of dietary antibiotics, studies have shown that antimicrobial usage can lead to antibiotic resistance, thus potentially impacting human health (Chen et al., 2008; Cameron and McAllister, 2016). Therefore, North American beef producers are challenged to raise beef cattle that are not fed antibiotics and ionophores, especially since they are now facing much tighter government regulations for adding antibiotics to livestock diets (FDA, 2017; Government of Canada, 2018). An alternative approach to feeding cattle with antibiotics is replacement with essential oils (EO) and organic acids (OA). Essential oils are plant-derived aromatic liquids that exert antimicrobial properties (Gyawali, Hayek, & Ibrahim, 2015). Past studies have shown that EO blends may have synergistic or additive antibacterial effects which in turn affect rumen fermentation and meat quality (Burt, 2004; Goñi et al; 2009; Prado et al., 2016). Meanwhile, OA can suppress fungal activity and maintain an acidic environment in the rumen to prevent disease (Castillo et al., 2004). Little is known about the effects of supplementing OA to beef cattle, and there are only a few research studies (Diao et al., 2015; Liu et al., 2017) that have explored the possible synergistic effects of EO and OA in pigs and poultry. Therefore, the objective of this study was to evaluate the effects of feeding essential oils and(or) benzoic acid to finishing cattle in an effort to replace monensin/tylosin. Parameters evaluated in this study were growth performance, carcass characteristics, meat quality, and

sensory properties for the *longissimus thoracis* (LT) muscle. It was hypothesized that feeding finishing cattle essential oils and(or) benzoic acid would improve growth performance and feed efficiency in a magnitude similar to that of feeding finishing cattle monensin and tylosin (with an improvement in growth performance to cattle not fed monensin and tylosin), while not affecting carcass characteristics, meat quality, or beef eating experience.

2. MATERIALS AND METHODS

All animal procedures in this study were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol #3706). Animals were received and managed in accordance with the Animal Utilization Protocol, which was approved based on guidelines and principles of the Canadian Council on Animal Care (1993).

All procedures in this study that involved human participants (sensory testing) were approved by the University of Guelph Human Ethics Committee REB Project #17-12-017. Written, informed consent was obtained from each participant before the start of screening.

2.1 CATTLE AND FACILITIES

Upon arrival to the University of Guelph Elora Beef Research Centre, sixty-eight crossbred steers (starting BW = 478 ± 33 kg) were tagged with electronic identification tags (High Performance HDX Ultra EID Tag; Allflex, Dallas, TX) and assessed to be in good health by research personnel. Steers were vaccinated according to the research facility's protocol and implanted with Synovex S (200 mg of progesterone, 20 mg of estradiol benzoate; Zoetis, Kalamazoo, Michigan, USA) on a common day before the beginning of the study. Steers were inspected for the presence of testicles and intact animals were not included in the study. The steers were used in a randomized complete block design, where blocks were assigned by starting

weight (light weight, medium weight, and heavy weight). There were 23 steers in the light weight block, 22 steers in the medium weight block, and 23 steers in the heavy weight block. Blocks were assigned before the start of the finishing period so that the steers could undergo an adaptation period to a high concentrate diet. This period of time consisted of an adaptation period of 43 days, which was used to transition steers to a high concentrate diet and train steers on the feeding stations for the Insentec feeding system. At the time of blocking (and the beginning of the adaptation period), allocation weights for block 1 steers ranged from 412 – 460 kg; allocation weights for block 2 steers ranged from 460 – 495 kg; and allocation weights for block 3 steers ranged from 495 – 537 kg. At the start of the finishing period, starting weights for block 1 steers ranged from 461.5 – 543.5 kg; starting weights for block 2 steers ranged from 509.5 – 564 kg; and starting weights for block 3 steers ranged from 515 – 631 kg. Average initial weight of all 68 cattle in the study was 539 ± 36 kg.

Steers were allocated into eight equal sized pens (9.14 m × 6.71 m) that included a 4.88 m × 6.71 m area bedded with wood shavings. Each pen was equipped with an automatic watering system and four Insentec feeding stations (Insentec, B.V., Marknesse, Netherlands). The Insentec feeding system arrangement allowed the assignment of two dietary treatments per pen with two Insentec feeding stations assigned per dietary treatment which recorded daily feed intakes for individual steers. Seven pens housed 8-10 steers per pen from two different dietary treatments (4-5 steers/dietary treatment/pen); only 4 steers on one dietary treatment (the control treatment) were housed in the eighth pen. An adaptation period of 43 days was provided before the study began to adjust the cattle from a high roughage diet to a high concentrate finishing diet. During the adaptation period, steers were trained on the Insentec feeding system and gradually adapted to the basal diet used for the study. Rations were delivered once daily to supply feed ad libitum.

2.2 TREATMENTS AND EXPERIMENTAL DESIGN

Within each of the three blocks used in this study, cattle were randomly assigned to 1 of 5 dietary treatments for the finishing period: 1) a negative control where no additives were included (**CON**); 2) a positive control with supplementation of monensin/tylosin (**M/T**), where monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN, USA), and tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a dry matter basis; 3) a proprietary blend of essential oils (**EO**) supplemented at 1 g/steer/day (Victus Liv, DSM Nutritional Products, Parsippany, NJ, USA); 4) benzoic acid (**BA**) provided at 0.5% dietary inclusion on a DM basis (VevoVitall; DSM Nutritional Products); and 5) a combination of a proprietary blend of essential oils and benzoic acid (**COMBO**). These additives were included at their respective targeted inclusion levels in the premix of a basal diet that consisted of high moisture corn, alfalfa silage, soybean meal, and white salt (**Table 3.1**). The blend of essential oils used in this study was a commercially available, proprietary blend containing thymol, eugenol, vanillin, guaiacol, and limonene. Dosage levels for the treatment ingredients were based on manufacturer recommendations.

Following adjustment to the designated finishing diet, starting BW were collected on the first two days of the finishing period (d 0 and d 1) and averaged for increased accuracy. Subsequently, all cattle were weighed once every 28-30 days throughout the study; however only starting and final weights were reported for this study. The feeding period concluded on d 98 of the study and final weights were determined by averaging the weights taken on d 97 and d 98 of the feeding trial. All steers were transported to a commercial beef packing plant in Ontario and slaughtered on d 98 of the finishing trial. Cattle weights along with the feed intake data collected by the Insentec system were used to calculate total weight gain (kg), average daily gain (kg/day),

dry matter intake (kg/day), and gain to feed ratio on an individual animal basis. Therefore, individual animal served as the experimental unit for all dependent variables.

2.3 CARCASS DATA COLLECTION

Steers were humanely handled and slaughtered (which included captive bolt stunning, followed by exsanguination) at a federally inspected meat processing facility following commercial industry standards and Canadian Food Inspection Agency (CFIA) inspection regulations. Individual animal ID was maintained throughout the slaughter process. Hot carcass weights were determined and recorded immediately before chilling. At approximately 3 d post-mortem, Canadian Beef Grading Agency (CBGA) graders evaluated the carcass at the 12th and 13th rib interface for quality and yield grades based on the Livestock and Poultry Carcass Grading Regulations (Canadian Food Inspection Agency, 1992). Camera data were collected for the last quadrant over the *longissimus* muscle on the 12th/13th rib interface for each steer using a commercial imaging system. The data were then used to determine the *longissimus* muscle area, fat thickness, marbling score, calculated yield grade, CFIA yield grade, and CFIA quality grade.

2.4 FEED ANALYSIS

Total mixed ration (TMR) feed samples from each of the five dietary treatments were collected ten times (every 8 to 10 days) during the feeding period. The TMR samples were stored at -20°C until analysis. Roughly 300 to 350 g of the as-fed TMR samples at each sampling date were weighed and then dried in a Hotpack Tru-temp forced air oven (Hotpack Canada Ltd, Waterloo, ON, Canada) at 60˚C for approximately 48 h. The dry matter content (%) of individual TMR feed samples was calculated using the equation $\left(\frac{Dry\ Weight}{Wet\ Weight}\right) x\ 100\%$. To calculate the daily dry matter intake for each steer, monthly averages of dry matter content for each dietary

treatment were first calculated. Those averages were then multiplied by the intake (kg) of each day in the corresponding month. Finally, daily dry matter intake values were averaged for all steers on an individual basis.

Dried TMR feed samples were ground using a Wiley Mill to pass through a 1 mm screen (Wiley Mill, Arthur H. Thomas; Philadelphia, PA, USA). The ground feed samples were then composited based on dietary treatment and then sent to a commercial feed analysis laboratory for further analysis using wet chemistry methods. Dry matter and ash were determined using AOAC methods 930.15 and 942.05 (AOAC, 2007). Crude protein content was determined using a LECO FP628 nitrogen analyzer (St. Joseph, MI) based on the AOAC (2007) method 968.06. Fiber fractions were determined using AOAC (2007) methods 13 and 15 respectively for neutral detergent fiber (NDF), and acid detergent fiber (ADF)/lignin using an Ankom 2000 fiber analyzer (Ankom Technology, Macedon, NY). Crude fat content was determined following AOAC method 920.39. Starch content was determined with AOAC method 996.11 (AOAC, 2006) using a Megazyme Total Starch Assay Kit (Megazyme, Chicago, IL). Mineral content was determined via aqua regia inductively coupled plasma optical emission spectrometry based on EPA 3050 and EPA 6010 methods.

2.5 SAMPLE PREPARATION

At 4 d post-mortem, bone-in beef ribs containing the *longissimus thoracis* (LT) (IMPS#107) from the right side of carcasses were collected and packaged at the commercial processing facility and delivered to the University of Guelph Meat Science Laboratory for further analyses. At 6 d post-mortem, the ribs were boned, trimmed of excess fat, and then fabricated into seven 2.54 cm thick LT steaks. Four steaks were used for this project and were

assigned for analyses in the following manner: Steak 1: determination of ultimate pH, instrumental color, and proximate composition (moisture and lipid content); Steak 2: determination of cooking losses and Warner-Bratzler shear force after 7 days of post-mortem ageing at ≤ 4˚C ; Steak 3: determination of cooking losses and Warner-Bratzler shear force after 14 days of post-mortem ageing at ≤ 4˚C; Steak 4: sensory evaluation after 7 days of post-mortem ageing at ≤ 4˚C.

2.6 MEAT QUALITY EVALUATION

Immediately after fabrication, steaks (steak 1) were allowed to bloom with exposure to oxygen in the air for at least 30 min to encourage color development. Afterwards, ultimate pH was evaluated in triplicate using a calibrated spear-tipped pH electrode (HI98163, Hanna Instruments; Mississauga, ON, Canada) with a thermocouple connected to an Accumet A71 pH meter (Fisher Scientific; Toronto, ON, Canada). A calibrated, handheld Chroma Meter CR-400 (Konica Minolta Sensing Americas, Inc.; Ramsey, New Jersey, USA) was used to measure objective color with illuminant D65 and 0˚ viewing angle settings. As per the Commission International de l'Eclairage (CIE, 1976), each measurement by the Chroma Meter was reported as L*, a*, and b*. L* represented luminosity, where a greater L* value indicated lighter meat color. a* measured the red to green color range, where a greater a* value indicated a greater level of redness. b* measured the blue to yellow color range, where a greater b* value indicated greater levels of blue. Chroma, a measure of color intensity, was calculated by the equation: $\sqrt{(a*)^2 + (b*)^2}$. Hue angle, a measure of distance in degrees from the true red axis of the CIE colour space, was calculated using the formula: tan-1$\left(\frac{b*}{a*}\right)$. Six measurements were taken per steak and then averaged to determine the L*, a*, b*, chroma, and hue values.

After the collection of pH and objective color measurements on steak 1 at 6 d post-mortem, steak 1 was vacuumed packaged and stored at ≤ -22˚C until further analyses. Steaks 2 and 3 were vacuum packaged and aged at ≤ 4˚C for 7 and 14 d post-mortem respectively, before being stored at ≤ -22˚C. Steak 4 was vacuum packaged and aged at ≤ 4˚C for 7 d post-mortem before being stored at ≤ -22˚C.

Moisture and lipid concentrations were determined based on modified air drying and Soxhlet extraction methods, respectively (AOAC, 2006; method 950.46 and method 991.36). In short, steaks were thawed overnight (15-20 h), trimmed of external subcutaneous fat, cubed, and homogenized in a food processor (KitchenAid model KHB23511CU; St. Joseph, MO, USA). Duplicate 5 g samples of the homogenate were weighed onto an aluminum weighing dish and covered with two #1 Whatman Qualitative filter papers (42 mm; GE Healthcare Life Sciences; Chicago, IL, USA). Next, the samples were dried in the Fisherbrand Isotemp drying oven (Thermo Fisher Scientific; Ottawa, ON, Canada) at 100˚C for at least 24 h and then weighed again to determine percent moisture. The dried samples were then placed in the Soxhlet extraction apparatus and washed multiple times for 4-5 h using approximately 200 mL of warm petroleum ether. Washed samples were placed into a 100˚C drying oven for a minimum of 24 h to evaporate the petroleum ether, and then weighed for lipid determination.

Cooking loss and Warner-Bratzler shear force values were determined as described by Streiter et al. (2012). Steaks were removed from the -22˚C freezer and thawed at ≤ 4˚C for 48 h prior to cooking. Steaks were then weighed and cooked on a preheated and greased Garland Grill set to a cooking surface temperature of 105˚C (ED-30B: Garland Commercial Ranges LTD; Mississauga, ON, Canada) to an internal temperature of 72˚C. A type K flexible high-temperature thermocouple was placed within the geometric center of the steak throughout the

cooking process to determine the steak temperature. Steaks were flipped at an internal temperature of 40˚C. Final weights were recorded immediately after cooking; steaks were then bagged and submerged into an iced water bath to stop the cooking process. Afterwards, steaks were chilled overnight at ≤ 4˚C. Six to eight, approximately 1.3 cm diameter by 2.5 cm long circular cores were removed from each steak parallel to muscle fibers (AMSA, 1995) using a drill press mounted corer. Cores were sheared perpendicular to muscle fibers using a TA-XT Plus Texture Analyzer (Texture Technologies Corp.; Scarsdale, NY, USA) fitted with a Warner-Bratzler blade at a crosshead speed of 3.3 $mm \cdot s^{-1}$.

2.7 SENSORY EVALUATION

Sensory analysis of LT steaks, which included the recruitment, screening, and training of panelists, was performed according to American Meat Science Association (2016) guidelines and Adhikari et al. (2011). The trained sensory panel consisted of 8 undergraduate and graduate students from the University of Guelph, which included 5 females and 3 males between the ages of 18-30. Panelists were trained over 10 consecutive days on the objective evaluation of beef tenderness, juiciness, chewiness, beef flavor intensity, and off-flavor intensity using a continuous 15-cm line scale with end anchors at 0 and 15 cm. Panelists were introduced to potential off-flavors in beef that may be present during actual testing such as sweet, salty, sour, bitter, bloody/serumy, metallic, liver-like, brown/roasted, rancid, chemical, grassy, spoiled flavors, as well as lemon, clove, and thyme flavor notes.

Following training, there was one evaluation session per day for nine days. The sensory evaluation consisted of nine sessions over a 2-week period. Seven randomly selected samples were served to 7-8 panelists each day with at least 1 sample from each dietary treatment.

