ESSENTIAL OIL EFFECTS ON RUMEN FERMENTATION, ANIMAL PERFORMANCE, AND MEAT QUALITY OF BEEF STEERS

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And hereby certify that in their opinion it is worthy of acceptance.

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ABSTRACT

Efficiency in ruminants has historically been improved by using antibiotics and ionophores to alter rumen fermentation. Nutritionists, however, have begun searching for alternative rumen modifiers due to the negative attention received by non-therapeutic antibiotic use. Plant extracts, like essential oils, are being explored as a potential alternative to alter fermentation and improve growth and efficiency in ruminants. Essential oils are naturally occurring, secondary metabolites that can be distilled or extracted from most plants and possess antimicrobial properties. Next Enhance® (**NE**, Novus International Inc.) is comprised of garlic (diallyl disulfide) and cinnamon (cinnamaldehyde) extracts; both have demonstrated the ability to modify fermentation. A series of experiments was conducted to determine how feeding NE affects *in vitro* fermentation, site and extent of nutrient digestion, feedlot performance, carcass traits, meat quality, and consumer sensory characteristics of LM steaks from beef steers. The first experiment in this thesis examined how feedlot steer performance and carcass traits were affected by NE feeding. ADG and G:F were improved early in the feeding period by 150 $mg \cdot hd^{-1} \cdot d^{-1}$ targeted NE inclusion. DMI, overall ADG, and overall G:F were not

affected by NE inclusion. All NE levels improved dressing percent, 12th rib backfat, LM area, and calculated USDA yield grade. Steers fed 150 mg·hd^{-1}·d^{-1} NE yielded carcasses worth nearly $30 more than control steers. LM steaks were obtained from five head/treatment and used to evaluate meat quality and consumer sensory characteristics. L*, a*, and b* color values were not affected by TRT on d 0 or 14. Cook loss percent was increased when low NE levels were fed but decreased by high NE doses. Warner-Bratzler shear force, and percent drip loss, moisture, and fat were not affected by NE inclusion. A consumer sensory panel reported no difference in beef steer LM steak organoleptic properties due to NE. NE inclusion at 150 – 300 mg·hd^{-1}·d^{-1} improved beef steer carcass traits and total carcass value while achieving feedlot performance, meat quality, and consumer acceptance not different from non-supplemented steers. A continuous culture fermentation experiment and a cannulated steer study were conducted to see if observed animal performance and carcass improvements could be explained by NE effects on ruminal fermentation or site and extent of nutrient digestibility. *In vitro*, NE inclusion at 15 – 120 mg·kg^{-1} DM increased nutrient digestibility, microbial N flow, and microbial efficiency. Total VFA, acetate, ammonia, and peptide production were not affected by NE inclusion *in vitro*. In cannulated steers, NE inclusion at 15 – 30 mg·kg^{-1} increased N degradation and decreased NDF digestibility in the rumen, while all NE levels increased microbial N production. Increased protein degradation and microbial N flow during lean tissue growth could contribute to the increased calf performance early in the feeding trial. If consistent results can be achieved, NE could be an alternative to ionophores.

CHAPTER 1

LITERATURE REVIEW

Introduction

Feed efficiency is a large factor in determining feedlot profitability (Pyatt et al., 2005; Cruz et al., 2010). Thus, nutritionists have long looked for methods to improve efficiency by manipulating rumen fermentation. One approach has been to use antibiotics and ionophores in diets to modify rumen fermentation. Due to negative perceptions of antibiotic use however, nutritionists have begun searching for alternative rumen modifiers. This has resulted in increased interest in using plant extracts, like essential oils (**EO**), to alter rumen fermentation (Benchaar et al., 2008a).

EO are bioactive plant compounds found in many plants that can be obtained via steam distillation or chemical extraction (Greathead, 2003; Calsamiglia et al., 2007; Hart et al., 2008). Previous studies have reported improved gain and efficiency in swine and poultry due to dietary EO inclusion (Jang et al., 2004; Cho et al., 2006; Janz et al., 2007; Yan et al., 2010). EO possess antimicrobial properties that are effective against Gram-positive and Gram-negative bacteria suggesting they could also be beneficial when included in ruminant diets (Helander et al., 1998; Dorman and Deans, 2000; Burt et al., 2004). However, their effects and the mode of action in the rumen are still unclear.

Before EO inclusion in feedlot diets can become common practice, it is necessary to understand how EO affect rumen fermentation and nutrient digestibility.

Essential Oil Background

Many plants produce bioactive compounds like saponins, tannins, essential oils (**EO**), and other phenolic compounds that have antimicrobial properties and have been shown to alter rumen fermentation. In the plant, these compounds often help protect the plant from bacterial, insect, and fungal attacks and typically contribute to the plant's flavor or smell, i.e. its "essence" (Levin, 1976; Cowan, 1999; Iason, 2005; Benchaar et al., 2008a; Hart et al., 2008; Patra, 2011). EO were originally researched to determine their role in reducing palatability in some plant species (Oh et al., 1968). For many years humans have used EO for their flavoring, scents, and preservative properties (Burt, 2004).

Although EO have an oily appearance they are considered volatile or ethereal oils rather than true lipids and are commonly extracted via steam distillation or solvent extraction (Greathead, 2003). EO can be extracted from many parts of the plant (leaves, stem, roots, seed, flower, etc.) but composition can vary greatly among different segments (Dorman and Deans, 2000). EO chemical composition can also be influenced by plant growth stage, plant health, and external factors like temperature, light, and moisture (Hart et al., 2008). Due to many influencing factors, EO chemical composition varies making it even more difficult to determine consistent effects due to EO inclusion.

EO are secondary plant metabolites that are alcohol, ester, or aldehyde derivatives of terpenoids and phenylpropanoids (Calsamiglia et al., 2007; Hart et al., 2008). Each group is synthesized through separate metabolic pathways using different primary metabolites as precursors (Figure 1.1; Calsamiglia et al., 2007). Terpenoids are

synthesized using acetyl-CoA via the deoxyxylulose or mevalonate metabolic pathway (Calsamiglia et al., 2007; Hart et al., 2008). Terpenoids are more numerous, diverse, and well documented than phenylpropanoids and are characterized by the basic five carbon, isoperene, (C_5H_8) structure that makes up their skeleton. Terpenoids can be further divided into subcategories based on the number of isoprene units in the skeleton: monoterpenoids and sesquiterpenoids. Monoterpenoids are the most common and contain two isoprene units ($C_{10}H_{16}$), while sesquiterpenoids contain three isoprene units ($C_{15}H_{24}$) and are less common (Dudareva et al., 2004).

Phenylpropanoids contain a three carbon chain bound to a six carbon aromatic ring (Calsamiglia et al, 2007; Hart et al., 2008). Phenylpropanoids are typically derived from phenylalanine via the shikimate metabolic pathway that is only functional in plants and microorganisms (Sangwan et al., 2001; Hart et al., 2008). These compounds are less common, but can be found in large percentages in some plants. There are reportedly over 1,000 monoterpenes and roughly 50 phenylpropanoids that occur naturally in plants (Lee et al., 2004).

EO have been shown to work against bacteria, protozoa, and fungi but their mode of action isn't clear (Dean and Ritchie, 1987; Cowan, 1999; Burt, 2004; Hart et al, 2008).

Mode of Action

Several theories have been suggested to explain the antimicrobial properties possessed by EO. The most widely accepted theory is that EO interact with bacterial cell membranes (Griffin et al., 1999; Dorman and Deans, 2000; Calsamiglia et al., 2007). Many EO are hydrophobic and lipophilic in nature and are able to interact with lipid cell

membranes, fuse with fatty acid chains comprising the membrane, and accumulate in the lipid bilayer of bacterial cells (Sikkema et al., 1994; Ultee et al., 1999; Calsamiglia et al., 2007).

EO accumulation between the fatty acid chains causes conformational changes in the cell membrane resulting in increased membrane instability and fluidity (Griffin et al., 1999). Thus, ion leakage occurs and ion gradients across the membrane are diminished. Bacteria counteract this by using ionic pumps to facilitate transport across the membrane, but this process diverts a great deal of energy and causes bacterial growth to decrease (Griffin et al., 1999; Ultee et al., 1999; Calsamiglia et al., 2007). This decreases bacterial populations and alters fermentation profile in the rumen.