In terms of product preparation, frozen and vacuum-packaged 2.54 cm thick LT steaks were thawed at ≤ 4˚C for 24 h prior to being cooked on a searing grill set to a cooking surface temperature of 204˚C (Model #25360, Hamilton Beach; Markham, Ontario, Canada). The steaks were flipped once at 40˚C and cooked to a final internal temperature of 68˚C. Cooked steaks were trimmed of their outside edges and then cut into 1-cm cubes. Each panelist was served two random cubes from each steak at ambient temperature and humidity in a 29.5 mL capped plastic cup. Sample cups were pre-labelled with a random 3-digit code. Panelists were seated in individual booths, under an overhead red light to prevent visual bias. Each person was served the seven samples in random order along with bottled water and unsalted crackers which served as palate cleansers. Panelists were instructed to cleanse their oral palates between samples.

Trained panelists evaluated tenderness, juiciness, and chewiness based on the definitions provided by the American Meat Science Association (2016) using the 15-cm line scale. The left anchor (score = 0) indicated extremely tough, very little juiciness, and not chewy. The right anchor (score = 15) indicated extremely tender, very high juiciness, and very chewy. Moreover, panelists evaluated beef flavor intensity and off-flavor intensity according to the definition by Adhikari et al. (2011), where 0 = very weak beef flavor and no off-flavor(s) while 15 = very intense beef flavor and the presence of off-flavor(s). Finally, panelists were asked to describe any off-flavor(s) if detected when tasting the samples. All responses were collected and recorded on a computer through Compusense version 5.8 software (Guelph, ON, Canada).

2.8 STATISTICAL ANALYSES

Data for growth performance, carcass characteristics, and meat quality traits were analyzed using a randomized complete block design with PROC GLIMMIX in SAS 9.4 (SAS

Institute Inc., Cary, NC, USA). Fixed effects included dietary treatment, block, and the dietary treatment by block interaction, while pen was a random effect. Individual steers were used as the experimental unit as feeding treatments were applied to each ID through the Insentec feeding system. Trained sensory panel results were also analyzed using a randomized complete block design with PROC GLIMMIX in SAS. For the sensory panel, day served as a random effect, while fixed effects included dietary treatment, panelist, and the dietary treatment by panelist interaction. The PDIFF option of the LSMEANS statement was used when comparing treatment means. Statistical differences were considered significant when the *P*-value was less than or equal to 0.05 and tendencies were considered when the *P*-value was greater than 0.05 and less than 0.10. The maximum standard error of the mean (SEM) was reported for each dietary treatment.

3. RESULTS AND DISCUSSION

3.1 STUDY RATIONALE

Antimicrobials are feed additives commonly used within the livestock industry for their ability to improve animal health and feed efficiency, thus helping lower production costs (Zawadzki el al., 2011; Lemos et al., 2012; de Oliveira Monteschio et al., 2017). Two categories of antimicrobials frequently used within the beef cattle industry are ionophores and macrolides. Ionophores are highly lipophilic molecules that can interact with the hydrophobic membrane phases of targeted bacteria and protozoa. They also possess polar regions which allow them to recognize and trap cations (McGuffey et al., 2001). Ionophores cause death of microorganisms through their attachment to rumen bacteria, protozoa and probably fungi, by facilitating the delocalization of ions across cell membranes (Mathison et al., 1998; McGuffey et al., 2001).

Monensin was the first ionophore discovered and later gained approval for use in beef cattle from the Food and Drug Administration in 1975. Monensin is the most popular feed additive among cattle producers. Monensin is used to increase feed efficiency through selective inhibition of gram-positive bacteria, control coccidiosis, and decrease the risk of certain digestive diseases such as acidosis (Goodrich et al., 1984; McGuffey et al., 2001; Lemos et al., 2012; Vyas et al., 2018). Tylosin, a macrolide that has a 16-membered lactone ring, is an antibiotic used to improve weight gain and prevent liver abscesses in beef cattle (Giguère, 2013). Despite their many functions, studies have shown that improper or excessive antimicrobial usage can lead to antimicrobial resistance, thus potentially impacting human health (Teuber, 2001; Chen et al., 2008; Cameron and McAllister, 2016). Antimicrobial resistance is a concern particularly for macrolides (e.g. tylosin) as they are in the same chemical family as antibiotics used for treating human bacterial infections (Inglis et al., 2005). As such, there is a growing interest and need for examining alternative antimicrobials in livestock production, such as essential oil blends and organic acids.

Essential oils (EO) are plant-derived aromatic liquids that can be obtained through fermentation, extraction or steam distillation (Burt, 2004; de Oliveira Monteschio et al., 2017). More importantly, EO exert antimicrobial properties by disrupting the cellular membrane to damage cell structure and function (Gyawali, Hayek, & Ibrahim, 2015). Essential oil components such as eugenol and thymol have been found to be effective against bacteria such as Listeria monocytogenes, Salmonella typhimurium, and Escherichia coli O157:H7 when added directly to meat products (Burt, 2004). Additionally, antimicrobial and antibacterial properties have been found in limonene, an organic compound found in citrus fruits, guaiacol from beechwood creosote, and vanillin, the major constituent in vanilla beans (Piccaglia et al., 1993; Liu et al.,

2011; Samii et al., 2016; Yang et al., 2017). Meanwhile, organic acids can suppress fungal activity and maintain an acidic environment in the rumen to prevent diseases (Castillo et al., 2004). Benzoic acid, a type of organic acid, possesses antibacterial and antioxidant properties, and can improve growth performance, nutrient digestibility, and microecological balance for growing pigs (Diao et al., 2015; Cheng et al., 2017).

3.2 ANIMAL PERFORMANCE

There were no interactions ($P \geq 0.29$) for dietary treatments and block during the 98 d finishing period for initial weight, final weight, total weight gain, average daily gain, and dry matter intake (**Table 3.2**). A block by dietary treatment interaction ($P = 0.05$) was present for G:F (**Fig. 3.1**). The interaction was due to differences in the effects of block (initial weight) on G:F for steers on CON and EO treatments versus all other treatments, whereas G:F values were similar ($P > 0.05$) across block (initial weight) for steers fed M/T, BA, and COMBO diets. For CON steers, G:F for steers from the lightweight block was greater ($P < 0.05$) than G:F values for steers from the medium and heavyweight blocks. In contrast, G:F values for EO steers from the light and medium weight blocks were greater ($P < 0.05$) than G:F for steers from the heavyweight block. There were no differences ($P \geq 0.12$) in initial weight, final weight, total weight gain, average daily gain, and dry matter intake among finishing diets. There was a dietary treatment effect ($P = 0.05$) for G:F, where steers fed the M/T diet had greater G:F compared with steers fed CON, EO, and COMBO diets. G:F for steers fed BA diets were intermediate and not different ($P > 0.05$) when compared to all other treatments.

Weight gain and dry matter intake were not affected regardless of diet fed. It was worth noting that M/T steers gained 17.4 kg (8.2%), 21.7 kg (10.2%), 21.5 kg (10.1%), and 23.2 kg

(10.9%) more total weight compared with CON, EO, BA and COMBO steers, respectively. While these differences were not statistically different, these numerical values would be meaningful to producers. These results match previous studies that have reported reduced DMI and greater ADG when cattle are fed diets containing monensin versus feeding diets without monensin (Potter et al., 1985; Duffield et al., 2012; Hales et al., 2017). Gain to feed results from this study were comparable to Meyer et al. (2009) where 468 crossbred finishing steers were fed essential oil blends, monensin, and(or) tylosin. The study reported G:F ratios between 0.151 to 0.156, with the greatest ratios for cattle fed monensin and tylosin. Benchaar et al. (2006) also reported a G:F ratio of 0.154 for 20 beef cattle that were fed an EO blend at 2 g/day, which was similar to the results observed in our study feeding EO with(without) benzoic acid.

3.3 CARCASS CHARACTERISTICS

There were no interactions ($P \geq 0.13$) between dietary treatment and block for any of the carcass characteristics measured in this study, with the exception of a dietary treatment by block interaction ($P = 0.02$) for marbling score (**Table 3.3**; **Fig. 3.2**). The interaction was due to differences in the effects of block (initial weight) on marbling score for steers on EO, BA, and COMBO treatments versus CON and M/T in which marbling score values were similar ($P > 0.05$) across block (initial weight) for each of the latter 2 dietary treatments. For the other dietary treatments, there was no consistent relationship between block (initial weight) and marbling score as marbling score for heavyweight EO steers was greater ($P < 0.05$) than marbling scores for light and medium weight blocks; marbling score was greater ($P < 0.05$) for medium vs. lightweight BA steers, while marbling score was greater ($P < 0.05$) for heavy vs. lightweight COMBO steers. There was only a tendency ($P = 0.07$) for dietary treatment to affect marbling score with the small marbling found in CON carcasses being numerically lower than the modest

marbling found in EO and BA carcasses. While Meyer et al. (2009) did not observe a treatment effect for marbling score, feeding EO in the current study produced higher ($P = 0.05$) quality grade carcasses compared to carcasses from the CON, M/T, and COMBO diets. Despite the statistical differences, carcasses from all five dietary treatments received the same quality grade of approximately 2 or Canada AAA (USDA Choice equivalence) when values were rounded to the nearest whole number. In a study conducted by Kung et al. (2008), lactating dairy cows supplemented with a blend of essential plant oils at 40 mg/ kg of fresh forage were found to have a lower molar proportion of acetate and a higher proportion of propionate versus when cows were not fed essential oils. In an in vitro study, Li et al. (2013) reported an increase in propionate concentration when essential oils were supplemented to batch cultures versus when they were not. The ability of essential oils to modify ruminal fermentation patterns is important and may be related to improvements in quality grade in the current study feeding EO as ruminal propionate is a precursor for glucose synthesis. As previously reported, glucose is the primary substrate for fatty acid synthesis for intramuscular fat deposition in beef cattle while acetate is the main precursor to fatty acid synthesis for subcutaneous fat (Chung et al., 2016; Kim et al., 2013; Park et al., 2018). Though the EO and BA carcasses received numerically higher marbling scores than CON carcasses, which was a positive, it could also be the reason why the feed efficiency of EO and BA steers were not better than the CON steers. This is because fat deposition requires more feed energy than muscle/protein deposition and thus as the cattle make more fat, the feed efficiency goes down. (Ricks et al., 1984).

Overall, most carcass characteristics were not influenced by dietary treatments in the current study. This was also the case for Meyer et al. (2009), where no differences were observed for hot carcass weight, dressing percentage, fat thickness, amount of kidney, pelvic, and heart fat,

marbling score, and *longissimus* area among cross-bred finishing steers that were fed no supplements, essential oil mixtures, essential oils and tylosin, and monensin and tylosin. Hot carcass weight, dressing percentage, LT area, fat thickness, calculated yield grade, and CFIA yield grade were not influenced ($P \geq 0.19$) by dietary treatment. CFIA quality grade differed ($P < 0.05$) among dietary treatments with the EO diet producing greater ($P \leq 0.05$) quality grade beef than CON, M/T, and COMBO diets.

Dietary treatment did not affect ($P = 0.19$) liver scores/abnormalities in a significant manner. Out of the 68 cattle in this study, 7 steers (4 from CON, 2 from EO, and 1 from COMBO) had liver abnormalities at the time of slaughter. This included 6 steers where their livers adhered to the gastrointestinal tract and(or) diaphragm. Incidence of liver abnormalities in the current study (10.3%) was less than the typical 12 to 32% liver abscess incidence rate found for cattle in commercial feedlots (Nagaraja and Chengappa, 1997). There were no liver abscesses observed in cattle fed monensin and tylosin in this study, which may have been expected in such a small group of cattle that were fed a compound that is specifically used to work against *Fusobacterium necrophorum* and *Arcanobacterium pyogenes*, two of the most frequent pathogens associated with development of liver abscesses (Amachawadi and Nagaraja, 2016; Elanco, 2019). There were also no liver abscesses found in cattle fed benzoic acid without essential oils in this study. Research on the effects of benzoic acid against the causative agents of liver abscesses are very limited, but it is known that organic acids are effective inhibitors of microbial growth and are common preservatives in the food industry (Biagi and Piva, 2007). Additionally, Biagi and Piva (2007) evaluated the effectiveness of different organic acids and found that benzoic acid can positively affect swine cecal microflora and limit bacterial activity with the production of less ammonia in an in vitro fermentation system. Two out of 13 cattle fed

EO had liver abscess issues in this study. The low incidence rate may be due to the antibacterial properties of essential oils, generated from their membrane and ion gradient disruption capabilities (Jacob et al., 2009). Overall, no steers were lost due to illness before slaughter and the high health status of the cattle in this study (as often is the case with university studies) was represented by the low incidence rate of liver abscesses.

3.4 MEAT QUALITY EVALUATION

Overall, there were no interactive effects ($P \geq 0.21$) observed for LT meat quality traits (**Table 3.4**). Additionally, dietary treatments did not impact ($P \geq 0.16$) ultimate pH, instrumental color (Minolta L*, a*, b*, chroma, and hue), Warner-Bratzler shear force (after both 7 and 14 d of post-mortem aging), and cooking loss. a* (redness), b* (yellowness), and chroma values differed ($P < 0.04$) among blocks, with lower values for LT from lightweight steers versus values for intermediate weight steers, respectively (data not shown). The pH values for LT steaks were within the normal range of 5.5 – 5.7 for the conversion of muscle to meat, such that animals were not under long-term stress leading up to slaughter and carcasses were adequately chilled (Scanga et al., 1998; Miller, 2007). Minolta L*, a*, and b* values for the LT steaks in this study were roughly similar compared with values reported by other studies (Wulf and Wise, 1999; Bohrer et al., 2014). Minolta L*, a*, chroma and hue values for the *longissimus* thoracis muscles in this study were lower than values reported by Preziuso and Russo (2004) on Chianina beef cattle, possibly due to study differences in age at slaughter or other production factors. Lower chroma values indicate duller color intensity, which may not be attractive to customers seeking cherry red beef (Streiter et al., 2012).

In terms of proximate composition, there were trends ($P \leq 0.07$) for dietary treatment to affect moisture and lipid content for LT steaks. *Longissimus thoracis* steaks from steers fed CON had more ($P < 0.05$) moisture than LT steaks from cattle fed MT, EO, and COMBO diets. Steaks from steers fed CON also had less lipid compared to steaks from steers fed other diets, which was similar to the reported marbling scores.

3.5 TRAINED SENSORY PANEL EVALUATION OF LT

To our knowledge, no other study has examined the effects of essential oils and(or) benzoic acid supplementation in finishing diets for cattle on sensory traits and thus studies evaluating other animal species were reviewed. Supplementation of broiler diets with oregano and garlic essential oils improved juiciness, flavor, and overall acceptability as found by Kirkpinar et al. (2014). Moreover, the addition of essential oil compounds, carvacrol and cinnamaldehyde into lamb finishing diets did not affect juiciness, flavor intensity and liking, as determined by 8 trained panelists (Chaves et al., 2008). Trained panelists were not able to detect differences in flavor and aroma profiles in pork from pigs fed various essential oils (rosemary, garlic, oregano or ginger) at 0.05% of the diet for the last 41 days before slaughter (Janz et al., 2006). Cheng et al. (2017) found that feeding oregano essential oil with benzoic acid to pigs did not improve tenderness, juiciness, flavor, or overall acceptance for LT muscle.

In the current study, there were no interactions ($P > 0.62$) between dietary treatment and panelist with statistical analysis of sensory data and therefore the main effect of panelist was removed from the statistical model. Tenderness, chewiness, juiciness, and beef flavor intensity scores for steaks differed ($P \leq 0.01$) among dietary treatments (**Table 3.5**). For tenderness, panelists reported the steaks from the CON and COMBO diets were slightly tougher ($P \leq 0.05$)

compared with other diets. Finishing steers fed the CON and COMBO diets also produced the most chewy ($P \leq 0.05$) steaks compared with the other three treatments. Moreover, CON steaks were less juicy ($P < 0.05$) than MT, EO and BA steaks. Dietary treatment differences in lipid content and marbling could have been responsible for some of these differences, as steaks from steers fed CON had less lipid content compared with all other treatments. Steaks with less marbling have been previously reported as less juicy (Savell et al., 1987; Thompson, 2004; Corbin et al., 2015). Beef flavor intensity for all treatments was perceived to be on the more intense side, with scores between 8 to 10 on the 15-cm line scale. Moreover, the trained taste panel reported BA steaks to have more intense ($P < 0.05$) beef flavor compared to CON and COMBO steaks. It was unclear why steaks from steers fed BA would have more flavor, and greater investigation here is warranted. In general, off-flavor was barely detectable for all samples and off-flavor intensity scores were not affected by dietary treatment ($P = 0.70$).

Overall, panelists reported steaks from CON and COMBO diets to be tougher and more chewy than steaks from cattle fed the remaining diets. This finding corresponded with the WBSF results for 7 d aged steaks, where CON and COMBO steaks had higher, but not significantly different, shear force values than the 3 other types of dietary treatments. According to Corbin et al. (2015), marbling levels play a large role in influencing tenderness, juiciness, and flavor scores as evaluated by consumers and trained panelists. Thus, lipid content and marbling could have been attributed to some of these differences, particularly in the CON steaks. Off-flavors were not often detected; however, the terms used most frequently were metallic and livery.

4. APPLICATIONS

The objective of this study was to evaluate the effects of feeding essential oils and(or) benzoic acid to finishing cattle on growth performance, carcass characteristics, meat quality, and sensory properties. Overall, results from this study found that supplementation of beef finishing diets with essential oils and(or) benzoic acid did not negatively affect most major growth performance, carcass characteristics, meat quality, or sensory traits when compared with cattle fed no additives or conventionally fed cattle supplemented with monensin and tylosin. However, feed efficiency was improved as expected when monensin and tylosin were provided in the diet, even when compared with cattle fed essential oils and(or) benzoic acid. This remains a major challenge with replacing antibiotics and ionophores in the diet with alternative antimicrobials as use of antibiotics and ionophores consistently improves feed efficiency, thus lowering cost of production and decreasing days on feed.

5. ACKNOWLEDGEMENTS

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7. TABLES

Table 3.1. Ingredient and nutrient composition (% DM basis) of beef finishing diets.

	Dietary Treatment				
	CON[1]	M/T[2]	EO[3]	BA[4]	COMBO[5]
Ingredient, % on a DM[6] basis					
High moisture corn	76.50	76.50	76.50	76.00	76.00
Alfalfa silage	15.30	15.30	15.30	15.30	15.30
Soybean meal	6.80	4.80	4.80	6.80	4.80
Feedlot premix without monensin[7]	1.10	–	1.10	1.10	1.10
Feedlot premix with monensin[7]	–	1.10	–	–	–
Tylosin premix	–	2.00	–	–	–
Essential oil premix	–	–	2.00	–	2.00
Benzoic acid premix	–	–	–	0.50	0.50
White salt	0.30	0.30	0.30	0.30	0.30
Calculated Analysis, % on a DM basis					
Dry matter, %	60.14	59.51	60.19	60.04	60.07
NE_m[8], Mcal/kg	2.04	2.04	2.05	2.04	2.05
NE_g[9], Mcal/kg	1.32	1.32	1.33	1.32	1.33
Starch, %	46.27	45.66	47.48	44.82	46.76
Crude protein, %	14.10	13.49	14.34	13.58	14.34
NDF[11], %	17.88	18.87	16.65	18.02	17.59
ADF[12], %	11.23	11.28	10.60	10.97	10.76
Crude Fat, %	3.25	3.47	3.60	3.62	3.39
Ash, %	4.54	4.66	4.47	4.40	4.70
Calcium, %	0.59	0.65	0.58	0.64	0.64
Magnesium, %	0.20	0.21	0.18	0.21	0.20
Phosphorus, %	0.35	0.32	0.33	0.33	0.41
Potassium, %	1.16	1.16	1.08	1.19	1.23
Sodium, %	0.19	0.19	0.21	0.20	0.23

Sulphur, %	0.17	0.16	0.16	0.16	0.18
Copper, µg/kg	12.36	15.18	11.64	13.19	13.82
Iron, µg/g	145.13	116.17	125.53	116.90	130.87
Manganese, µg/g	48.04	44.30	47.12	51.16	52.92
Zinc, µg/g	52.14	74.84	66.05	56.49	58.07

[1]CON = control diet; no additional supplement was provided.

[2]M/T = monensin and tylosin supplemented diet; monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN) while tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a DM basis.

[3]EO = essential oils supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day.

[4]BA = benzoic acid supplemented diet; benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[5]COMBO = essential oils and benzoic acid supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day and benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[6]DM = dry matter.

[7]Feedlot premix with(without) monensin includes the following minerals and vitamins: calcium, phosphorus, sodium, magnesium, potassium, sulphur, cobalt, iodine, iron, copper, fluorine, manganese, zinc, Vitamin A, Vitamin D, Vitamin E.

[8]NE_M = net energy for maintenance.

[9]NE_G = net energy gain**.**

[10]TDN = total digestible nutrients.

[11]NDF = neutral detergent fiber.

[12]ADF = acid detergent fiber.

Table 3.2. Effects of replacing monensin and tylosin in beef finishing diets with alternative feed ingredients on cattle growth performance.

	Dietary Treatment (Trt)						*P*-values for ANOVA		
Item	CON[1]	M/T[2]	EO[3]	BA[4]	COMBO[5]	SEM[6]	Treatment	Block	Block*Trt
Steers, number	14	14	13	13	14				
Starting weight, kg	534.0	543.6	536.5	536.0	544.9	6.58	0.67	<0.001	0.95
Final weight, kg	729.1	757.2	725.8	726.1	735.6	11.23	0.23	<0.001	0.88
Total weight gain, kg	195.1	213.6	189.3	190.2	190.7	7.53	0.12	0.67	0.40
Average daily gain, kg/day	1.99	2.18	1.93	1.94	1.95	0.08	0.12	0.67	0.40
Dry matter intake, kg/day	12.54	12.27	12.41	11.75	12.44	0.43	0.70	0.10	0.29
Gain to feed ratio (G:F)	0.161[b]	0.178[a]	0.157[b]	0.166[ab]	0.157[b]	0.006	0.05	0.07	0.05

[a,b]Least square means lacking a common superscript letter within a row are different ($P \leq 0.05$).

[1]CON = control diet; no additional supplement was provided.

[2]M/T = monensin and tylosin supplemented diet; monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN), and tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a DM basis.

[3]EO = essential oils supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day.

[4]BA = benzoic acid supplemented diet; benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[5]COMBO = essential oils and benzoic acid supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day and benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[6]Maximum SEM (standard error of the mean) was reported.

Table 3.3. Effects of replacing monensin and tylosin in beef finishing diets with alternative feed ingredients on cattle carcass characteristics.

Item	Dietary Treatment (Trt)					SEM[6]	*P*-values for ANOVA		
	CON[1]	M/T[2]	EO[3]	BA[4]	COMBO[5]		Trt	Block	Block*Trt
Steers, number	14	14	13	13	14				
Hot carcass weight, kg	427.9	444.4	423.7	424.1	435.1	7.10	0.19	<0.0001	0.83
Dressing percentage, %	61.12	61.18	60.79	60.82	62.13	0.69	0.62	0.46	0.91
Longissimus muscle area, cm^2	96.92	98.88	93.55	95.74	98.04	2.28	0.49	0.96	0.69
Backfat thickness, mm	19.3	18.3	19.4	20.4	18.2	1.53	0.84	0.82	0.13
Calculated yield grade[7]	3.81	3.76	3.95	3.94	3.71	0.23	0.92	0.17	0.63
Marbling score[8]	Sm_{59}^{b}	Mt_{24}^{ab}	Mt_{43}^{a}	Mt_{48}^{a}	Mt_{05}^{ab}	24	0.07	0.38	0.02
CFIA yield grade[9]	1.82	2.17	2.37	2.22	2.28	0.22	0.43	0.20	0.40
CFIA quality grade[10]	2.28^{a}	2.30^{a}	1.92^{b}	2.07^{ab}	2.28^{a}	0.11	0.05	0.27	0.15
Liver Scores[11]	0.70	0.00	0.50	0.00	0.20	0.20	0.19	0.69	0.71
No liver abscesses	10	13	11	13	13				
1-2 small abscesses	1	0	0	0	0				
2-4 large abscesses or multiple small abscesses	0	0	0	0	0				
Liver adhered to gastrointest-inal tract	3	0	2	0	1				

[a,b]Least square means lacking a common superscript letter within a row are different ($P \leq 0.05$).

[1]CON = control diet; no additional supplement was provided.

[2]M/T = monensin and tylosin supplemented diet; monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN) while tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a DM basis.

[3]EO = essential oils supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day.

[4]BA = benzoic acid supplemented diet; benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[5]COMBO = essential oils and benzoic acid supplemented diet; a proprietary blend of essential oil containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day and benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[6]Maximum SEM (standard error of the mean) was reported.

[7]Calculated yield grade: Calculated USDA yield grade = 2.50 + (2.50 × adjusted fat thickness, in) + (0.20 × 2.5%) + (0.0038 × Hot Carcass Weight, pounds) − (0.32 × ribeye area, in^2).

[8]Marbling score: Sm^{00} = $Small^{00}$; Mt^{00} = $Modest^{00}$.

[9]CFIA yield grade: 1 = CFIA yield grade 1; 2 = CFIA yield grade 2; 3 = CFIA yield grade 3.

[10]CFIA quality grade: 1.0 = Prime; 2.0 = AAA; 3.0 = AA.

[11]Liver scores were reported as observational data.

Table 3.4. Effects of replacing monensin and tylosin in beef finishing diets with alternative feed ingredients on meat quality evaluation of *longissimus thoracis.*

	Dietary Treatment (Trt)						*P*-values for ANOVA		
Item	CON[1]	M/T[2]	EO[3]	OA[4]	Combo[5]	SEM[6]	Treatment	Block	Block*Trt
Steers, number	13	13	11	12	14				
pH	5.52	5.49	5.50	5.49	5.48	0.01	0.24	0.92	0.21
Minolta L* (lightness)	37.54	39.29	38.93	38.82	39.08	0.67	0.29	0.49	0.50
Minolta a* (redness)	21.36	21.39	21.54	21.90	21.60	0.48	0.92	0.04	0.71
Minolta b* (yellowness)	8.17	8.75	8.65	8.96	8.88	0.33	0.38	0.03	0.80
Chroma[7]	22.90	23.13	23.24	23.68	23.39	0.55	0.85	0.03	0.76
Hue[8]	20.80	22.12	21.78	22.20	22.24	0.53	0.19	0.25	0.60
Moisture content, %	71.78[a]	70.45[b]	70.08[b]	70.86[ab]	70.39[b]	0.47	0.07	0.69	0.56
Lipid content, %	4.34[b]	6.25[a]	6.50[a]	5.98[a]	6.03[a]	0.59	0.06	0.53	0.29
7 d aged, WBSF[9], kg	4.16	3.63	3.33	3.54	3.73	0.34	0.38	0.98	0.88
7 d aged, cooking loss, %	19.08	19.46	18.19	19.04	19.61	0.70	0.61	0.03	0.89
14 d aged, WBSF[9], kg	3.41	3.04	2.78	3.25	3.02	0.19	0.16	0.87	0.82
14 d aged, cooking loss, %	21.35	19.62	21.43	21.13	19.33	1.00	0.30	0.54	0.83

[a,b]Least square means lacking a common superscript letter within a row are different ($P < 0.05$).

[1]CON = control diet; no additional supplement was provided.

[2]M/T = monensin and tylosin supplemented diet; monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN) while tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a DM basis.

[3]EO = essential oils supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day.

[4]BA = benzoic acid supplemented diet; benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[5]COMBO = essential oils and benzoic acid supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day and benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[6]Maximum SEM (standard error of the mean) was reported.

[7]Chroma = square root of $((a^{*2}) + (b^{*2}))$.

[8]Hue = $Tan^{-1}(b^*/a^*)$.

[9]WBSF = Warner-Bratzler shear force.

Table 3.5. Effects of replacing monensin and tylosin in beef finishing diets with alternative feed ingredients on sensory evaluation of *longissimus thoracis.*

	Dietary Treatment (Trt)						*P*-value
Item	CON[1]	M/T[2]	EO[3]	BA[4]	COMBO[5]	SEM[6]	Trt
Steaks, number	13	13	11	12	14		
Tenderness[7]	8.70[b]	10.39[a]	10.40[a]	10.50[a]	9.40[b]	0.34	<0.001
Chewiness[7]	6.20[a]	4.60[b]	4.35[b]	4.50[b]	5.48[a]	0.41	<0.001
Overall Juiciness[7]	8.10[c]	8.90[ab]	9.65[a]	9.40[a]	8.58[bc]	0.38	0.0007
Beef Flavor Intensity[7]	9.19[bc]	9.25[abc]	9.78[ab]	9.91[a]	8.91[c]	0.26	0.01
Off-Flavor Intensity[7]	0.07	0.23	0.18	0.16	0.18	0.09	0.70

[a,b,c]Least square means lacking a common superscript letter within a row are different ($P < 0.05$).

[1]CON = control diet; no additional supplement was provided.

[2]M/T = monensin and tylosin supplemented diet; monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN) while tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a DM basis.

[3]EO = essential oils supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day.

[4]BA = benzoic acid supplemented diet; benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[5]COMBO = essential oils and benzoic acid supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day and benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

[6]Maximum SEM (standard error of the mean) was reported.

[7]Traits are measured on a 15-cm line scale which included the following: tenderness: 0 = extremely tough to 15 = extremely tender; chewiness: 0 = not chewy to 15 = extremely chewy; overall juiciness: 0 = very little juiciness to 15 = very high juiciness; beef flavor intensity: 0 = very weak beef flavor detected to 15 = very intense beef flavor; off flavor intensity: 0 = no off flavors detected to 15 = very intense off flavor.