This method of action should be more effective against Gram-positive bacteria because they lack the protective, hydroliphic outer layer possessed by Gram-negative bacteria and EO can interact directly with the cell membrane (Chao et al., 2000; Burt, 2004; Calsamiglia et al., 2007). However, EO are able to exert their antimicrobial properties on both Gram-negative and Gram-positive bacteria (Helander et al., 1998; Dorman and Deans, 2000; Burt, 2004; Benchaar et al., 2008a). Due to low molecular weights, EO compounds are able cross the protective cell wall possessed by Gram-negative bacteria by slowly diffusing through the outer lipopolysaccharide layer or through membrane proteins and reach the inner lipid bilayer (Griffin et al., 1999; Dorman and Deans, 2000; Calsamiglia et al., 2007).

EO interaction with the cell membrane also interferes with protein and electron transport, phosphorylation, and some membrane-bound enzyme dependent reactions

(Doorman and Dean, 2000; Benchaar et al., 2008a). Juven et al. (1994) suggested some EO compounds are able to interact with some enzymes and other biologically active compounds present in the cell. Both phenolic and nonphenolic compounds typically interact with proteins via hydrogen bridges and ionic interactions; however, nonphenolic compounds react via various functional groups (alcohol, aldehyde, ester, etc.) they possess rather than a phenolic ring. Aldehyde compounds are thought to deactivate proteins and enzymes using alkylation and cross bridges (Ouattara et al., 1997). Cinnamaldehyde (**CIN**; Figure 1.2) specific mode of action is unknown, but its antimicrobial activity is thought to be linked to the reactivity of its carbonyl group (Wendakoon and Sakaguchi, 1995; Helander et al., 1998). Helander et al. (1998) reported that unlike other EO, CIN had no effect on membrane stability, but interacted with membrane proteins. This interaction is thought to denature membrane proteins, increase membrane permeability, and cause cell constituents to coagulate (Juven et al., 1994; Gustafson and Bowen, 1997). This coagulation eventually causes the cell to lyse and die (Burt, 2004).

EO may also work by inhibiting hyper-ammonia producing (**HAP**) bacteria in the rumen. HAP bacteria are not present in large quantities in the rumen, but have a very high deamination activity and generate much of the ammonia produced in the rumen (Russell et al., 1988; Wallace, 2002; Patra, 2011). Thus a reduction in HAP bacteria could cause decreased amino acid (**AA**) deamination, decreased ammonia concentrations, and increased protein utilization efficiency in the rumen (Wallace et al., 2002).

Garlic oil (**GAR**) is thought to function differently than most other EO because it is a complex mixture of compounds, including diallyl disulfide (Figure 1.3) found in the

plant or produced due to changes that occur during extraction and processing (Calsamiglia et al., 2007). Feldberg et al. (1988) suggested GAR may exhibit its antimicrobial properties by inhibiting protein synthesis in the cell. Gebhardt and Beck (1996) suggested GAR inhibits hydroxymethylglutaryl-CoA reductase activity which reduces the production of the cholesterol and other isoprenoids responsible for membrane stability. By inhibiting isoprenoid production cells become unstable and eventually die. Busquet et al. (2005a,b) suggested this is how GAR and its active compounds directly inhibit *Archaea* microorganisms in the rumen and reduce methane production. However, several other studies suggest its ability to interact with sulfhydryl groups found in other active compounds is responsible for its antimicrobial activity (Reuter et al., 1996; Ross et al., 2001; Busquet et al., 2005a).

Multiple theories have been suggested to explain EO antimicrobial activities. However, since many compounds and functional groups comprise EO it is likely that multiple modes of action, rather than a single method, are exploited to exert their antimicrobial properties on rumen microbes.

Essential Oils and Digestibility

Organic Matter Digestibility

Organic Matter (**OM**) digestibility appears to be largely unaffected by EO inclusion. Blended EO inclusion had no effect on true OM digestibility *in vitro* (Castillejos et al., 2005, 2007) or total tract digestibility (Benchaar et al., 2006; Meyer et al., 2009). Diallyl disulfide and GAR inclusion had no effect on apparent total tract digestibility (Klevenhusen et al., 2011). GAR and CIN have also been reported to

increase true rumen digestibility, but have no effect on total tract OM digestibility (Yang et al., 2007; Benchaar et al., 2008a; Yang et al., 2010a).

Nitrogen Digestibility

There have been mixed results when evaluating how N digestibility is affected by EO inclusion. Some studies support the theory that EO reduce protein digestibility, but others report EO had no effect or actually increased N degradation. When beef heifers were fed CIN at 0, 400, 800, and 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$, true rumen and total tract digestibility decreased linearly (Yang et al., 2010a). GAR and diallyl disulfide fed at 312 $mg \cdot L^{-1}$ also decreased protein degradation *in vitro*, while low levels had no effect (Busquet et al., 2005b). Yang et al. (2010a) and Busquet et al. (2005c) reported EO had antimicrobial properties because increasing EO concentration decreased fermentation activity.

Other *in vitro* studies report N degradation in the rumen increased with blended EO inclusion (Castillejos et al., 2005, 2007). *In vivo,* CIN had no effect on rumen or total tract digestibility (Benchaar et al., 2008a). In dairy cows fed GAR or juniper berry extract rumen digestibility increased, but had no effect on total tract digestibility (Yang et al., 2007). Peppermint (Aldo et al., 2003) and mixed EO (Benchaar et al., 2006) also had no effect on total tract digestibility.

Fiber Digestibility

NDF digestibility *in vitro* was decreased by GAR and diallyl disulfide inclusion (Busquet et al., 2005b), but was not affected by blended EO inclusion (Castillejos et al., 2005, 2007). *In vivo,* rumen and total tract digestibility linearly decreased in heifers fed 0,

400, 800, and 1,600 mg·hd^{-1}·d^{-1} CIN. Heifers fed 1,600 mg·hd^{-1}·d^{-1} CIN had a greater than 12% reduction in rumen digestibility and total tract digestibility was reduced by over 10% (Yang et al., 2010a). Conversely, there was no difference in rumen and total tract NDF digestibility observed when GAR and juniper berry extracts (Yang et al., 2007) or CIN (Benchaar et al., 2008b) were included in dairy cow diets.

Essential Oils on Rumen Fermentation

Ammonia production

EO main effect in the rumen is a reduction in protein and starch degradation (Hart et al., 2008). When protein degradation in the rumen decreases and HAP bacteria are inhibited, AA deamination and ammonia production should decrease. This shift in fermentation would improve nitrogen utilization efficiency in the rumen and be nutritionally beneficial to the animal (Wallace et al., 2002).

This theory is supported by CIN (Busquet et al., 2006), diallyl disulfide (Busquet et al., 2005b), and GAR inclusion (Cardozo et al., 2004) reducing ammonia concentration *in vitro*. GAR also reduced ammonia concentrations in growing lambs (Chaves et al., 2008a; Klevenhusen et al., 2011). However, other studies report feeding GAR to dairy cattle (Yang et al., 2007) and CIN to growing lambs (Chaves et al., 2008b) had no effect on rumen ammonia concentrations. Conversely, increased ammonia production was observed when CIN was included in growing lamb (Chaves et al., 2008a) and finishing steer diets (Yang et al., 2010a). EO consistently reduced ammonia concentrations *in vitro,* but did not have the same beneficial effects *in vivo*. This suggests rumen microbiota may

become acclimated to EO inclusion during longer duration experiments and EO may not be able to improve N utilization efficiency in the live animal.

pH

Most feedlot animals are fed high starch, low fiber diets that decrease rumen pH. Fibrolytic bacteria are sensitive to low rumen pH while amylolytic bacteria are more acid-tolerant (Russell and Dombowki, 1980; Hoover, 1986; Wolin and Miller, 1988). As a result, decreased rumen pH from feeding high starch diets decreases fibrolytic bacteria numbers and increases amylolytic bacteria populations. Fibrolytic bacteria generally produce acetate and butyrate while amylolytic bacteria are responsible for most propionate production in the rumen, thus the altered microbial population increases propionate and decreases acetate production (Russell and Dombowki, 1980; Hoover, 1986; Wolin and Miller, 1988; Brockman, 1993). This fermentation is more energy efficient because reducing acetate:propionate (**AP**) decreases carbon lost as methane (Wolin and Miller, 1988).