8. FIGURES

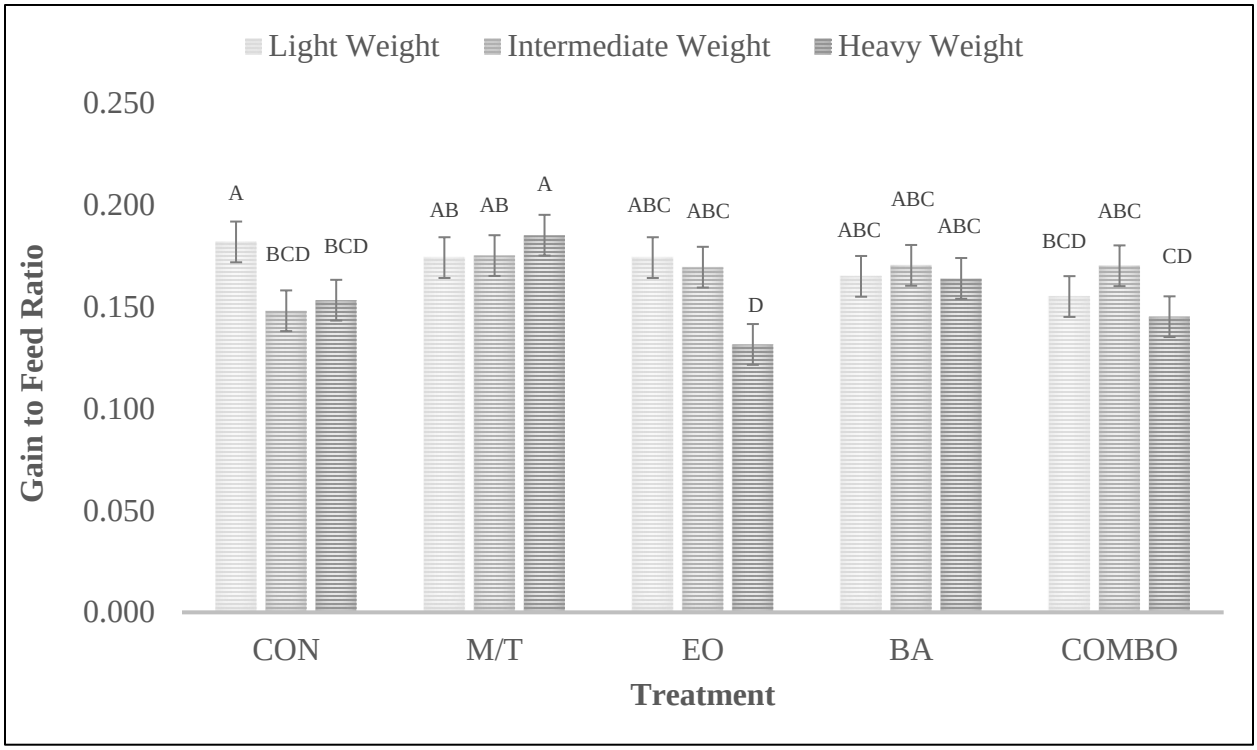

Figure 3.1. Interactive effects between treatment and block (initial weight) for G:F. [a,b,c]Least square means lacking a common superscript letter are different ($P < 0.05$). CON = control diet; no additional supplement was provided; M/T = monensin and tylosin supplemented diet; monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN), and tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a DM basis; EO = essential oils supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day; BA = benzoic acid supplemented diet; benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis; COMBO = essential oils and benzoic acid supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day and benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis.

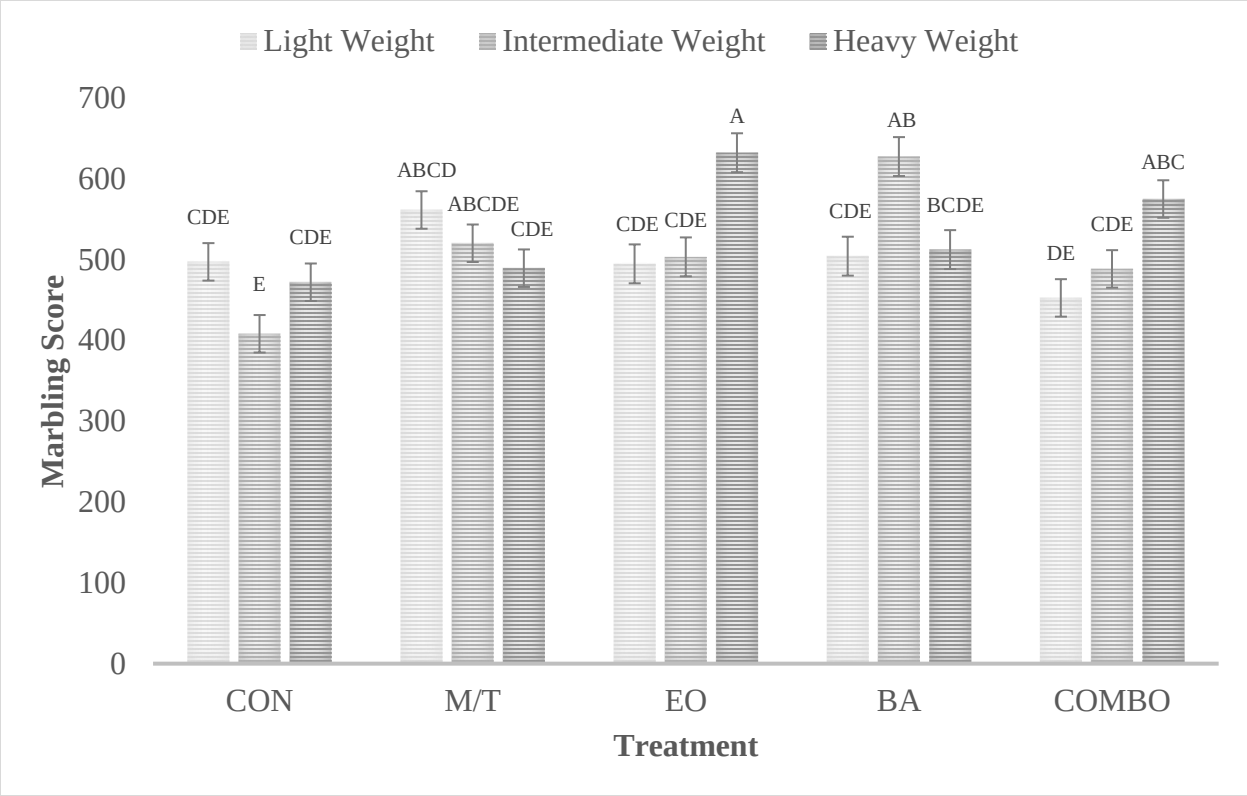

Figure 3.2. Interactive effects between treatment and block (initial weight) for marbling score. [a,b,c]Least square means lacking a common superscript letter are different ($P < 0.05$). CON = control diet; no additional supplement was provided; M/T = monensin and tylosin supplemented diet; monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN), and tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a DM basis; EO = essential oils supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day; BA = benzoic acid supplemented diet; benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis; COMBO = essential oils and benzoic acid supplemented diet; a proprietary blend of essential oils containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) was provided at 1.0 g/steer/day and benzoic acid (DSM Nutritional Products; Parsippany, NJ) was provided at 0.5% dietary inclusion on a DM basis. Marbling score: Sl^{00} = $Slight^{00}$ = 300; Sm^{00} = $Small^{00}$ = 400; Mt^{00} = $Modest^{00}$ = 500; Md^{00} = $Moderate^{00}$ = 600; $SlAb^{00}$ = $Slightly\ Abundant^{00}$ = 700.

Chapter 4

Running head: Feeding cattle essential oil and benzoic acid

The effect of feeding finishing cattle essential oils and(or) benzoic acid for replacing antibiotics on meat color and oxidative stability of ribeye steaks and ground beef [1]

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For this research chapter, the primary author (Lydia Wang) planned & conducted experiments, collected & analyzed data, and prepared the manuscript draft.

ABSTRACT: The objective of this study was to investigate and compare the effects of finishing cattle supplemented with essential oils and(or) benzoic acid versus monensin/tylosin on the color characteristics and oxidative stability of beef during a simulated retail display period. *Longissimus thoracis* (LT) and *semimembranosus* (SM) muscles were collected from crossbred steers (n = 63; BW = 542 ± 35 kg) that were randomly assigned to 1 of 5 dietary treatments: (1) control (**CON**) diet (no antibiotics fed); (2) monensin/tylosin (**M/T**) diet (monensin supplemented at 33 mg/kg on dry matter (DM) basis; tylosin supplemented at 11 mg/kg on DM basis); (3) essential oils (**EO**) diet (supplemented at 1.0 g/steer/day); (4) benzoic acid (**BA**) diet (supplemented at 0.5% on DM basis); and (5) combination (**COMBO**) diet (essential oils supplemented at 1.0 g/steer/day and benzoic acid supplemented at 0.5% on DM basis). The LT muscle was fabricated into 2.5-cm steaks, and the SM muscle was used to manufacture lean (no additional fat) and regular (subcutaneous rib fat from the same carcass added at a targeted level of 25%) ground beef patties. Instrumental color and visual surface discoloration were evaluated daily during the shelf life study. Lipid oxidation for the three beef products was estimated using thiobarbituric reactive substances (TBARS). before and after storage The simulated retail display periods were terminated at approximately 60% discoloration for the study populations, which were 12 days for LT steaks and 7 days for ground beef. L*, a*, b*, and visual discoloration scores prior and after display for steaks and ground beef from steers fed the M/T diet were not different ($P > 0.05$) from beef products from steers fed EO or BA diets. TBARS values for beef products were not affected ($P > 0.05$) by dietary treatment. Results from this study indicated that essential oils and(or) benzoic acid in finishing cattle diets produced beef products with similar color and oxidative stability traits compared to beef from cattle that were supplemented with monensin and tylosin or not fed any additional feed additives (control fed).

Key words: antibiotics, beef, benzoic acid, essential oils, lipid oxidation, retail display

1. INTRODUCTION

Ionophores, and specifically monensin are the most used non-nutritional feed additives for feedlot cattle operations (Samuelson et al., 2016). Benefits from their use in cattle include enhanced efficiency of nitrogen utilization and improved energy metabolism resulting in greater gain to feed and a decreased risk of digestive diseases (McGuffey et al., 2001). Another common feed additive for cattle is tylosin, which is a medically important antimicrobial used to improve weight gain and prevent liver abscesses (Giguère, 2013). For consumers, there is a growing interest in purchasing meat from animals that were not fed antibiotics (Centner, 2016; Lovelace et al., 2018). In addition, government health agencies are increasing their efforts for combatting antibiotic residues and resistance through stricter usage policies (FDA, 2017; Government of Canada, 2018). Therefore, the beef industry seeks to incorporate non-antibiotic feed additive alternatives to be fed to finishing cattle.

Essential oils (EO) are aromatic liquids extracted from plant materials that are known to have antimicrobial and antifungal properties (Jayasena and Jo, 2013; Gyawali, Hayek, & Ibrahim, 2014). When applied to products such as minced beef, chicken breast, and beef patties, EO lower total bacterial plate counts due to antioxidant and antimicrobial effects; this consequently improves product quality and shelf life stability of meat products (Dzudie et al., 2004; Solomakos et al., 2008; Fratianni et al., 2010). de Oliveira Monteschio et al. (2017) reported that the inclusion of EO in finishing diets of feedlot heifers produced *longissimus thoracis* steaks with greater antioxidant capacity and lower lipid oxidation levels. Organic acids (OA) also have antimicrobial and possible bactericidal effects through their ability to decrease external pH (Lucera et al., 2012). Benzoic acid is one of the oldest and most popular preservatives among the different types of organic acids, with documented uses and success in

food, beverages, and household items. With very limited studies examining the singular or interactive effects of EO and OA when incorporated in animal feed, the objective of this study was to investigate and compare the effects of finishing cattle feed supplemented with essential oils and(or) benzoic acid versus monensin/tylosin on the color characteristics and oxidative stability of beef (*longissimus thoracis* steaks and ground beef) during a simulated retail display period.

2. MATERIALS AND METHODS

All animal procedures in this study were approved by the University of Guelph Animal Care Committee Animal Utilization Protocol #3706. Animals were received and managed in accordance with the Animal Utilization Protocol, which was approved based on guidelines and principles of the Canadian Council on Animal Care (1993).

2.1 CATTLE AND FACILITIES

Upon arrival to the University of Guelph Elora Beef Research Centre, sixty-eight crossbred steers (initial BW = 539 ± 36 kg) were tagged with electronic identification tags (High Performance HDX Ultra EID Tag; Allflex, Dallas, TX) and assessed to be in good health by research personnel. Steers were vaccinated according to the research facility's protocol and implanted with Synovex S (200 mg of progesterone, 20 mg of estradiol benzoate, Zoetis, Kalamazoo, Michigan, USA) on a common day before the beginning of the study. Steers were inspected for the presence of testicles and intact animals were not included in the study. The steers were used in a randomized complete block design, where blocks were assigned by starting weight (light weight, medium weight, and heavy weight). Blocks were assigned before the start of the finishing period so that the steers underwent a training period on the feeding system

(Insentec, B.V., Marknesse, Netherlands) used in this study. At the time of blocking, weights for block 1 steers ranged from 412 – 460 kg; weights for block 2 steers ranged from 460 – 495 kg; and weights for block 3 steers ranged from 495 – 537 kg. At the start of the finishing period (when treatment diets began 6 weeks after the time of blocking), weights for block 1 steers ranged from 461.5 – 543.5 kg; weights for block 2 steers ranged from 509.5 – 564 kg; and weights for block 3 steers ranged from 515 – 631 kg.

Steers were allocated into eight equal sized pens (9.14 m × 6.71 m) that included a 4.88 m × 6.71 m area bedding with wood shavings. Each pen was equipped with an automatic watering system and four Insentec feeding stations (Insentec, B.V., Marknesse, Netherlands). The Insentec feeding system arrangement allowed the assignment of two dietary treatments per pen with two Insentec feeding stations assigned per dietary treatment which recorded daily feed intakes for individual steers. Seven pens housed 8-10 steers per pen from two different dietary treatments (4-5 steers/dietary treatment/pen); only 4 steers on one dietary treatment were housed in the eighth pen. An adaptation period of 43 days was provided before the study began to adjust the cattle from a high roughage diet to a high concentrate finishing diet. During the adaptation period, steers were trained on the Insentec feeding system and gradually adapted to the basal diet of the study which consisted of high moisture corn, alfalfa silage, soybean meal, and white salt. Rations were delivered once daily to supply feed *ad libitum*.

2.2 FINISHING DIET TREATMENTS

Within each of the three blocks used in this study, steers were randomly assigned to 1 of 5 dietary treatments based on finishing diet: 1) a negative control where no additives were included (**CON**); 2) a positive control with supplementation of monensin/tylosin (**M/T**), where

monensin was supplemented at 33 mg/kg (Rumensin; Elanco Animal Health, Greenfield, IN, USA), and tylosin was supplemented at 11 mg/kg (Tylan; Elanco Animal Health) on a dry matter basis; 3) a proprietary blend of essential oils (**EO**) supplemented at 1 g/steer/day (Victus Liv, DSM Nutritional Products, Parsippany, NJ, USA); 4) benzoic acid (**BA**) provided at 0.5% dietary inclusion on a DM basis (VevoVitall; DSM Nutritional Products); and 5) a combination of a proprietary blend of essential oils and benzoic acid (**COMBO**). These additives were included in the premix for a basal diet that consisted of high moisture corn, alfalfa silage, soybean meal, and white salt.