EO may also be able to improve fermentation efficiency if they can reduce pH and thus decrease AP. CIN decreased pH in growing lambs (Chaves et al., 2008b) and beef heifers (Yang et al., 2010a). However, most data are contradictory as several *in vitro* and *in vivo* studies report pH was unaffected by GAR (Busquet et al., 2005b; Chaves et al., 2008a; Klevenhusen et al., 2011), diallyl disulfide (Busquet et al., 2005b; Yang et al., 2007; Klevenhusen et al., 2011), CIN (Chaves et al., 2008a, 2011), juniper berry extract (Yang et al., 2007; Chaves et al., 2008a), and mixed EO (Meyer et al., 2009) inclusion. Based on the data, it appears EO do not function by reducing rumen pH.

Volatile Fatty Acids

As previously stated, reducing acetate production and AP decreases carbon lost as methane and improves fermentation efficiency. EO effects on volatile fatty acid (**VFA**) production are unclear. Some studies suggest EO have no effect on total VFA production (Cardozo et al., 2004; Yang et al., 2007; Chaves et al., 2008b; Klevenhusen et al., 2011). Others report total VFA production is increased by CIN (Yang et al., 2010a), GAR (Chaves et al., 2008a), and blended EO inclusion (Castillejos et al., 2005). Conversely, research reports total VFA production decreased due to CIN (Chaves et al., 2011) or GAR and diallyl disulfide (Busquet et al., 2005b) inclusion in ruminant diets.

Reports about EO effects on individual VFA production and AP are similarly conflicting. Acetate proportion decreased, propionate proportion increased, and AP decreased when EO were included in the diet (Busquet et al., 2004, 2005b; Yang et al., 2010a). However, Castillejos et al. (2005) reported decreased propionate production, increased acetate production, and increased AP when blended EO were included in continuous culture diets at 1.5 $mg \cdot L^{-1}$. Others observed no difference in individual VFA proportions and AP due to EO inclusion (Cardozo et al., 2004; Yang et al., 2007; Chaves et al., 2008b; Chaves et al., 2011; Klevenhusen et al., 2011). Cardozo et al. (2005) observed decreased AP and increased butyrate proportions when oregano, capsicum, GAR, and yucca were included in the diet which is consistent with fermentation profiles of methane inhibitors (Chalupa et al., 1980; Martin and Macy, 1985). This implies these specific EO may function and improve efficiency by reducing rumen methane production and carbon lost as methane.

Methane Production

Some EO can reduce enteric methane production without negatively impacting digestibility or VFA production. Busquet et al. (2005) reported that at 300 $mg \cdot L^{-1}$ GAR and diallyl disulfide had no effect on digestibility, but reduced methane production by 74 and 69% respectively in a batch culture. Cardozo et al. (2004) reported GAR inclusion in continuous culture diets reduced methane production. Others reported thymol (Evans and Martin, 2000) and clove and fennel (Patra et al., 2005) reduced methane production, but also negatively affected digestibility or VFA production. EO effects on *in vivo* methane production have not been assessed at length, but methane production was not affected by mixed EO inclusion in beef cattle diets for 21 d (Beauchemin and McGinn, 2006) or GAR and diallyl disulfide inclusion in sheep diets for 23 d (Klevenhusen et al., 2011).

Microbial N Production and Efficiency

Microbial efficiency (**MOEFF**) is defined as the grams of microbial N produced per kilogram of OM fermented. Microbial N production and MOEFF were both improved by GAR and juniper berry EO inclusion in dairy cow diets (Yang et al., 2007). An increase in microbial N production was also observed in beef heifers fed 0, 400, 800, and 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ CIN, but MOEFF was not affected (Yang et al., 2010a). *In vitro* studies also reported no difference in MOEFF due to GAR and diallyl disulfide (Busquet et al., 2005b) or blended EO inclusion (Castillejos et al., 2007). Increased microbial protein production would increase protein available to the animal in the small intestine and potentially cause increased animal growth and/or efficiency.

Feed Intake and Animal Performance

Feed Intake

EO effects on feed intake are variable and may be affected by EO type and dose (Patra, 2011). Dry matter intake (**DMI**) was not influenced by CIN (Chaves et al., 2008a,b, 2011), GAR (Yang et al., 2007; Chaves et al., 2008a; Klevenhusen et al., 2011), diallyl disulfide (Klevenhusen et al., 2011), juniper berry (Yang et al., 2007; Chaves et al., 2008a), and mixed EO inclusion (Benchaar et al., 2006; Meyer at al., 2009).

EO dose may be critical in determining how feed intake is affected. Feed intake may be stimulated by EO inclusion at low doses while high levels may reduce palatability and be detrimental to intake. When CIN was fed to beef steers at 0, 400, 800, or 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ DMI tended to increase quadratically where 400 and 800 $mg \cdot hd^{-1} \cdot d^{-1}$ resulted in increased DMI while 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ had the lowest intake (Yang et al., 2010b). Yang et al. (2010a) reported that DMI decreased linearly in beef heifers fed 0, 400, 800, or 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ CIN

Average Daily Gain

Generally EO improve ADG. Growing lamb ADG was improved by at least 15% when fed 200 $mg \cdot kg^{-1}$ diet DM CIN or juniper berry extract (Chaves et al., 2008a). ADG was also improved in growing lambs by CIN and carvacrol (Chaves et al., 2008b) and in beef steers by mixed EO inclusion (Meyer et al., 2009). When beef steers were fed 0, 400, 800, and 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ CIN, ADG quadratically improved during the first 28 d, but did not differ during the rest of the feeding period, suggesting rumen microbes may

become adapted to EO during long feeding periods. This may help explain why Benchaar et al. (2006) observed no difference in beef cattle ADG due to mixed EO inclusion.

Feed Efficiency

Feed cost is a major portion of beef production costs, meaning profits can increase dramatically with improved feed efficiency. Feed to gain was improved by nearly 10% in growing lambs fed CIN, GAR, or juniper berry (Chaves et al., 2008a) and CIN or carvacrol extracts (Chaves et al., 2008b). Meyer et al. (2009) also reported improved efficiency with mixed EO inclusion in beef steer diets. However, overall G:F did not differ in beef steers fed CIN (Yang et al., 2010b). Conversely, Benchaar et al. (2006) reported mixed EO inclusion at 2 $g \cdot d^{-1}$ reduced G:F by 5%, but 4 $g \cdot d^{-1}$ improved G:F by over 10%, suggesting response to EO may be dose dependent.

Carcass Traits, Meat Quality, and Sensory Characteristics

There has been limited research looking at the carcass traits of ruminants fed EO during finishing and even less examining meat quality and sensory traits of meat from those animals. Carcass traits appear to be largely unaffected by EO inclusion in ruminant diets. Yang et al. (2010b) reported CIN inclusion in beef steer diets at 0, 400, 800, and 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ increased dressing percent, LM area, and 12^{th} rib backfat, but had no effect on HCW, marbling score, or USDA quality grade. Feeding a blend of thymol, eugenol, vanillin, guaiacol, and limonene EO had no effect on beef steer HCW, dressing percent, 12^{th} rib backfat, LM area, USDA yield grade, and marbling score when at 1 $g \cdot hd^{-1} \cdot d^{-1}$ (Meyer et al., 2009).

EO effects on meat quality of ruminants have not been studied. However in swine, rosemary, GAR, oregano, and ginger inclusion at 0.05% of the diet (Janz et al., 2007) and a blend of thyme, rosemary, and oregano extracts fed 0.01% of the diet (Yan et al., 2010) did not affect meat quality. No difference in juiciness, lamb flavor intensity, overall tenderness, overall palatability, and flavor desirability was observed in lambs fed 0, 100, 200, and 400 $mg \cdot kg^{-1}$ DM CIN. However, off-flavor intensity was decreased in lambs fed 100 and 400 $mg \cdot kg^{-1}$ compared to CON lambs (Chaves et al., 2011). Chaves et al., (2008b) reported CIN and carvacrol inclusion at 200 $mg \cdot kg^{-1}$ diet DM had no effect on juiciness, lamb flavor intensity, off-flavor intensity, mouth coating residue, overall palatability, and flavor desirability of lamb meat. Juiciness, lamb flavor intensity, overall tenderness, overall palatability, and flavor desirability of lamb sirloin patties were not influenced by CIN, GAR, or juniper berry extract inclusion at 200 $mg \cdot kg^{-1}$ DM. Off-flavor intensity decreased with CIN inclusion but was not altered by GAR and juniper berry extract inclusion (Chaves et al., 2008a). Beneficial differences in off-flavor intensity were observed and it was concluded that EO inclusion can be fed to ruminants without altering consumer acceptance of meat products from those animals.

Effect of pH on Essential oil effectiveness

Cardozo et al. (2005) concluded EO inclusion in high pH environments, like those found feeding high roughage diets, was not beneficial. At pH 7.0 oregano, CIN, cinnamon, capsicum, and anise extract inclusion increased AP and decreased fermentation energy efficiency. However, Cardozo et al. (2005) also determined that in low pH environments, like those found when feeding high concentrate diets, capsicum, CIN, GAR, and yucca extracts could be beneficial to beef production. When pH was held

at 5.5, capsicum, GAR, CIN, and yucca inclusion reduced AP and increased total VFA production. Cinnamon, GAR, capsicum, and CIN inclusion also reduced ammonia production, suggesting decreased AA deamination in the rumen. Skandamis and Nychas (2000) also reported bacteria cells appear to be more susceptible to EO at low pH.

Essential Oils and Ionophores

Ionophores alter ion movements across biological membranes (Schelling, 1984). Monensin (**MO**) is a carboxylic polyether ionophore that selectively inhibits Gram-positive bacteria and alters VFA ratios in the rumen (Haney and Hoehn, 1967; Schelling, 1984). MO increases propionate production and reduces acetate and butyrate proportions, thus increasing energy and nitrogen metabolism efficiency in the rumen (Richardson et al., 1976; Prange et al., 1978; Schelling, 1984).

While EO and MO result in similar fermentation modifications, synergistic effects could occur when MO and EO are fed in combination due to potentially different modes of action. When beef steers were fed Next Enhance (a combination of diallyl disulfide and CIN), MO and tylosin, or a combination treatment, Next Enhance had no influence on feedlot performance and carcass characteristics with or without MO and tylosin inclusion. There was also no interaction between Next Enhance and the Monensin and tylosin treatment (Bittner et al., 2013).

While no synergistic effects were observed in Bittner et al. (2013) study, it appears EO can be fed to achieve performance not different from MO. Dairy cows fed GAR or juniper berry extract had increased rumen OM and N digestibility compared to

cows fed MO, but DMI, total tract OM, NDF, and N digestibility, ammonia and microbial N production, and VFA production did not differ (Yang et al., 2007).

In beef cattle fed mixed EO or MO, DMI was decreased by MO, but ADG, G:F, and DM, OM, and N digestibility were not affected (Benchaar et al., 2006). Beef steers fed CIN had DMI, ADG, G:F, HCW, dressing percent, LM area, marbling scores, and USDA quality grades that did not differ from steers fed MO (Yang et al., 2010b).

Conclusion

Due to the variability in EO composition and the broad range of EO and doses that have been studied, EO have been reported to have varied and contradictory effects on rumen fermentation. However, most EO consistently inhibited rumen fermentation when fed at high doses, thus confirming their antimicrobial activities and ability to manipulate rumen fermentation. EO appear to be more effective when included in low pH environments, like those found when feeding high starch diets, like those commonly fed in feedlots. However, in longer duration *in vivo* EO effects appear to diminish over time suggesting rumen bacteria may become acclimated to EO inclusion.

Both CIN and GAR have demonstrated the ability to alter fermentation by reducing rumen N and NDF digestibility, decreasing ammonia N production, shifting to more favorable VFA production, and increasing microbial N production. The observed improvements in fermentation caused improved ADG and feed efficiency in ruminants, CIN also increased dressing percent and LM area in beef steers, and although not extensively studied, meat quality and consumer acceptance appear to be unaffected by CIN and GAR inclusion.

CIN and GAR were beneficial when included in ruminant diets and once positive results can be obtained consistently *in vivo*, they could prove to be viable alternatives to ionophores.

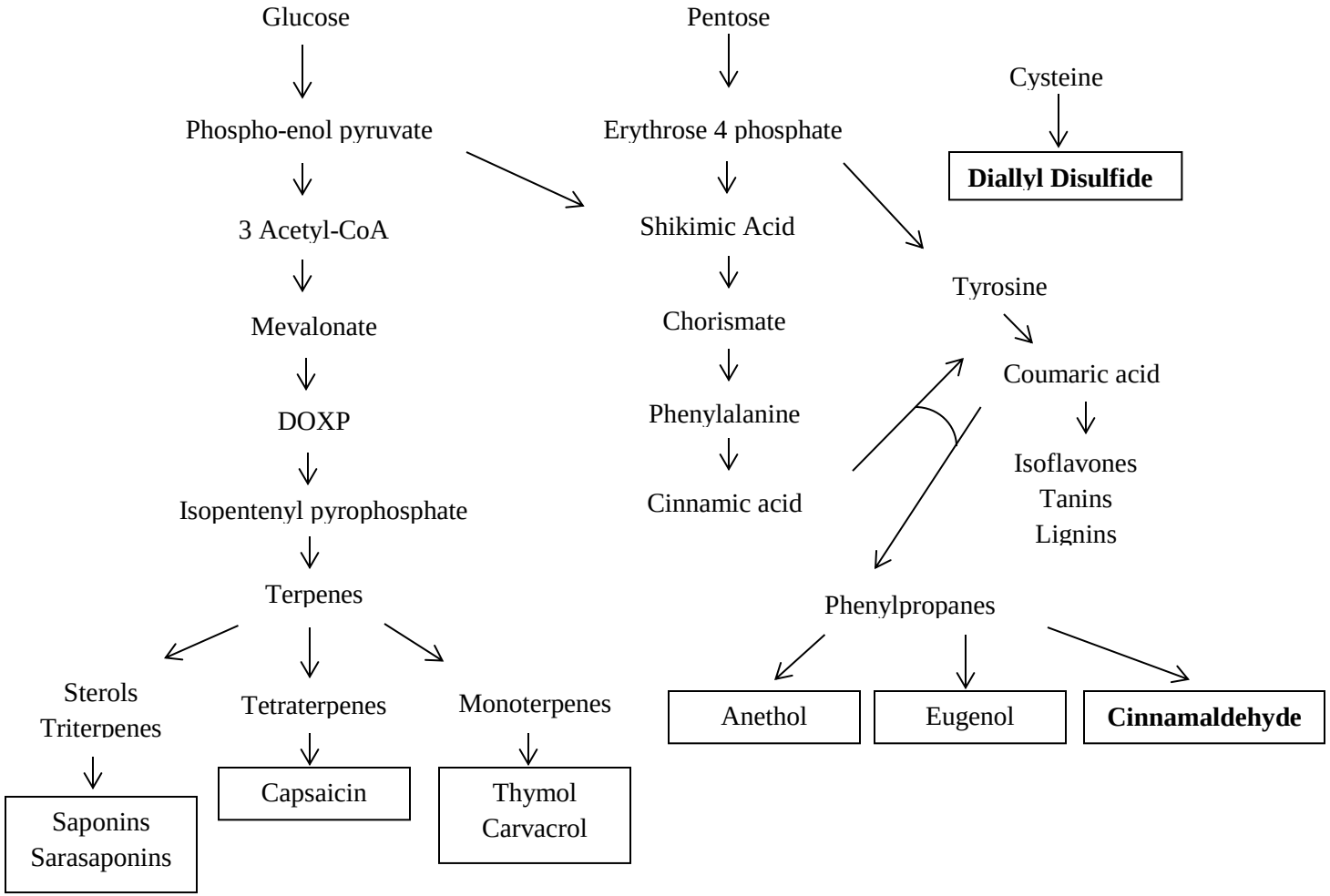

Figure 1.1. Metabolic pathways for the biosynthesis of the main plant extract active components. Adapted from Calamiglia et al., 2007.