2.3 CARCASS AND SAMPLE COLLECTION

Steers were humanely handled and slaughtered (which included captive bolt stunning, followed by exsanguination) at a federally inspected meat processing facility based on commercial industry standards and Canadian Food Inspection Agency (CFIA) inspection regulations. Individual animal ID was maintained throughout the slaughter process. Five carcasses were lost due to sample collection errors. At 4 d post-mortem, beef bone-in ribs (IMPS#107) containing the *longissimus thoracis* (LT) and beef inside rounds (IMPS #168) including the *semimembranosus* (SM) from the right side of carcasses were collected and packaged at the commercial processing facility and delivered to the University of Guelph Meat Science Laboratory for further analyses.

2.4 PREPARATION OF *LONGISSIMUS THORACIS* STEAKS

At 6 d post-mortem, the ribs were boned, trimmed of excess fat, and then fabricated into seven 2.54 cm thick steaks. Four steaks were used for this project and were assigned for analyses in the following manner: Steak 1: proximate composition (moisture and lipid content); Steak 2: d

0 lipid oxidation using the TBARS assay; Steak 3 and 4: retail simulated shelf life stability evaluation and afterwards, lipid oxidation using the TBARS assay. The excess fat from the ribs was vacuum-packaged, and blast frozen at -30°C.

2.5 PREPARATION OF LEAN AND REGULAR GROUND BEEF PATTIES

Upon arrival at the University of Guelph Meat Science Laboratory, the inside rounds were vacuum packaged, and blast frozen at -30°C. Due to shelf space limitations, the 63 inside rounds were separated into 4 batches, such that each batch would be processed into patties on a different day. At least two inside rounds from each of the 5 treatments were represented in each batch. The inside round (SM, adductor, and other associated muscles) and subcutaneous fat originating from beef bone-in rib were thawed for approximately 5 days at 4°C, then manually cut into cube sized pieces. The inside round was prepared such that all visible fat and connective tissues were trimmed. 4.54 kg of the cubed beef were fed through the Salvinox E130 3 mm grinding plate attachment of a Sirman Master 90 Y12 meat grinder (Sirman USA, Franklin Park, IL, USA), to formulate lean patties. All of the grinding equipment was cleaned between each animal to prevent the mixing of samples form different animals. To formulate the regular patties, 3.40 kg of the lean inside round muscle and 1.14 kg of rib fat were cubed, combined, and fed through the grinder and formed into patties. Each patty weighed approximately 115 g and was 10 cm in diameter and 1.3 cm in depth. Out of each 4.54 kg batch, 6 patties were collected and used for this study. Patties were assigned for analyses in the following manner: Patties 1 and 2: proximate composition (moisture and lipid content) and d 0 lipid oxidation using the TBARS assay; Patties 3-6: retail simulated shelf life stability evaluation and afterwards, lipid oxidation using the TBARS assay.

2.6 PROXIMATE COMPOSITION ANALYSES

Moisture and lipid concentration were determined based on modified air drying and Soxhlet extraction methods, respectively (Association of Official Analytical Chemists, 2006, method 950.46; Association of Official Analytical Chemists, 2006, method 991.36). In short, 63 steaks (previously assigned as Steak 1) were thawed overnight (15-20 h), trimmed of external subcutaneous fat, cubed, and homogenized in a food processor (KitchenAid model KHB23511CU; St. Joseph, MO, USA). Duplicate 5 g samples of the homogenate were weighed onto an aluminum weighing dish and covered with 2 (42 mm) #1 Whatman Qualitative filter papers (GE Healthcare Life Sciences, Chicago, IL, USA). Next, the samples were dried in the Fisherbrand Isotemp drying oven (Thermo Fisher Scientific, Ottawa, ON, Canada) at 100˚C for at least 24 h then weighed again to determine percent moisture content. The dried samples were then placed in the Soxhlet extraction apparatus and washed multiple times for 4-5 h using approximately 200 mL of warm petroleum ether. Washed samples were placed into the 100°C drying oven for a minimum of 24 h to evaporate the petroleum ether, and then weighed for lipid determination. Proximate composition analyses for ground beef were performed the same way as steaks except there was no additional homogenization with a food processor.

2.7 SHELF LIFE STABILITY UNDER SIMULATED RETAIL DISPLAY CONDITIONS

Immediately after the LT steaks were portioned, two steaks (steaks 3 & 4) and an animal identification tag were placed on top of a meat soaker pad (Tite-Dri Industries, Boynton Beach, FL, USA) within a Styrofoam tray (Genpak 1005, Genpak, Mississauga, Ontario). The steaks were then tightly overwrapped with 60-gauge meat wrapping film (Western Plastics, Calhoun GA, USA) using the Avantco WM-18 single roll film wrapping machine (Avantco Equipment,

USA). Next, the trays were laid out onto two multi-level meat display cases. Each tier of the display case was at equal distance to each other, and each level was illuminated with two 1.22 m long LED lights (52 watts, 1850 lumens, and color temperature of 4000K, 1612.5 to 2152 lux). Trays were shuffled once every 24 h such that a more even amount of illuminance was applied to all samples. Objective color and surface discoloration (% metmyoglobin formation) were evaluated daily until the whole study population (63 trays) reached an average surface discoloration of 60%. Objective color was evaluated using a calibrated, handheld Minolta Chroma meter (Konica Minolta Sensing Americas, Inc, Ramsey, New Jersey, USA) on illuminant D_{65} and 0° viewing angle settings. As per the Commission International de l'Eclairage (CIE, 1976), each measurement by the Chroma Meter were reported as L* a* b*. Chroma, a measure of color intensity, was calculated by the equation $\sqrt{(a*)^2 + (b*)^2}$. Hue angle, a measure of distance in degrees from the true red axis of the CIE colour space, was calculated using the formula, $\tan^{-1}\left(\frac{b*}{a*}\right)$. Two measurements per steak or four measurements per tray were collected and then averaged to determine the L* a* b*, chroma, and hue values for each animal. Surface discoloration (%) was evaluated with two trained evaluators on each day of the shelf life study based on a modified version of the AMSA (2012) Meat Color Measurement Guidelines. Discoloration values for each animal were the average of both panelists' assessments. At the end of the study, the trays were vacuumed packaged, then stored at -22°C until further analysis (TBARS assay).

Shelf life evaluation was conducted the same way for ground beef patties as the steaks except for one minor change. Two trays of two patties (four patties for each animal) were used for shelf life evaluation instead of just one tray and two steaks. Final objective color results were

from the average of eight measurements (4 readings per tray) and surface discoloration was reported from the average of four responses by two panelists. With more data points collected for each animal, a better representation of the ground beef color can be obtained.

2.8 THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) ASSAY

In total, two steaks from 63 animal were used for the TBARS assay. This included 1 steak that did not undergo the simulated retail display period (day 0), and 1 steak that was used in the retail display study (day 12). Only one out of the two available steaks (steaks 3 & 4) from the shelf life study was used for the determination of TBARS and this steak was chosen at random. D 0 steaks (steak 2) were vacuum packaged, boxed-up and frozen at -22°C at 6 d post-mortem after whole rib sections were received at the University of Guelph Meat Science Laboratory and portioned. Styrofoam trays with d 12 steaks overwrapped with meat wrapping film were vacuum packaged, boxed-up then frozen at -22°C after the 12-d simulated retail display period. All steaks remained in the freezer until it was needed for TBARS analysis. To conduct the analysis, steaks had to first be thawed overnight (15-20 h) in a dark, ≤ 4°C cooler, trimmed of external subcutaneous fat, cubed, and ground in a food processor such that homogenized samples were obtained. The prepared samples were packed in tightly closed-up Whirl-Pak bags and again frozen until it was needed for the rest of the TBARS steps. A modification of the TBARS method described by Leick et al. (2010) was used to evaluate the degree of lipid oxidation for this study. The procedure was modified as the samples were thawed at room temperature for 2-3 hours prior to use (versus no thawing period used by Leick et al.) such that duplicate 5 g samples could be collected. Moreover, 1 additional ID (duplicate 5 g samples) was selected from the previous 20 IDs analyzed and was ran as the spiked samples for every 20 duplicate TBARS samples (versus every 10 duplicate TBARS samples by Leick et al.)

such that percent recovery could be calculated. A plate reader (Synergy HT; BioTek Instruments, Inc, Winooski, VT, USA) was used to read the absorbance of the samples, blanks, and standards at 530 nm. TBARS were expressed as mg MDA/kg of meat as well as mg MDA/ g of fat, where the lipid content was previously determined from the proximate analysis conducted on steak 1. Factors of increase in TBARS between d 0 and 12 samples were also calculated for each animal; this was done by dividing the d 12 TBARS values by their corresponding d 0 TBARS values.

D 0 ground beef patties from each ID and targeted fat level (patties 1 and 2) were vacuumed packaged and stored in boxes at -22°C as soon as they were fabricated from the inside rounds. The other four patties (patties 3-6) made were immediately used for shelf life color evaluations and stored at 4°C for 7 days. After this 7-d simulated retail display period, Styrofoam trays of ground beef patties overwrapped with meat wrapping film were vacuum packaged, boxed-up, then frozen at -22°C until further use. The TBARS procedure was conducted for the ground beef samples in a similar manner to the steaks. However, there were three differences. First, only one of the four patties that had undergone the simulated retail display period was chosen at random and used for TBARS determination. Second, the patties were considered homogenous already since they were manufactured as a ground product; therefore, the duplicate 5 g samples were weighed right after they were thawed at room temperature for 2-3 hours. Finally, lipid content was measured using the remaining portion of d 0 patties used for the TBARS assay.

2.9 STATISTICAL ANALYSIS

Data for objective and subjective color evaluation were analyzed using PROC GLIMMIX of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) with repeated measures on day. Fixed effects

included treatment, day, the treatment by day interaction, and block. Batch was deemed a random effect. Individual steers served as the experimental unit. TBARS data were analyzed as a randomized complete block design using PROC GLIMMIX. Fixed effects included treatment, block, and the treatment by block interaction, while pen was a random effect. Individual steers were once again the experimental units. Differences among least squares means were identified using LSMEANS and treatment means were compared using the PDIFF option. Statistical differences were considered significant at $P \leq 0.05$. The maximum standard error of the mean (SEM) was reported for each parameter displayed in the tables.

3. RESULTS

3.1 INSTRUMENTAL COLOR AND VISUAL DISCOLORATION EVALUATION

Lightness (L*) values for LT steaks, measured with the Minolta colorimeter differed ($P \leq 0.04$) among treatments on days 1 and 5 of the shelf life study (**Table 4.1**). On both days, pairwise comparisons revealed that CON steaks had lower L* values or were darker compared with the other four treatments. L* measured on d 2-4 and d 7-12 of the study were not different ($P > 0.06$) among treatments. Overall, L* increased ($P < 0.0001$) as storage time under simulated retail conditions increased. Between the start and the end of the study, the CON steaks experienced the greatest numerical increase in L* compared with the other treatments (2.39 units), while the MT steaks only experienced a numerical increase of 0.93 units in L*, which was the lowest out of the five treatments On d 11 of the study, CON and EO steaks had the lowest a* (redness) values and were less ($P \leq 0.01$) red than MT steaks. The amount of redness was not different among treatments for the other 11 days ($P \geq 0.12$) of the shelf life study period. On average, all steaks maintained the same amount of redness intensity up to d 8, after which the a* values steadily decreased approximately 38-40% at the end of the storage period, with respect to

initial values. Yellowness (b*) values for steaks were not different ($P \geq 0.10$) during d 1-5 and d 8-12 of the storage period. On d 6, b* values for steaks from steers fed the CON diet were lower ($P \leq 0.001$) compared to yellowness values for steaks from steers supplemented with steaks from cattle fed MT, EO and COMBO diets. b* values were also significantly different between CON and MT steaks on d 7, where the CON steaks had the lowest b* values. There were no differences ($P > 0.11$) in chroma for most of the shelf life study. Similar to a* values, chroma for steaks decreased approximately 38-40% between the first and last day of the study with lower ($P \leq 0.01$) chroma values on d 11 between steaks from steers fed MT diet versus chroma values for steaks from steers fed CON and EO diets. There were minor fluctuations in hue across dietary treatments throughout the display period, with similar values across dietary treatments at the beginning and end of the experiment. Dietary treatment affected hue values on d 6 and d 12, where the CON steaks had lower hue values than MT, EO and COMBO steaks ($P < 0.02$). Surface discoloration on steaks appeared least ($P \leq 0.02$) on M/T and OA steaks on d 11 of storage but were not different ($P > 0.10$) among treatments on the final day of the shelf life study.

L*, a*, b*, and chroma values for lean patties were not affected ($P > 0.10$) by dietary treatments during 7 days of display under simulated retail conditions. Hue values numerically increased over the storage period because there was a greater degree of a numerical decrease for a* than b* values over the 7 d of storage. Differences were observed ($P \leq 0.05$) among treatments for lean patties on d 7, where the MT patties had higher hue values than CON, BA, and COMBO patties. Surface discoloration for lean ground beef patties began primarily on d 4 for all dietary treatments (**Table 4.2**). COMBO patties on average had lower ($P < 0.03$) amounts of surface discoloration on d 7 and were less discolored than MT and EO lean patties.

There were no effects ($P \geq 0.17$) of dietary treatments on objective measures of color (L*, a*, b*, hue, chroma values) measured on regular beef patties throughout 7 d of storage. Similar to lean ground beef data, surface discoloration for regular patties became apparent on d 4 (**Table 4.3**). On d 5, regular ground beef fabricated from steers supplemented with both EO and BA were perceived to be less discolored ($P < 0.01$) than regular ground beef made from steers that were finished on the other four dietary treatments. On d 6, COMBO patties were significantly less discolored ($P = 0.02$) than CON, MT, and EO patties. However, at the end of the simulated retail display, trained panelists found the amount of discoloration on regular ground beef to be similar ($P \geq 0.16$) across dietary treatments.

3.2 LIPID OXIDATION (TBARS)

For LT steaks and both types of ground beef, there were no significant treatment*block interaction effects ($P > 0.33$) observed for TBARS values in units of mg MDA/kg of meat when measured before and after the simulated shelf life study period (**Table 4.4**). Similarly, TBARS (mg MDA/kg of meat) values for all meat products were not affected by dietary treatments ($P > 0.37$). When the fat content of steaks and ground beef were factored into determination of TBARS, an interaction effect was observed for d 12 LT steaks ($P \leq 0.03$) (**Table 4.5**). However, main effects of treatment and block did not have a significant impact for d 7 TBARS values for lean and regular ground beef samples. Besides the treatment x block interaction for d 12 steaks, there were no interaction or treatment effects on TBARS for all other product and measurement day combinations ($P \geq 0.06$). While TBARS values for LT and ground beef increased 4.25 to 6.45 times after being stored under simulated retail display conditions, there were no treatment or interaction effects ($P > 0.30$) for the factor of increase (**Table 4.5**). Notably, the factor of

increase for TBARS on average was numerically greater for lean ground beef than regular ground beef.

4. DISCUSSION

Numerous studies have demonstrated the effectiveness of the feed additives, monensin and tylosin and their ability to consistently improve animal health and feed efficiency. When feedlot cattle experience lower incidences of disease and better rates of gain, the length of the feeding period can be shortened with a decrease in production costs (Meschiatti et al., 2019). For meat and meat products, spoilage can occur at various steps in the production chain due to their susceptibility to lipid oxidation, microbial contamination, and autolytic enzymatic spoilage. Besides being perishable in nature, shelf life can also be shortened due to intrinsic factors such as pH and water activity (Dave and Ghaly, 2011). Thus, it is critical to practise proper preservation and handling techniques to push back expiry dates and prevent cases of foodborne illness caused by spoilage microorganisms associated with meat products (Jayasena and Jo, 2013). For the food industry, physical treatments such as freezing and high pressure processing or chemical treatments such as the addition of nitrites and sulfites are common methods to inactivate bacteria. However, alternative preservation techniques are now being investigated and preferred by consumers over traditional methods and use of synthetic additives due to increasing demands for meat products that are marketed as low in sodium or clean-labelled (Hyldgaard et al., 2012; Jayasena and Jo, 2013).

One alternative that is rising in popularity is the application of essential oils (EO) as natural preservatives. Essential oils are aromatic and volatile liquids obtained through expression, extraction, or steam distillation from various plant materials (Burt, 2004; Hyldgaard

et al., 2012). EO are regarded as secondary metabolites, and the major phenolic constituents found in the oils are known to possess wide ranges of antimicrobial and antioxidant properties (Brenes and Roura, 2010; Tajkarimi et al., 2010; Jayasena and Jo, 2013). The antimicrobial activity of EO is mainly attributed to its composition, the chemical structure of major constituent components and functional groups such as the hydrophilic hydroxyl groups of phenolic compounds, as well as interactions between major and minor constituents within the plant oils (Dorman and Deans, 2000; Hyldgaard et al., 2012). According to Burt (2004), EO can damage cell walls and membrane proteins to allow greater cell membrane permeability and consequently a loss of cellular constituents. Additionally, EO can disrupt the proton motive force and are capable of impairing enzyme systems and destroying the genetic material of bacterial cells (Burt, 2004; Solomakos et al., 2008). The proprietary EO blend used in this study included the components thymol, eugenol, guaiacol, limonene, and vanillin. Thymol, found in thyme (*Thymus vulgaris*) or oregano (*Origanum vulgare*), and eugenol, found in cloves (*Syzygium aromaticum*), are phenolic compounds with known antibacterial properties (Lucera et al., 2012; Jayasena and Jo, 2013). They are known to be effective against bacteria such as *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella enteritidis,* and *Campylobacter jejuni* (Smith-Palmer et al., 1998; Burt, 2004). Limonene, an organic compound found in citrus fruits and rosemary (*Rosmarinus officinalis*), guaiacol from beechwood creosote, and vanillin, the major constituent in vanilla beans also have antimicrobial and antibacterial properties (Piccaglia et al., 1993; Bozin et al., 2007; Liu et al., 2011; Samii et al., 2016; Yang et al., 2017). Meanwhile, benzoic acid (BA), a type of organic acid used by the food industry as a preservative for acidic products, was also used in the current study. Past research has revealed that feeding BA can improve growth performance, nutrient digestibility, and microecological

balance in the gastrointestinal tract for growing pigs (Diao et al., 2015; Cheng et al., 2017). Additionally, use of BA can acidify the cytosol of yeast and fungi, depleting ATP as an energy source for the microorganisms, and ultimately preventing growth of those targeted microorganisms (Ghaly et al., 2010). The present study examined the effects of these plant-based additives within finishing cattle diets on the shelf life stability of beef cuts from the LT and SM muscles.

L* (lightness) values for LT steaks ranged from 43 to 48 units, with values increasing over storage times for all treatments. This was in accordance with Franco et al. (2012), who reported LT steaks to have an L* increase from 40 to 43 units after 14 days of storage in a dark chamber at 4˚C. An increase in L* is known to be correlated with a decrease in fresh beef quality, including the presence of undesirable odors and greater microbial counts associated with microbial growth (Insausti et al., 2008). Only on day 1 and 4 of the simulated retail display period, L* was significantly lower for the CON treatment than steaks from the other 4 treatments. In evaluating 1, 7 and 14 d aged LT steaks in a shelf life study, de Oliveira Monteschio et al. (2017) found that only d 7 LT steaks from steers finished on predominantly soybean and corn had greater L* values than steaks from steers finished on basal diets supplemented with clove EO (2 g/animal/day), and a eugenol + thymol + vanillin active principal blend (2 g/animal/day). The much higher inclusion level of EO, use of vacuum packaging instead of a poly-vinyl film overwrap, and lack of simulated retail display cases overhead lighting during storage by de Oliveira Monteschio et al. may have led to the different L* values observed. Consumers desire a cherry red color when they are purchasing beef from the retail case. Thus, changes in a* (redness) and b* (yellowness) are important to examine as they provide an indication of browning and changes in myoglobin concentrations and its redox state in meat

(Luciano et al., 2009). The a* values for all dietary treatments in the present study decreased between 37-41% over time as steaks became more greenish-brown. Besides day 11, where M/T steaks had a higher a* value than CON, EO and COMBO steaks, dietary treatments did not impact redness stability over storage. de Oliveira Monteschio et al. (2017) also reported a decrease in a* over 14 days of wet aging; however, this decrease was much lower than the present study as their products were vacuumed packed. de Oliveira Monteschio et al. (2017) observed a better-preserved redness in steaks from cattle fed EO (clove and rosemary, each supplied at 1.33 g/animal/day) and an active principle blend (eugenol + thymol + vanillin at 1.33 g/animal/day) compared to the control on d 14. This was not seen in the current study as there were no differences between the CON and EO steaks on d 12. Our EO inclusion rate at 1.0 g/animal/day was lower than de Oliveira Monteschio et al. and may be the reason why no improvement in a* stability was observed. Initial and final b* values did not differ across treatments in the present study. Steak b* values in this study were much lower than the normal average range of 9-13 (Page et al., 2001). This may be because the steaks were packaged for retail display immediately after fabrication, leaving minimal time for bloom, resulting in a more purplish color from increased formation of surface deoxymyoglobin (Page et al., 2001). b* values though, are generally less useful in determining color stability than a* values and are often not reported in studies regarding fresh meat color (Page et al., 2001; Cardoso et al., 2016). Chroma, a measure of color intensity, decreased 37-41% over time similar to the decrease in a* values. Lower chroma values indicate a paler color, which is more undesirable to consumers and may negatively impact purchase decisions (Cardoso et al., 2016). Dietary treatment did not impact chroma except on d 11 of retail display, where CON and EO steaks appeared duller than M/T steaks. Hue, the measure of color shade and calculated based on a* and b*, was less

affected over time than chroma. Hue was less intense on the last day of retail display for CON beef than beef from cattle fed M/T, EO, and COMBO, indicating the comparable effectiveness of alternative additives in preserving color. Surface discoloration on steaks appeared least on M/T and OA steaks on d 11 of storage but did not differ among treatments on the final day of the shelf life study.

Trained panelists perceived lean ground beef patties made from inside rounds from cattle fed M/T and EO diets as more discolored than ground beef prepared from cattle fed the COMBO diet (**Table 4.2**). M/T beef had significantly greater hue angle values compared to the CON, OA, and COMBO beef and this reflected the greater perceived deviation from the desired red color consumers prefer, since a greater hue angle is associated with a more yellow color (McLellan, Lind, and Kime, 1994). Similarly, for regular ground beef, trained panelists also observed the least amount of surface discoloration on the COMBO patties on d 5, and those patties were less discolored than CON, M/T, and EO patties on d 6 of display. However, this result differs from the objective instrumental measurements, where values were not different among treatments throughout storage. The lower amounts of surface discoloration observed with the COMBO patties suggest there could be synergistic effects between EO and BA, whose antioxidant compounds slows down the oxidative processes that converts myoglobin to metmyoglobin (Sirocchi et al., 2017). Unlike the ground beef, no improvement in color stability was seen with the COMBO steaks. As a comminuted meat product, ground beef was exposed to more pro-oxidants during processing than steaks, which made them more prone to protein oxidation (Soladoye et al., 2015). Perhaps by experiencing more stresses during fabrication, the effect of EO and BA on minimizing surface discoloration was more pronounced.

Lipid oxidation of beef products was estimated using TBARS values in the present study since malondialdehyde (MDA) is often the most abundant distinct aldehyde produced during secondary lipid oxidation in foods (Reitznerova et al., 2017). TBARS values for LT steaks were similar among treatments and initial weight block at approximately 0.10 mg MDA/kg of meat on d 0. This was lower than values reported by de Oliveira Monteschio et al. (2017) with TBARS value of approximately 0.25 mg MDA/kg of meat on d 1. The differences in oxidation susceptibility between studies could be attributed to the cattle breed used (Falowo et al., 2014). *Bos indicus* Nellore heifers used by de Oliveira Monteschio et al. have been found to have higher amounts of intramuscular fat compared to *Bos Taurus* cattle, and heifers have more external fat, marbling, and higher polyunsaturated fatty acids: saturated fatty acid ratio than steers (Bressan et al., 2011; Venkata Reddy et al., 2015). Greater amounts of polyunsaturated fatty acids are known to be linked to lower color and lipid stability (Bressan et al., 2011). Moreover, beef from Holstein steers have been found to be more oxidative than beef from crossbred steers due to having a lower content of nicotinamide adenine dinucleotide (NAD), which is positively correlated with oxygen uptake and more meat discoloration, as well as possible genetic selection pressures that may have affected metabolic muscle characteristics (Faustman and Cassens, 1991). Day 12 MDA values (0.51 mg MDA/kg meat) in the present study for EO steaks were the same as the TBARS values for d 14 steaks from heifers supplemented with an active principal blend (4 g/animal/day) or an active principal blend (2 g/animal/day) + clove EO (2 g/animal/day) reported by de Oliveira Monteschio et al. (2017); however, MDA values for control beef in the latter study had significantly higher MDA values, thus demonstrating the positive effects of the eugenol, thymol and vanillin blend antioxidant potential. Similar to lipid oxidation results for the steaks, lipid oxidation increased over time for both kinds of ground beef

as expected due to its exposure to light and oxygen and the fact that raw beef contains high amounts of iron, myoglobin, and unsaturated fatty acids, all of which decreases oxidative stability (Amaral, da Silva, and Lannes, 2018). Again, treatment and block did not affect TBARS values. Initial TBARS values were greater for ground beef patties than steaks since exposure to oxygen and heat during ground beef patty preparation accelerated the lipid oxidation process. Although the d 7 lean and regular patties had an average TBARS value of 1.34 mg MDA/kg, flavor may still be acceptable as the oxidized flavor in beef is normally detected by the general population until 2.0 mg MDA/kg (Greene and Cumuze, 1981). Since lipid content varied across the three beef products, there was a need to statistically analyze TBARS values by factoring in the % lipid, such that each beef product was assessed on a similar basis. However, TBARS values were not affected by treatment nor block when fat content was accounted for.

5. CONCLUSION

In summary, use of alternatives to antibiotics for beef production did not impact L* a* b*, chroma, and visual surface discoloration of LT steaks at the beginning and end of the simulated retail display study. Dietary treatment also did not affect L* a* b*, and chroma values for lean and regular ground beef patties. In addition, lipid oxidation values for all three meat products made were not affected by dietary treatment. Although no improvements in shelf life stability were observed, feeding finishing cattle essential oils and(or) benzoic acid could be an adequate replacement to antibiotics when considering beef color stability and lipid oxidation in the retail meat case.

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7. TABLES

Table 4.1. Dietary treatment effects on color traits for longissiumus thoracis (rib) steaks during simulated retail display study.

Item		Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
Steers, number		13	13	11	12	14	
L* (lightness)	day						
	1	43.13[a]	45.68[b]	44.88[b]	44.92[b]	44.93[b]	0.04
	2	43.59	45.10	44.76	44.59	44.77	0.43
	3	44.37	46.50	45.24	45.26	45.98	0.11
	4	44.45	46.36	45.14	45.49	45.66	0.22
	5	43.84[a]	45.96[b]	46.10[b]	45.85[b]	45.80[b]	0.04
	6	44.52	45.89	46.14	45.43	45.84	0.33
	7	44.08	46.38	45.55	44.63	44.91	0.06
	8	43.61	45.32	44.76	44.78	45.01	0.30
	9	44.75	46.80	46.85	46.59	46.17	0.08
	10	46.47	47.34	47.44	46.46	47.51	0.53
	11	44.00	45.15	44.50	44.73	45.49	0.42
	12	45.52	46.61	46.45	45.96	46.55	0.64
	SEM[7] (trt)	0.59	0.59	0.64	0.61	0.57	
a* (redness)	day						
	1	20.70	21.03	21.30	21.49	21.05	0.84
	2	20.05	20.94	20.95	20.76	20.80	0.69
	3	20.51	21.05	21.52	20.82	21.08	0.71
	4	20.34	20.68	21.20	20.65	20.74	0.83
	5	20.67	20.84	20.66	20.55	20.59	1.00

	day	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
	6	20.33	21.42	21.76	20.94	21.29	0.33
	7	20.36	21.42	21.24	21.10	20.86	0.59
	8	20.83	21.51	21.99	21.15	21.49	0.57
	9	18.71	19.72	18.87	19.13	19.68	0.47
	10	17.65	19.45	18.67	19.04	18.80	0.12
	11	15.12[a]	17.25[b]	15.14[a]	16.49[ab]	16.10[ab]	0.01
	12	12.35	12.83	12.94	13.12	13.15	0.78
	SEM[7] (trt)	0.49	0.49	0.53	0.51	0.47	
b* (yellowness)	day						
	1	5.27	5.42	5.77	5.84	5.98	0.24
	2	4.68	5.44	5.28	5.51	5.18	0.17
	3	5.02	5.71	5.71	5.17	5.68	0.16
	4	4.02	4.67	4.60	4.46	4.58	0.40
	5	4.72	4.90	4.74	4.80	4.90	0.98
	6	5.21[a]	6.19[bc]	6.73[c]	5.80[ab]	6.22[bc]	0.001
	7	4.46[a]	5.52[b]	5.18[ab]	4.82[ab]	4.94[ab]	0.05
	8	6.18	6.61	7.00	6.39	6.71	0.25
	9	4.17	4.81	4.56	4.36	5.07	0.10
	10	4.31	5.02	4.67	4.70	5.22	0.11
	11	3.57	4.37	3.84	4.00	4.40	0.11
	12	3.06	3.98	3.73	3.54	3.87	0.10
	SEM[7] (trt)	0.26	0.26	0.28	0.27	0.25	
Chroma[8]	day						
	1	21.37	21.74	22.08	22.30	22.13	0.73
	2	20.61	21.64	21.61	21.49	21.45	0.60

	day	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
	3	21.13	21.83	22.28	21.47	21.85	0.62
	4	20.74	21.21	21.70	21.14	21.26	0.80
	5	21.22	21.43	21.21	21.12	21.18	1.00
	6	21.01	22.32	22.81	21.75	22.21	0.16
	7	20.85	22.13	21.88	21.66	21.45	0.47
	8	21.74	22.52	23.09	22.11	22.52	0.46
	9	19.18	20.31	19.43	19.63	20.34	0.39
	10	18.20	20.11	19.27	19.63	19.53	0.11
	11	15.56[a]	17.81[b]	15.65[a]	16.99[ab]	16.72[ab]	0.01
	12	12.77	13.51	13.51	13.62	13.78	0.68
	SEM[7] (trt)	0.51	0.51	0.56	0.53	0.50	
Hue[9]	day						
	1	14.21	14.35	15.12	15.05	15.19	0.67
	2	13.06	14.56	14.12	14.81	13.89	0.29
	3	13.68	15.08	14.79	13.85	15.04	0.29
	4	11.14	12.71	12.18	12.11	12.39	0.43
	5	12.75	13.16	12.83	13.07	13.31	0.96
	6	14.22[a]	16.03[b]	17.14[b]	15.36[ab]	16.07[b]	0.02
	7	12.26	14.43	13.54	12.77	13.24	0.12
	8	16.43	16.98	17.53	16.73	17.26	0.75
	9	12.56	13.59	13.54	12.63	14.49	0.13
	10	13.79	14.28	13.87	13.64	15.33	0.25
	11	13.45	14.21	14.52	13.76	15.72	0.07
	12	14.26[a]	18.03[c]	16.55[bc]	15.37[ab]	16.98[bc]	0.0001
	SEM[7] (trt)	0.60	0.60	0.65	0.62	0.58	

Visual Discoloration[6]	day	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
	1	0.00	0.00	0.00	0.00	0.00	1.00
	2	0.00	0.00	0.00	0.00	0.00	1.00
	3	0.00	0.00	0.00	0.00	0.00	1.00
	4	0.00	0.00	0.00	0.00	0.00	1.00
	5	0.08	0.00	0.00	0.00	0.00	1.00
	6	0.08	0.00	0.00	0.00	0.00	1.00
	7	0.15	0.00	0.32	0.00	0.11	1.00
	8	0.69	0.31	1.32	0.38	0.32	1.00
	9	4.23	0.81	2.82	0.71	1.00	0.95
	10	11.38	3.85	10.09	6.50	5.89	0.58
	11	46.58[b]	30.58[a]	44.68[b]	36.25[ab]	40.61[b]	0.02
	12	70.88	59.81	64.09	63.96	57.68	0.10
	SEM[7] (trt)	3.63	3.63	3.95	3.78	3.50	

[a,b,c]Least square means lacking a common superscript letter within a row are different ($P < 0.05$).