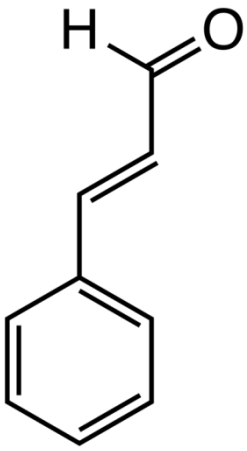

Figure 1.2. Cinnamaldehyde chemical structure.

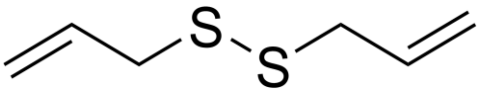

Figure 1.3. Diallyl disulfide chemical structure.

CHAPTER 2

FEEDING NEXT ENHANCE® 300 IMPROVED BEEF STEER GROWTH PERFORMANCE AND CARCASS MEASUREMENTS

ABSTRACT

Essential oils are secondary plant metabolites that exhibit antimicrobial properties and can alter rumen fermentation. Next Enhance® 300 (**NE**, Novus International, Inc.) is composed of essential oils from cinnamon (cinnamaldehyde) and garlic oil (diallyl disulfide). Both have been shown to modify rumen fermentation, suggesting NE could improve feedlot animal performance and carcass traits. Ninety-eight crossbred steers (n = 98; BW = 413 ± 37.7 kg) were used in a randomized, complete block design to evaluate NE feeding on feedlot performance and carcass traits. Steers were blocked by initial BW and randomly assigned to treatment (**TRT**), with five replicate pens per TRT. Corn-based dietary TRT consisted of 0 (**CON**), **150**, **300**, and **600** mg·hd^{-1}·d^{-1} NE. NE inclusion had no ($P \geq 0.44$) effect on BW at any measured time point. ADG was not affected ($P \geq 0.17$) by NE inclusion from d 0 – 84 or overall, but from 85 – finish CON steers tended ($P = 0.08$) to gain more steers fed NE. DMI did not differ ($P \geq 0.12$) due to TRT at any time point. G:F was increased ($P = 0.05$) from d 29 – 56 in steers fed NE, but was not affected ($P \geq 0.18$) by NE inclusion at any other time point. Steers fed NE had increased dressing percent (**DP**; $P = 0.02$), decreased 12th rib backfat (**BF**; $P = 0.01$), and decreased ($P = 0.02$) calculated USDA yield grade compared to CON steers. HCW, LM area, LM

area/45.4 kg, marbling, price/45.4 kg, and total carcass value were not affected ($P \geq 0.21$) by TRT. However, steers fed 150 yielded carcasses worth nearly $30 more than CON steers due to improved HCW, DP, BF, and LM area. Dietary inclusion of 150 – 300 $mg \cdot hd^{-1} \cdot d^{-1}$ NE can improve carcass traits of beef steers while achieving feedlot performance not different from non-supplemented steers.

INTRODUCTION

Antibiotics and ionophores are commonly used to promote growth and efficiency in feedlot animals. However, interest in using plant extracts, like essential oils (EO), has increased due to their potential to modify rumen fermentation and serve as an alternative to the antibiotics commonly used today (Benchaar et al., 2008a). EO are secondary metabolites or volatile oils that can be distilled from many plants (Calsamiglia et al., 2007). EO possess antimicrobial properties that are effective against both Gram-negative and Gram-positive bacteria suggesting they can inhibit rumen bacterial growth and modify fermentation (Helander et al., 1998; Dorman and Deans, 2000; Burt, 2004).

Several studies have been conducted using dairy cattle or *in vitro* fermentation to determine the effect of various EO on rumen microbial fermentation and animal performance (Busquet et al., 2005 b,c; Cardozo et al., 2005; Castillejos et al., 2006; Yang et al., 2007). However, limited information exists on the growth, efficiency, and carcass characteristics of cattle fed EO during the finishing period.

Next Enhance 300® (**NE**, Novus International, Inc.) is a combination of EO extracted from garlic (diallyl disulfide) and cinnamon (cinnamaldehyde; **CIN**), both of

which have been shown to modify rumen fermentation. Busquet et al. (2004) reported CIN increased the proportion of propionate and reduced ammonia N concentration while garlic oil increased propionate and butyrate proportions and decreased acetate production compared to controls. CIN is thought to work by increasing bacteria cell membrane permeability through protein denaturation and causing cell constituents to coagulate (Juven et al., 1994; Gustafson and Bowen, 1997). Garlic oil may function by inhibiting production of cholesterol and other isoprenoids responsible for membrane stability (Gebhart and Beck, 1996; Busquet et al., 2005b) or by its ability to interact with sulfhydryl groups found in other active compounds (Reuter et al., 1996; Ross et al., 2001; Busquet et al., 2005b). Burt (2004) proposed combining EO with different modes of action may result in synergistic effects that could further improve rumen fermentation. This suggests that since CIN and garlic oil are thought to function differently, feeding them in combination could improve fermentation and nutrient utilization efficiency and further improve animal performance compared to feeding them separately.

Therefore, the objective of this study was to determine how feeding increasing levels of NE affected feedlot steer growth performance and carcass traits.

MATERIALS AND METHODS

Experimental Design

All animals were handled in accordance with University of Missouri Animal Care and Use Committee guidelines. Ninety-eight crossbred steers (413 ± 37.7 kg) were used in a randomized, complete block design. Steers were sourced from three producers and delivered to the University of Missouri Beef Research and Teaching Farm near

Columbia, MO. Steers were adapted to the high concentrate diet and GrowSafe feed intake system for at least 14 d prior to the start of the feeding trial. After acclimation, one day weights (d 0) were collected to determine initial body weight (**BW**). Cattle were blocked by BW into five blocks and randomly assigned to treatment (**TRT**). Dietary TRT included: 0 (**CON**; n = 25), **150** (n = 24), **300** (n = 25), and **600** (n = 24) $mg \cdot hd^{-1} \cdot d^{-1}$ NE. Each block x TRT group of four to five steers was randomly assigned to one of twenty pens, with five replicate pens per TRT. Steers were housed in bedded concrete pens partially covered by roof. Steers were allowed *ad libitum* access to feed and water for the duration of the experiment; diet composition is presented in Table 2.1. All diets were formulated to meet or exceed all nutrition requirements for beef cattle as described by the National Research Council (NRC, 2000).

Animals were harvested when greater than 60% of steers were deemed finished by visual appraisal of external fat cover. Two day consecutive BW were collected after 114 days on feed and averaged to determine final body weight. Steers were shipped to a commercial abattoir (JBS Swift & Co., Grand Island, NE) for harvest and carcass data collection on d 118.

Carcass Data Collection

The heaviest steer from each pen (n=20; five steers/TRT) were harvested at the University of Missouri abattoir for carcass data collection and meat quality analysis on d 86, 100, 107, and 113. Steers were harvested in groups of 4-5 with all TRT represented on each kill date. HCW, 12^{th} rib backfat (**BF**; 3/4 off the midline), LM area (**LMA**),

marbling scores, USDA quality grade, dressing percent (**DP**), and calculated USDA yield grade (**YG**) were determined.

HCW, DP, BF, LMA, marbling score, USDA quality grade, and YG measurements for all remaining steers (n=78) were collected at the commercial abattoir on d 119.

Statistical Analysis

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute, Cary, NC, U.S.A.). The model included TRT as a fixed effect and the random effects of block and the block*TRT interaction. DMI, ADG, and G:F were analyzed as repeated measures in time using compound symmetry as the variance-covariance error structure. Carcass traits were also analyzed to determine effects of harvest date. LSMeans comparisons were made using Fisher's Least Significance Difference and a contrast statement was used to assess the effects of NE (CON vs. all levels of NE).

RESULTS

The time*TRT and harvest date*TRT interactions were not significant for any variable analyzed.

Intake and Growth Performance

BW ($P \geq 0.19$) and DMI ($P \geq 0.16$) did not differ across TRT at any measured time point (Table 2.2). ADG on d 28, 56, 84, and overall did not different ($P \geq 0.17$) among TRT, but CON steer ADG tended to be greater ($P = 0.08$) than steers fed NE from

d 85 until finish. G:F was improved by NE inclusion on d 56 ($P = 0.05$) but was otherwise not affected.

Carcass Characteristics

Steers fed NE had increased dressing percent ($P = 0.02$), decreased 12th rib backfat (**BF**; $P = 0.01$), and decreased ($P = 0.02$) calculated USDA yield grade compared to CON steers (Table 2.3). HCW, LM area, LM area/45.4 kg, marbling, price/45.4 kg, and total carcass value were not affected ($P \geq 0.21$) by TRT. However, steers fed 150 yielded carcasses worth nearly $30 more than CON steers due to improved HCW, DP, BF, and LM area. Dietary inclusion of 150 – 300 mg·hd^{-1}·d^{-1} NE can improve carcass traits of beef steers while achieving feedlot performance not different from non-supplemented steers.

DISCUSSION

Much of the available work on EO has been completed using dairy cattle or *in vitro* fermentation and continuous culture models (Cardozo et al., 2004; Busquet et al., 2005 b,c; Cardozo et al., 2005). There have been few studies looking at EO effects on finishing steer performance and carcass traits. Furthermore, completed studies report inconsistent and inconclusive results.

EO effects on DMI are unclear. In two studies using cannulated heifers DMI decreased due to EO inclusion (Cardozo et al., 2006; Yang et al., 2010a). In another study, Yang et al., (2010b) reported CIN inclusion caused a quadratic increase in overall DMI. However, our findings that DMI was not different due to NE inclusion ($P \geq 0.12$) at

any measured time point is similar other studies that reported EO had not effect on DMI (Chaves et al., 2008b; Meyer et al., 2009; Chaves et al., 2011).

Although ADG was not different ($P \geq 0.17$) across TRT on d 28, 56, or 84, steers fed 150 had numerically greater ADG than all other TRT at d 28 and 56. However, this improvement was gone by d 84 and from d 85 – finish steers fed NE tended to have decreased ($P = 0.08$) ADG compared to CON steers. Nevertheless, overall ADG was not different due to NE inclusion ($P = 0.32$). Yang et al. (2010b) observed a similar, numerical improvement in ADG early in the feeding period when CIN was fed to steers at 400 and 800 $mg \cdot hd^{-1} \cdot d^{-1}$, but again overall ADG did not differ among TRT. The improved ADG of 150 steers, although not significant on d 56 or 84, can be considered economically relevant as it contributed to 150 steers having the greatest final BW and HCW.

Similar to ADG results, 150 steer G:F on d 28 was numerically improved compared to all other TRT. On d 56 NE inclusion caused increased ($P = 0.05$) G:F, as all NE TRT had improved G:F compared to CON. NE did not affect ($P \geq 0.18$) efficiency during the rest of the feeding period or overall G:F. These findings are similar to studies where EO had no effect on overall efficiency (Yang et al., 2010b; Meyer et al., 2009; Chaves et al., 2011), but contradict Benchaar et al. (2006) who reported EO improved feed efficiency.

NE inclusion increased DP ($P = 0.02$), with 300 steers dressing more than 2% greater than CON steers. In other studies, DP has been reported as greater and not different in animals fed EO. CIN fed to beef steers at 400 to 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ tended to

cause a linear increase in DP (Yang et al., 2010b). However, feeding a blend of thymol, eugenol, vanillin, guaiacol, and limonene EO had no effect on beef steer DP when fed at 1.0 $g \cdot hd^{-1} \cdot d^{-1}$ (Meyer et al., 2009).

In the same study where CIN tended to increase DP, numerical increases in LMA and BF were also observed (Yang et al., 2010b). Similar results were found in this study as LMA and LMA/45.4 kg were numerically increased in all NE TRT compared to CON. However, their data contrasts our findings that increasing NE significant decrease (P = 0.02) in BF. In the current study, NE caused a decrease (P = 0.02) in YG due to NE steers having reduced BF, increased DP, and increased LM area compared to CON steers. This contrasts the findings of Meyer et al. (2009) who reported EO had no effect on YG.

Although price/45.4 kg did not differ (P > 0.27), steers fed 150 yielded carcasses worth greater than $30 more than CON due to improved HCW, DP, BF, LMA, and YG.

The reason CON steers had improved G:F and steer fed CON, 300, and 600 had increased ADG from d 85 until finish is unclear. We hypothesize that it was due in part to 150 steers having increased performance early in the feeding phase and consequently being further along in their physiological growth curve by d 84. This resulted in these cattle being heavier at d 84 and having numerically decreased efficiency and gain during the last feeding period. In an attempt to maintain similar days on feed for all TRT, steers fed CON, 300, and 600 were able achieve similar final BW to steers fed 150 as they were lighter and still had more growth and performance potential left.

It is also important to note that no liver abscesses were observed in the 20 steers harvested at the University of Missouri abattoir.

CONCLUSION

NE dietary inclusion at 150 – 300 mg·hd^{-1}·d^{-1} improved DP, BF, LMA, YG, and total carcass value while achieving feedlot performance not different from non-supplemented steers. The data from this study suggests that similar to feeding other rumen modifiers, alternative harvest endpoints should be considered when feeding EO. Animals should be harvested at a similar carcass endpoint rather than similar DOF.

Table 2.1. Ingredient and nutrient composition of diets (DM basis) fed to feedlot steers.

Item	Amount
Ingredient, %	
Corn	60.2
Hay	8.1
DDGs	16.3
AminoPlus	8.0
Soyhulls	4.0
Vitamins & Minerals	2.7
Analyzed Composition	
DM, %	88.0
OM, %	93.6
CP, %	15.4
NDF, %	24.3
ADF, %	10.3

Table 2.2. Growth and performance characteristics of feedlot steers fed Next Enhance®. [1]

	Treatment					*P*-value	
Item	CON	150	300	600	SEM	Treatment	CON v. NE
No. of steers	25	24	25	24			
Days on Feed	113	111	112	113	1.6	0.86	0.52
Body Weight, kg							
Initial	411	416	408	418	18.6	0.46	0.77
d 28	450	460	444	452	18.6	0.46	0.82
d 56	482	495	479	487	18.6	0.46	0.58
d 84	523	532	515	516	18.6	0.46	0.87
Final	569	572	560	557	18.4	0.19	0.44
ADG, kg							
d 0 – 28	1.38	1.64	1.28	1.25	0.113	0.12	0.98
d 29 – 56	1.16	1.27	1.26	1.25	0.111	0.12	0.39
d 57 – 84	1.44	1.38	1.29	1.14	0.114	0.12	0.17
d 85 – Finish	1.71	1.42	1.60	1.46	0.113	0.12	0.08
Overall	1.41	1.41	1.36	1.24	0.069	0.20	0.32
DMI, kg/d							
d 0 – 28	12.54	12.80	12.10	11.76	0.710	0.17	0.65
d 29 – 56	12.75	12.25	11.75	11.43	0.706	0.17	0.19
d 57 – 84	13.23	13.05	12.74	12.13	0.706	0.17	0.41
d 85 – Finish	13.92	12.94	13.04	12.42	0.715	0.17	0.12
Overall	12.92	12.69	12.25	11.79	0.459	0.16	0.12
G:F							
d 0 – 28	0.111	0.127	0.106	0.106	0.0067	0.56	0.76
d 29 – 56	0.092	0.105	0.107	0.106	0.0065	0.56	0.05
d 57 – 84	0.110	0.103	0.102	0.094	0.0068	0.56	0.18
d 85 – Finish	0.119	0.116	0.126	0.123	0.0068	0.56	0.72
Overall	0.111	0.112	0.111	0.105	0.0048	0.63	0.73

[1]Any steers with a negative ADG were excluded from all calculations during that feeding period.

Table 2.3. Carcass characteristics of beef steers fed Next Enhance®.