[1]No additional supplement.

[2]Supplementation of monensin/tylosin: supplementation of 33 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN), and 11 mg/kg tylosin (Tylan; Elanco Animal Health) on a DM basis.

[3]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ). Provided at 1.0 g/steer/day.

[4]Supplementation of benzoic acid (DSM Nutritional Products; Parsippany, NJ). Provided at 0.5% dietary inclusion on a DM basis.

[5]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) and benzoic acid (DSM Nutritional Products, Parisippany, NJ). Essential oil provided at 1.0 g/steer/day and benzoic acid provided at 0.5% dietary inclusion on a DM basis.

[6]Visual discoloration was based on a percentage scoring system, whereas 0 indicated 0% surface discoloration and 100 indicated 100% surface discoloration.

[7]The SEM (standard error of the mean) for each dietary treatment (trt) was reported.

[8]Chroma = square root of ((a*^2) + (b*^2)).

[9]Hue = Tan^{-1}(b*/a*).

Table 4.2. Dietary treatment effects on color traits for lean ground beef patties during simulated retail display study.

Item		Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
Lean ground beef, number		13	13	11	12	14	
L* (lightness)	day						
	1	44.78	46.33	45.40	45.96	46.45	0.27
	2	45.74	47.26	46.84	46.59	46.62	0.51
	3	47.45	48.24	47.27	48.35	48.42	0.57
	4	47.06	48.04	47.67	47.40	48.27	0.63
	5	46.24	47.68	47.28	46.92	47.49	0.48
	6	46.56	47.79	47.04	47.09	47.45	0.69
	7	46.96	48.60	47.90	47.76	48.32	0.38
	SEM[7] (trt)	0.61	0.61	0.67	0.64	0.59	
a* (redness)	day						
	1	21.26	21.48	22.18	21.43	21.34	0.79
	2	18.67	19.06	19.52	19.27	19.09	0.87
	3	17.59	17.56	17.80	17.96	18.13	0.93
	4	15.79	16.18	16.48	16.22	15.60	0.79
	5	14.87	15.50	15.31	15.32	15.31	0.94
	6	13.44	13.49	13.48	13.65	14.15	0.86
	7	11.82	11.32	11.73	12.03	12.23	0.79
	SEM[7] (trt)	0.53	0.53	0.58	0.55	0.51	
b* (yellowness)	day						
	1	9.33	10.30	10.82	9.85	10.04	0.10
	2	8.08	8.80	9.46	8.90	9.11	0.14
	3	7.98	8.32	8.09	8.70	8.91	0.36

			Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
		4	6.82	7.29	7.56	7.11	7.27	0.75
		5	6.94	7.86	7.61	7.47	7.85	0.41
		6	7.18	7.74	7.63	7.47	7.95	0.66
		7	6.92	7.71	7.27	6.98	7.53	0.52
		SEM[7] (trt)	0.38	0.38	0.41	0.39	0.37	
Chroma[8]	day							
		1	23.24	23.85	24.72	23.61	23.61	0.51
		2	20.36	21.02	21.71	21.25	21.18	0.61
		3	19.36	19.46	19.59	19.99	20.23	0.79
		4	17.24	17.79	18.18	17.74	17.25	0.77
		5	16.45	17.41	17.15	17.11	17.24	0.80
		6	15.30	15.63	15.57	15.69	16.28	0.80
		7	13.79	13.81	13.91	14.03	14.43	0.93
		SEM[7] (trt)	0.58	0.58	0.63	0.60	0.56	
Hue[9]	day							
		1	23.58	25.47	25.73	24.57	25.03	0.64
		2	23.35	24.64	25.83	24.76	25.43	0.53
		3	24.24	25.27	24.34	25.75	26.15	0.63
		4	23.26	24.07	24.66	23.63	24.92	0.78
		5	25.02	26.84	26.53	26.28	27.19	0.63
		6	28.24	30.04	30.05	29.09	29.43	0.73
		7	30.85[a]	34.74[b]	32.62[ab]	30.67[a]	31.81[a]	0.05
		SEM[7] (trt)	1.04	1.04	1.13	1.08	1.00	
Visual Discoloration[6]								

day	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
1	0.00	0.00	0.00	0.00	0.00	1.00
2	0.19	0.19	0.00	0.63	0.54	1.00
3	0.96	1.10	0.68	1.77	4.38	0.97
4	4.52	6.73	2.95	12.08	8.93	0.63
5	21.25	24.71	20.26	29.58	29.02	0.44
6	31.25	40.99	39.09	30.10	28.66	0.16
7	54.52[ab]	67.31[b]	61.83[b]	56.98[ab]	48.57[a]	0.03
SEM[7] (trt)	4.29	4.29	4.66	4.46	4.13	

[a,b]Least square means lacking a common superscript letter within a row are different ($P < 0.05$).

[1]No additional supplement.

[2]Supplementation of monensin/tylosin: supplementation of 33 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN), and 11 mg/kg tylosin (Tylan; Elanco Animal Health) on a DM basis.

[3]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ). Provided at 1.0 g/steer/day.

[4]Supplementation of benzoic acid (DSM Nutritional Products; Parsippany, NJ). Provided at 0.5% dietary inclusion on a DM basis.

[5]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) and benzoic acid (DSM Nutritional Products, Parisippany, NJ). Essential oil provided at 1.0 g/steer/day and benzoic acid provided at 0.5% dietary inclusion on a DM basis.

[6]Visual discoloration was based on a percentage scoring system, whereas 0 indicated 0% surface discoloration and 100 indicated 100% surface discoloration.

[7]The SEM (standard error of the mean) for each dietary treatment (trt) was reported.

[8]Chroma = square root of ((a*^2) + (b*^2)).

[9]Hue = Tan^{-1}(b*/a*).

Table 4.3. Dietary treatment effects on color traits for regular ground beef patties during simulated retail display study.

Item		Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
Regular ground beef, number		13	13	11	12	14	
L* (lightness)	day						
	1	49.75	50.55	49.98	50.60	50.76	0.86
	2	50.78	51.41	50.60	51.31	51.28	0.93
	3	49.70	51.09	50.39	50.11	50.61	0.76
	4	50.85	52.44	51.33	51.79	52.24	0.58
	5	51.15	51.42	50.98	51.96	52.19	0.77
	6	51.51	52.16	51.61	52.31	52.19	0.93
	7	51.84	52.44	52.31	52.88	53.26	0.72
	SEM[7] (trt)	0.77	0.77	0.83	0.80	0.74	
a* (redness)	day						
	1	23.77	23.22	24.40	23.38	23.65	0.70
	2	21.30	20.56	21.87	20.92	21.29	0.63
	3	18.97	18.59	19.96	18.79	18.76	0.54
	4	16.68	16.30	17.54	16.45	17.73	0.29
	5	14.07	14.60	15.24	14.25	15.84	0.17
	6	11.44	11.65	11.85	12.06	13.22	0.20
	7	10.16	9.96	9.94	10.70	10.59	0.84
	SEM[7] (trt)	0.58	0.58	0.63	0.60	0.56	
b* (yellowness)	day						
	1	12.52	12.51	13.62	12.57	13.05	0.19
	2	11.16	11.08	11.98	11.39	11.70	0.43
	3	9.72	10.01	10.41	9.88	9.87	0.77

	day	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
	4	8.76	9.16	9.45	9.18	9.67	0.49
	5	9.40	9.38	9.52	9.41	9.82	0.90
	6	9.25	9.44	9.34	9.08	9.94	0.53
	7	9.17	9.23	9.17	9.25	9.56	0.94
	SEM[7] (trt)	0.37	0.37	0.40	0.39	0.36	
Chroma[8]	day						
	1	26.89	26.41	27.98	26.57	27.04	0.40
	2	24.06	23.39	24.97	23.84	24.32	0.43
	3	21.36	21.16	22.54	21.28	21.23	0.47
	4	18.90	18.78	19.95	18.92	20.24	0.23
	5	17.04	17.46	18.05	17.24	18.71	0.22
	6	14.88	15.15	15.26	15.28	16.67	0.18
	7	13.82	13.75	13.71	14.33	14.46	0.83
	SEM[7] (trt)	0.57	0.57	0.62	0.60	0.55	
Hue[9]	day						
	1	27.34	28.20	29.03	28.27	28.78	0.92
	2	27.45	28.26	28.62	28.22	28.79	0.96
	3	27.18	28.15	27.41	27.59	27.55	0.99
	4	27.93	29.59	28.25	30.16	28.52	0.74
	5	35.12	33.21	32.48	34.82	32.15	0.39
	6	40.57	39.53	39.22	38.40	37.70	0.59
	7	41.04	43.23	43.40	42.79	43.01	0.73
	SEM[7] (trt)	1.32	1.32	1.43	1.37	1.27	
Visual Discoloration[6]							

day	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	*P*-value
1	0.00	0.00	0.00	0.00	0.00	1.00
2	0.00	0.19	0.00	0.00	0.00	1.00
3	1.44	5.58	1.02	6.25	1.52	0.84
4	19.90	18.17	18.64	22.08	10.71	0.35
5	53.94[b]	51.63[b]	47.16[b]	47.92[b]	33.30[a]	0.004
6	70.00[b]	70.58[b]	70.68[b]	63.44[ab]	54.55[a]	0.02
7	90.00	91.35	89.77	86.44	78.48	0.16
SEM[7] (trt)	4.12	4.12	4.47	4.28	3.97	

[a,b]Least square means lacking a common superscript letter within a row are different ($P < 0.05$).

[1]No additional supplement.

[2]Supplementation of monensin/tylosin: supplementation of 33 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN), and 11 mg/kg tylosin (Tylan; Elanco Animal Health) on a DM basis.

[3]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ). Provided at 1.0 g/steer/day.

[4]Supplementation of benzoic acid (DSM Nutritional Products; Parsippany, NJ). Provided at 0.5% dietary inclusion on a DM basis.

[5]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) and benzoic acid (DSM Nutritional Products, Parisippany, NJ). Essential oil provided at 1.0 g/steer/day and benzoic acid provided at 0.5% dietary inclusion on a DM basis.

[6]Visual discoloration was based on a percentage scoring system, whereas 0 indicated 0% surface discoloration and 100 indicated 100% surface discoloration.

[7]The SEM (standard error of the mean) for each dietary treatment (trt) was reported.

[8]Chroma = square root of ((a*^2) + (b*^2)).

[9]Hue = Tan^{-1}(b*/a*).

Table 4.4.

Effects of replacing monensin and tylosin in beef finishing diets with natural ingredients on TBARS values (mg MDA/ kg meat) for ribeye steaks and ground beef before and after a simulated retail display period.

Item	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	SEM[6]	Trt	Block	Trt x Block
Steers, number	13	13	11	12	14			*P*-value	
LT (rib)									
d 0 TBARS, mg MDA/kg meat	0.11	0.12	0.10	0.08	0.12	0.02	0.65	0.76	0.59
d 12 TBARS, mg MDA/kg meat	0.53	0.50	0.51	0.52	0.49	0.06	0.99	0.34	0.34
Lean Ground Beef									
d 0 TBARS, mg MDA/kg meat	0.27	0.27	0.24	0.25	0.26	0.03	0.92	0.90	0.33
d 7 TBARS, mg MDA/kg meat	1.48	1.25	1.30	1.27	1.31	0.15	0.79	0.65	0.53
Regular Ground Beef									
d 0 TBARS, mg MDA/kg meat	0.32	0.30	0.24	0.27	0.31	0.03	0.37	0.39	0.97
d 7 TBARS, mg MDA/kg meat	1.41	1.25	1.28	1.32	1.49	0.18	0.77	0.50	0.42

[1]No additional supplement.

[2]Supplementation of monensin/tylosin: supplementation of 33 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN), and 11 mg/kg tylosin (Tylan; Elanco Animal Health) on a DM basis.

[3]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ). Provided at 1.0 g/steer/day.

[4]Supplementation of benzoic acid (DSM Nutritional Products; Parsippany, NJ). Provided at 0.5% dietary inclusion on a DM basis.

[5]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) and benzoic acid (DSM Nutritional Products, Parisippany, NJ). Essential oil provided at 1.0 g/steer/day and benzoic acid provided at 0.5% dietary inclusion on a DM basis.

[6]The maximum SEM (standard error of the mean) was reported.

Table 4.5.

Effects of replacing monensin and tylosin in beef finishing diets with natural ingredients on TBARS values (mg MDA/ g of fat) for ribeye steaks and ground beef before and after a simulated retail display period.

Item	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	SEM[6]	Trt	Block	Trt x Block
Steers, number	13	13	11	12	14			*P*-value	
LT (rib)									
d 0 TBARS, mg MDA/g fat	2.92	1.91	1.83	1.73	2.12	0.44	0.24	0.97	0.19
d 12 TBARS, mg MDA/g fat	14.56[a]	8.31[b]	8.85[b]	9.19[b]	10.01[b]	1.76	0.06	0.39	0.03
Factor of Increase between d 0 & d 12	5.09	5.26	5.65	6.45	4.82	0.73	0.49	0.91	0.97
Lean Ground Beef									
d0 TBARS, mg MDA/g fat	2.81	2.50	1.97	2.40	2.85	0.47	0.63	0.94	0.09
d7 TBARS, mg MDA/g fat	16.45	11.72	10.72	11.63	15.45	2.97	0.49	0.91	0.14
Factor of Increase b/tw d0 & d7	6.20	4.81	5.93	5.50	5.74	0.71	0.61	0.74	0.44
Regular Ground Beef									
d0 TBARS, mg MDA/g fat	1.51	1.67	1.12	1.33	1.40	0.18	0.22	0.63	0.77
d7 TBARS, mg MDA/g fat	6.85	6.82	5.57	6.66	6.75	1.00	0.87	0.90	0.06
Factor of Increase b/tw d0 & d7	4.61	4.25	5.44	5.98	5.52	0.86	0.45	0.58	0.30

[1]No additional supplement.

[2]Supplementation of monensin/tylosin: supplementation of 33 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN), and 11 mg/kg tylosin (Tylan; Elanco Animal Health) on a DM basis.

[3]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ). Provided at 1.0 g/steer/day.

[4]Supplementation of benzoic acid (DSM Nutritional Products; Parsippany, NJ). Provided at 0.5% dietary inclusion on a DM basis.

[5]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) and benzoic acid (DSM Nutritional Products, Parisippany, NJ). Essential oil provided at 1.0 g/steer/day and benzoic acid provided at 0.5% dietary inclusion on a DM basis.

[6]The maximum SEM (standard error of the mean) was reported.

Chapter 5: Conclusions and Future Works

Feed additives are routinely used for finishing beef cattle for various purposes. Monensin, an ionophore can alter rumen fermentation, improve feed efficiency, and prevent coccidiosis. Tylosin phosphate, an antibiotic is used to reduce and prevent incidences of liver abscesses. However, due to growing antimicrobial resistance concerns, new regulations limiting the use of antibiotics in livestock production and increased consumer interest in natural beef products (raised without antibiotics and hormonal growth promotants), there is an increased need for research on alternative feed additives with similar or improved effectiveness as compared to using antibiotics. The objective of this study was to investigate the effects of replacing monensin and tylosin with essential oils and(or) benzoic acid in finishing cattle diets. Growth performance, feed efficiency, and carcass characteristics of finishing steers were evaluated. Additionally, meat quality and eating attributes of *longissimus thoracis* (LT) muscle were investigated. Finally, LT steaks and two different targeted fat levels of ground beef made from the inside round were examined for their colour and oxidative stability after being stored under simulated retail display conditions.

The results of the first study (Chapter 3) found that supplementation of beef finishing diets with essential oils and(or) benzoic acid did not affect most major growth performance, carcass, and meat quality traits when compared with cattle fed no additives or conventionally fed cattle supplemented with monensin and tylosin. However, feed efficiency was improved for steers that were fed monensin/tylosin, confirming once again their effectiveness and why they are so commonly used in feedlot cattle production. Eating quality were best for steaks from steers fed the monensin/tylosin, essential oils, or benzoic acid diet. Trained panelists were also unable to pick up any off-flavours present in the beef samples. The results of the second study

(Chapter 4) found no influence by dietary treatment on the color and oxidative stability of steak and ground beef products. Although the alternative treatments did not improve various animal performance and meat quality attributes, most results were comparable among diets with monensin/tylosin supplementation. This suggests the viability of replacing ionophores/antibiotics with essential oils and(or) benzoic acid in finishing cattle diets.

Many different essential oils and organic acids exist on the market. Future studies could examine and compare the effectiveness and costs of using different oil blends and organic acids on steers finished on commercial feedlot conditions. Modes of action by the alternative ingredients within the rumen ecosystem is also not well understood, and thus more research is needed to understand how essential oils and organic acids affect the rumen digestive physiology, microbiota, and immunology. Different delivery systems form administering essential oils may also be needed as they are volatile compounds and can often be evaporated or lost before reaching the rumen without losing their antimicrobial activities. Microbial profiles could also be characterized for the steaks and ground beef before, during, and after the storage period as another way of evaluating shelf life stability. Finally, future studies can conduct consumer panels for the beef products to determine overall acceptability and liking, as well as gain insights on consumer habits such as preferred method of cooking and willingness to pay.

Chapter 6: Appendix

6.1 TRAINED SENSORY EVALUATION PANEL RECRUITMENT ADVERTISEMENT

UNIVERSITY of GUELPH

PARTICIPANTS NEEDED!

WHO: Regular beef consumers

WHAT: Researchers in the Food Science Department are investigating the impact of different finishing diets on the eating quality (tenderness, juiciness, flavour) of cooked beef and are looking to recruit subjects for a trained taste panel.

WHEN: Selected panelists will need to be available from 11:30 – 12:30pm EVERY DAY from October 18 to November 16. There will be a maximum time commitment of 5 hours per week.

COMPENSATION: $10/session

If you are interested, please contact Lydia Wang at lwang06@uoguelph.ca for more information.

This project (REB# 17-12-017) has been reviewed by the Research Ethics Board for compliance with federal guidelines for research involving human participants.

6.2 CONSENT FORM

UNIVERSITY of GUELPH
ONTARIO AGRICULTURAL COLLEGE
Department of Food Science

Replacing antibiotics in beef cattle production with essential oils and organic acids may improve quality and fatty acid composition of beef products

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Department of Food Science
University of Guelph
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lwang06@uoguelph.ca

This project is supported with funds from the Weston Seeding Food Innovation grant. This project requires the consumption of beef and is limited to panelists that participate in the training sessions and are between 18 and 50 years old. 8 panelists will be chosen from the pool of available sensory panelists who have performed the best in the two previous screening sessions. The chosen panelists will then be trained for 10 sessions on evaluating tenderness, chewiness, juiciness and off-flavours. Following training, panelists will participate in 9 sensory sessions to evaluate 63 beef samples. Each training and sensory session will take approximately 1 hour/session and there will be a total of 19 sessions per panelist.

Potential risk of harm or danger includes trouble swallowing, choking, and allergic reactions to allergic or have been advised to avoid salt, citric acid, caffeine, sugar, chocolate, peanuts or nut products, gelatin, dextrin, dextrose, meat or dairy. Panelists will be provided compensation, in a cheque, at a rate of $10/session, at the end of the study.

Panelists may withdraw from the study at any point during the study without prejudice to pre-existing entitlements. Names will not be published. This project has been reviewed by the Research Ethics Board for compliance with federal guidelines for research involving human participants.

I, _____, have agreed to participate in this study as a member of the trained sensory panel that will be trained to evaluate beef samples based on the following criteria, but not limited to, flavour, juiciness and texture. If I have any issues or problems during this study I will direct them to the study principal investigator, Benjamin Bohrer, or the graduate student researcher in charge, Lydia Wang. I understand that compensation will be provided at a rate of $10/hour in the form of a cheque at the end of the study. I understand that I do not waive any legal rights by taking part in this study. I understand that once I leave the sensory lab at the end of the project, the data are not linked to identifiers and cannot be removed. A participant can request that the data to be destroyed upon withdrawal from the study.

Signature: _____

Date: _____

If you have questions regarding your rights and welfare as a research participant in this study (REB# 17-12-017), please contact: Director, Research Ethics; University of Guelph; reb@uoguelph.ca; (519) 824-4120 (ext. 56606).

6.3 SCREENING QUESTIONNAIRE

Name: _____
Preferred method of contact (please circle one): Email or Phone
Email Address or Phone Number: _____

1. Are you available to attend training sessions 11:30am – 12:30pm, Monday to Friday, from October 22nd to November 2nd and testing sessions from Monday to Friday 11:30am – 12:30pm from Nov 5th to Nov 16th (minus Nov 6th)?

   _____

a) Do you have any foreseeable scheduling conflicts with the above schedule?

   _____

2. Do you have any of the following?

   Food allergies (if yes, specify) _____ Oral or Gum Disease _____

   Hypoglycemia _____ Hypertension _____ Diabetes _____

3. Do you take any medications which affect your senses, especially taste and smell?

   _____

4. Are you currently on a restricted diet? If yes, please explain.

   _____

5. What is (are) your favourite food(s)?

   _____

6. What is (are) your least favourite food(s)?

   _____

7. Have you been a taste panelist before? If so, which food(s) did you evaluate?

   _____

8. Please rate your ability to distinguish smell and tastes.

	SMELL	TASTE
Better than average	____	____
Average	____	____
Worse than average	____	____

9. Please rate your ability to identify texture characteristics (i.e. crispy, crunchy, hard, tender, etc.) in foods.

Better than average	____
Average	____
Worse than average	____

10. How would you describe the difference between flavour and texture?

_____

_____

11. How would you describe the difference between flavour and aroma?

_____

_____

12. Describe some noticeable flavours in meat and/or meat products (can be burgers, sausages, bacon, steaks etc).

_____

_____

13. What are some textural properties of meat and/or meat products that you eat? (name and describe)

_____

_____

14. How often do you eat beef on a weekly or monthly basis?

Monthly_____ Weekly _____

15. What kind of meat do you eat most often i.e. Chicken, pork, beef, fish, etc.

_____

16. When you eat beef, how do you usually prepare it? i.e. grilling (steak, burgers); oven roasting (roast beef, stew); frying (steak, burgers, stir fry)

_____

_____

17. How do you prefer your steak to be prepared? (Please circle all the following degrees of doneness that apply) (please use degree of doneness photos in your assessment)

Blue Rare (seared on outside, completely red throughout)
Rare (seared on outside with at least 75% of the centre as red)
Medium Rare (seared on outside with 50% red centre)
Medium (seared on outside with 25% pink centre; no red)
Medium Well (only a slight hint of pink present)
Well Done (100% brown on the inside)

18. When offered meat, please name any degree of doneness that you will refuse to eat.

_____

6.4 SENSORY ATTRIBUTES DEFINITIONS

1. **Tenderness** – the force required by the molar (back) teeth to chew the beef. It is measured after four chews (0 being very tough and 15 being very tender)

2. **Juiciness** – the overall impression of the amount of moisture released by the sample after ten chews or before swallowing (0 being very little and 10 being very high)

3. **Chewiness** – the energy (force plus time) required to chew the sample as it is prepared for swallowing (at nine chews) (0 being not chewy and 15 being very chewy

4. **Beef Flavour Intensity** – the amount of full meaty flavour present after eight chews (0 being very weak and 15 being very intense)

5. **Browned/roasted** – the aromatic associated with the outside of grilled or broiled beef (seared but not blackened/burnt); A round, full aromatic generally associated with beef suet (raw beef fat from around the joints and kidneys) that has been broiled

6. **Bloody/serumy** – an aromatic associated with blood on cooked meat products; closely related to metallic

7. **Metallic** – the impression of slightly oxidized metal, such as iron, copper and silver spoons

8. **Livery** – aromatics associated with cooked organ meat/liver

9. **Rancid** – aromatics commonly associated with oxidized fat and oils; may include cardboard, painty, varnish, and fishy

10. **Spoiled** – presence of inappropriate aromatics and flavors that are commonly associated with the products; a foul taste and/or smell that indicates the product is starting to decay and putrefy

11. **Chemical** – the aromatics associated with garden hose, hot Teflon pan, plastic packaging and petroleum-based product such as charcoal liter fluid

12. **Floral** – sweet, light, slightly perfume impression associated with flowers

6.5 EXAMPLE OF A BEEF EVALUATION FORM

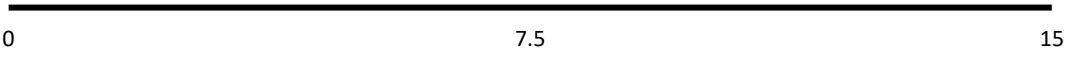
Name: _____

Date: _____

Please evaluate the following coded samples following the definitions provided. Place a **vertical line** on the 15 cm horizontal line for each attribute to indicate your perception. Complete the evaluation of one sample for all attributes before proceeding to the next sample. **DO NOT** compare the samples, evaluate each one individually. **Please cleanse your palate with water and soda crackers in between samples.**

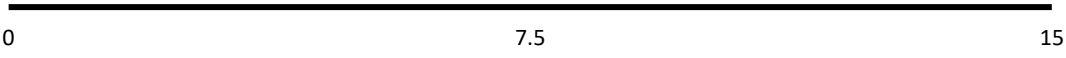
Sample Number: 101

TENDERNESS



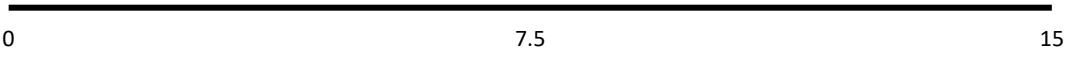
Very Tough Very Tender

CHEWINESS



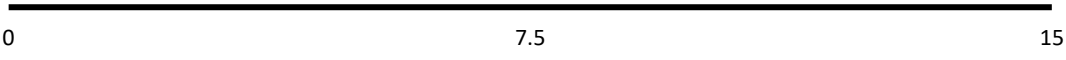
Not Chewy Very Chewy

JUICINESS



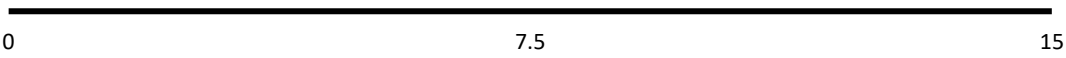
Very Little Very High

BEEF FLAVOUR



Very Weak Very Intense

OFF-FLAVOUR



Very Weak Very Intense

If any off-flavours, please describe:

_____

6.6 PROXIMATE COMPOSITION OF BEEF STEAKS FABRICATED FROM THE *LONGISSIMUS THORACIS* MUSCLE, AND GROUND BEEF MANUFACTURED FROM THE *SEMIMEMBRANOSUS* MUSCLE

Item	Con[1]	M/T[2]	EO[3]	BA[4]	Combo[5]	SEM[6]	Trt	Block	Trt x Block
							P-value		
LT (rib)									
Moisture content, %	71.78	70.45	70.08	70.86	70.39	0.47	0.07	0.69	0.56
Lipid content, %	4.34	6.25	6.50	5.98	6.03	0.59	0.06	0.53	0.29
Lean Ground Beef									
Moisture content, %	69.36	68.05	67.31	67.72	67.93	0.63	0.16	0.43	0.02
Lipid content, %	10.14	11.55	12.03	11.92	11.51	0.81	0.41	0.52	0.01
Regular Ground Beef									
Moisture content, %	60.00	62.13	59.24	61.18	59.86	0.86	0.09	0.02	0.23
Lipid content, %	21.86	18.90	23.09	20.75	22.44	1.17	0.07	0.03	0.10

[1]No additional supplement.

[2]Supplementation of monensin/tylosin: supplementation of 33 mg/kg monensin (Rumensin; Elanco Animal Health, Greenfield, IN), and 11 mg/kg tylosin (Tylan; Elanco Animal Health) on a DM basis.

[3]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ). Provided at 1.0 g/steer/day.

[4]Supplementation of benzoic acid (DSM Nutritional Products; Parsippany, NJ). Provided at 0.5% dietary inclusion on a DM basis.

[5]Supplementation of a proprietary blend of essential oil containing containing thymol, eugenol, vanillin, guaiacol, and limonene (DSM Nutritional Products; Parsippany, NJ) and benzoic acid (DSM Nutritional Products, Parisippany, NJ). Essential oil provided at 1.0 g/steer/day and benzoic acid provided at 0.5% dietary inclusion on a DM basis.

[6]The maximum SEM (standard error of the mean) was reported.