	Treatment					*P*-value	
Item	CON	150	300	600	SEM	Treatment	CON v. NE
HCW, kg	337	341	332	328	12.6	0.38	0.58
Dressing %	62.2[b]	63.2[ab]	64.3[a]	62.9[b]	0.57	0.03	0.02
BF, cm	1.44[a]	1.24[b]	1.12[b]	1.25[b]	0.107	0.05	0.01
LM area, cm^2	75.7	78.5	80.3	76.6	2.29	0.35	0.22
LM area/45.4 kg	1.66	1.73	1.77	1.69	0.050	0.35	0.22
Marbling[1]	545	559	535	518	26.7	0.58	0.76
Calculated USDA yield grade	3.43[a]	3.14[ab]	2.83[b]	3.12[ab]	0.188	0.04	0.02
Price, $/45.4 kg	196.77	198.40	198.96	197.33	1.15	0.50	0.27
Carcass Value, $	1,453	1,482	1,438	1,414	60.3	0.21	0.35

[1]400 = Small[0], 500 = Modest[0]

[a,b] Means in a rows that lack a common superscript differ ($P \leq 0.10$).

CHAPTER 3

CARCASS TRAITS, MEAT QUALITY, AND CONSUMER SENSORY CHARACTERISTICS OF LONGISSIMUS STEAKS FROM BEEF STEERS FED NEXT ENHANCE 300®

ABSTRACT

Ninety-eight crossbred steers (BW = 413 ± 37.7) were used in a randomized, complete block design to evaluate Next Enhance® 300 (**NE**, Novus Intl. Inc.) feeding on carcass characteristics, meat quality, and consumer sensory characteristics of LM steaks. Steers were blocked by initial BW and randomly assigned to treatment (**TRT**), with five replicate pens per TRT. Corn-based dietary TRT consisted of 0 (**CON**), **150, 300**, and **600** mg·hd^{-1}·d^{-1} NE. Five steers/TRT (n = 20) were harvested at the University of Missouri abattoir. Steers fed NE had increased dressing percent (**DP**; $P = 0.02$), decreased 12th rib backfat (**BF**; $P = 0.01$), and decreased ($P = 0.02$) calculated USDA yield grade compared to CON steers. HCW, LM area, LM area/45.4 kg, marbling, price/45.4 kg, and total carcass value were not affected ($P \geq 0.21$) by TRT. However, steers fed 150 yielded carcasses worth nearly $30 more than CON steers due to improved HCW, DP, BF, and LM area. TRT did not affect ($P \geq 0.13$) L*, a*, or b* objective color values at d 0 or d 14. A consumer sensory panel, 55 participants, was conducted using one steak per steer, with 4-5 panelists evaluating each sample. There were no differences ($P > 0.12$) d TRT for cook loss, Warner-Bratzler Shear Force, drip loss, percent moisture,

percent fat, or consumer opinion of overall like, liking of tenderness, juiciness, and flavor and level of tenderness, juiciness, and flavor. Feeding NE improved dressing percent, 12th rib backfat, LM area, and yield grade, and did not negatively affect meat quality or organoleptic properties of LM steaks from beef steers.

INTRODUCTION

Interest in using plant extracts, like essential oils (**EO**), in ruminant nutrition has increased in recent years due to their antimicrobial properties, ability to modify rumen fermentation, and potential to be alternatives to antibiotics and ionophores commonly fed today (Benchaar et al., 2008a).

Several studies have been conducted using dairy cattle or *in vitro* fermentation to determine the effect of EO on rumen microbial fermentation and subsequent animal performance (Cardozo et al., 2004; Busquet et al., 2005 b,c; Cardozo et al., 2005). However, limited information exists on the carcass traits, meat quality, and sensory characteristics of animals fed EO during the finishing period.

Next Enhance® 300 (**NE**, Novus International Inc.) is a combination of EO extracted from garlic (diallyl disulfide) and cinnamon (cinnamaldehyde; **CIN**). Both have been shown to modify rumen fermentation and increase propionate and total VFA production (Yang et al., 2010; Cardozo et al., 2005). These results suggest fermentation could be positively altered and subsequent carcass characteristics of feedlot steers could be improved by feeding these EO in combination.

Therefore, this project was designed to determine how feeding increasing levels of NE to beef steers affects carcass characteristics, meat quality, and sensory traits of LM steaks.

MATERIALS AND METHODS

Animals and Diets

All animals were handled in accordance with University of Missouri Animal Care and Use Committee guidelines. Ninety-eight crossbred steers (413 ± 37.7 kg) were used in a randomized, complete block design. Steers were adapted to a high concentrate diet and the GrowSafe feed intake system for at least 14 d prior to the project starting. After the acclimation period, one day weights (d 0) were collected to determine initial body weight (**BW**). Cattle were blocked by BW into five blocks and randomly assigned to treatment (**TRT**). Dietary TRT included: 0 (**CON**; n = 25), **150** (n = 24), **300** (n = 25), and **600** (n = 24) $mg \cdot hd^{-1} \cdot d^{-1}$ NE. Each block x TRT group of four to five steers was randomly assigned to one of twenty pens, with five replicate pens per TRT. Steers were housed in bedded concrete pens partially covered by roof. Steers were allowed *ad libitum* access to feed and water for the duration of the experiment; diet composition is shown in Table 3.1.

Animals were harvested when greater than 60% of steers were deemed finished by visual appraisal of external fat cover. Two day consecutive BW were collected after 114 days on feed and averaged to determine final BW. Steers were shipped to a commercial abattoir (JBS Swift & Co., Grand Island, NE) for harvest and carcass data collection on d 118.

Carcass Data Collection

The heaviest steer from each pen (n=20; five steers/TRT) were harvested at the University of Missouri abattoir for carcass data collection and meat quality analysis on d 86, 100, 107, and 113. Steers were harvested in groups of 4-5 with all TRT represented on each kill date. HCW, 12th rib backfat (**BF**; 3/4 off the midline), LM area, marbling scores, USDA quality grade, dressing percent (**DP**), and USDA yield grade (**YG**) were determined.

HCW, DP, BF, LM area, marbling score, USDA quality grade, and YG measurements for all remaining steers (n=78) were collected at the commercial abattoir on d 119.

Meat Quality Analysis

Objective Color Measurements

At 96 h post mortem (d 0) the left side of each carcass at the University of Missouri abattoir was ribbed and allowed to bloom for 45 min. Surface objective color measurements were taken using a Minolta Chroma Meter CR-410 (Minolta Camera Co., Osaka, Japan), calibrated using a white tile standard, with a D65 light source and a 10 degree observer. The lightness (L*), redness (a*), and yellowness (b*) were measured at three locations on the LM surface and averaged.

After color analysis, a four rib section was vacuum packaged and stored under refrigeration at 4º C. On d 14 rib sections were removed from the vacuum packaging and allowed to bloom for 45 min. Aged color measurements were taken and two steaks (2.54

cm thick) were removed for further quality analysis. Two additional steaks were frozen for later use during the consumer sensory panel.

Drip Loss

Drip loss was determined using a method adapted from Barton-Grade et al. (1993). External fat and connective tissue were removed from 10 g subsample of LM. The subsample was weighed and then suspended by a hook, inside a cup, within a plastic bag. Bags were suspended from a rod, making sure that the sample was not touching the cup or the bag, and refrigerated at 4º C for 24 h. After 24 h samples were reweighed and drip loss was calculated as, drip loss % = [(initial sample wt. – final sample wt.) / initial sample wt.] * 100.

Fat and Moisture Analysis

The remainder of the steak used for drip loss analysis was frozen at -5º C until used to determine fat and moisture percentages. Proximate analysis was completed using a CEM Moisture/Solids Analyzer and Smart Trac Rapid Fat Analysis system (CEM Corp., Matthews, NC, USA).

Cook Loss

Cook loss and Warner-Bratzler shear force (**WBSF**) values were determined using the second steak. Raw steaks were weighed and cooked on an open top griddle (National Presto Industries, Inc., Eau Claire, WI) to an internal temperature of 35º C. Steaks were then turned and cooked to a medium degree of doneness and final internal temperature of 71º C. Internal temperature was measured using a hand held thermometer

with a copper-constantan Type-T wire thermocouple (HH-21, Omega Engineering, Stamford, CT). After cooling, steaks were reweighed and cooking loss was calculated, where cooking loss % = [(raw wt. – cooked wt.) / raw wt.] * 100.

Warner-Bratzler Shear Force

WBSF was conducted according to AMSA (1995) research guidelines for fresh meat instrumental tenderness measurements. Steaks previously used to determine cook loss were wrapped in foil and refrigerated for 24 h at 4º C when six 1.27 cm cores were removed from each steak parallel to the muscle fiber using an automated coring device. Cores were sheared perpendicular to the muscle fiber with a United STM Smart-1 Test System SSTM-500 (United Calibration Corp., Huntington Beach, CA). WBSF test speed was 250 mm/min. WBSF was determined as the peak force in kilograms required to completely shear each core. Values for all six cores were averaged and converted from kilograms of force to newtons.

Consumer Sensory Panel

A consumer (n = 55) sensory panel was conducted using one steak per steer, with 4-5 panelists evaluating each sample. The panel was approved exempt by the Institutional Review Board and panelists were recruited from the Columbia, MO area using a list of previous panel participants, posted flyers, and word of mouth. Consumers received $10 for their participation. Panelists were given initial written and verbal instruction on how to complete the ballot. Consumers were asked to give their opinions on overall liking, liking of tenderness, juiciness, and flavor and level of tenderness, juiciness, and flavor using a 10 pt. hedonic scale, 1 = like extremely and 10 = dislike extremely.

Steaks were cooked on a convection conveyor oven (XLT 1823E-TS, Wolfe Electric, Inc. Wichita, KS) for 13-15 minutes to achieve a final internal temperature of 71º C. Cooked steaks were assigned a random number, placed in covered foil pans labeled with the random number, and kept warm until serving. Steaks were cut into 1 x 1 x 2.54 cm^3 cubes, placed in individual sample cups identified with the random number, and served to consumers. All samples were served warm and consumers received two cubes of each sample. Consumers recorded their opinions on a pre-labeled ballot with the corresponding sample number. All dietary TRT were represented in each sampling group.

Statistical Analysis

Carcass and meat quality data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute, Cary, NC, U.S.A.). The model included TRT as a fixed effect and the random effects of block and the block*TRT interaction. Color measurements were analyzed as repeated measures in time using compound symmetry as the variance-covariance error structure. LSMeans comparisons were made using Fisher's Least Significance Difference and a preplanned contrast statement was used to assess the effects of NE (All NE levels vs. CON).

Consumer panel responses were also analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute, Cary, NC, U.S.A.). The model included TRT as a fixed effect and the random effects of block and the block*TRT interaction. LSMeans comparisons were made using Fisher's Least Significance Difference and orthogonal contrast statements were used to determine linear and quadratic effects.

RESULTS

Carcass Characteristics

Steers fed NE had increased dressing percent ($P = 0.02$), decreased 12th rib backfat (**BF**; $P = 0.01$), and decreased ($P = 0.02$) calculated USDA yield grade compared to CON steers (Table 3.2). HCW, LM area, LM area/45.4 kg, marbling, price/45.4 kg, and total carcass value were not affected ($P \geq 0.21$) by TRT. However, steers fed 150 yielded carcasses worth nearly $30 more than CON steers due to improved HCW, DP, BF, and LM area.

Meat Quality Characteristics

L*, a*, and b* objective color values did not differ ($P \geq 0.13$) due to TRT on d 0 or 14 (Table 3.3). WBSF, drip loss, cook loss, percent moisture, and percent fat were not different across TRT ($P \geq 0.12$).

Consumer Sensory Panel

Consumer evaluation showed no difference ($P > 0.12$; Table 3.4) due to TRT for overall like, liking of tenderness, juiciness, and flavor and level of tenderness, juiciness, and flavor of LM steaks from steers fed NE.

DISCUSSION

At this point there have been few studies looking at the carcass traits of ruminants fed EO and even less examining the meat quality characteristics and sensory traits of steaks from those animals. Completed studies report results that are inconsistent and inconclusive.

NE inclusion increased DP (P = 0.02), with 300 steers dressing more than 2% greater than CON steers. In other studies, DP has been reported as greater and not different in animals fed EO. CIN fed to beef steers at 400 to 1,600 mg·hd^{-1}·d^{-1} tended to cause a linear increase in DP (Yang et al., 2010b). However, feeding a blend of thymol, eugenol, vanillin, guaiacol, and limonene EO had no effect on beef steer DP when fed at 1.0 g·hd^{-1}·d^{-1} (Meyer et al., 2009).

In the same study where CIN tended to increase DP, numerical increases in LMA and BF were also observed (Yang et al., 2010b). Similar results were found in this study as LMA and LMA/45.4 kg were numerically increased in all NE TRT compared to CON. However, their data contrasts our findings that increasing NE significant decrease (P = 0.02) in BF. In the current study, NE caused a decrease (P = 0.02) in YG due to NE steers having reduced BF, increased DP, and increased LM area compared to CON steers. This contrasts the findings of Meyer et al. (2009) who reported EO had no effect on YG.

Although price/45.4 kg did not differ (P > 0.27), steers fed 150 yielded carcasses worth greater than $30 more than CON due to improved HCW, DP, BF, LMA, and YG.

L*, a*, and b* objective color measurements on d 0 and 14 did not differ ($P \geq$ 0.13) across TRT. In objective color measurements, L* is a measurement of lightness where 0 = black and 100 = white, a* indicates redness and greenness, and b* values indicate yellowness and blueness. As a result, beef L* values aren't a major concern and are more critical in pork due to the occurrence of pale, soft, and exudative meat. In beef, a* values are considered the most important as desirable lean tissue is bright, cherry red in color. Observed differences were minimal and are not supported in the literature.

These differences would not be detectable to the average consumer and indicate NE had no negative effect on beef color.

WBSF, drip loss, cook loss, percent moisture, and percent fat were not affected by TRT ($P \geq 0.12$). These findings are similar to previous studies where pigs were fed a rosemary, garlic, oregano, or ginger EO or oleoresins mixture and no difference in WBSF, percent drip loss, or percent cook loss was observed in (Janz et al.,2007). NE had no negative effect on tenderness and all steaks were considered tender according to Shackelford et al. (1991) who suggested 4.6 kg (45.09 N) of force was considered the beef tenderness threshold.

The sensory panel reported no difference ($P > 0.12$) due to TRT for overall like, liking of tenderness, juiciness, and flavor and level of tenderness, juiciness, and flavor of LM steaks. NE had no negative effect on consumer perception, as responses below 6.0 are on the like side of the hedonic scale and finding no difference among TRT is considered a positive outcome. These findings are consistent with the lack of observed TRT effect on objective meat quality measurements like marbling score, WBSF, drip loss, cook loss, percent moisture, and percent fat. Previous research shows that EO have no effect on lamb and pork sensory evaluation (Chaves et al., 2008 a, b; Chaves et al., 2011; Yang et al., 2010). .

CONCLUSION

Dietary inclusion at 150 – 300 $mg \cdot hd^{-1} \cdot d^{-1}$ NE can improve beef steer DP, BF, calculated USDA yield grade, and overall carcass value without negatively influencing meat quality, organoleptic properties, or consumer opinion.

Table 3.1. Ingredient and nutrient composition of diets (DM basis) fed to feedlot steers.

Item	%
Ingredient, %	
Corn	60.2
Hay	8.1
DDGs	16.3
AminoPlus	8.0
Soyhulls	4.0
Limestone	2.2
Vitamins & Minerals	0.5
Analyzed Composition	
DM, %	88.0
OM, %	93.6
CP, %	15.4
NDF, %	24.3
ADF, %	10.3

Table 3.2. Carcass characteristics of beef steers fed Next Enhance®.

	Treatment					*P*-value	
Item	CON	150	300	600	SEM	Treatment	CON v. NE
HCW, kg	337	341	332	328	12.6	0.38	0.58
Dressing %	62.2[b]	63.2[ab]	64.3[a]	62.9[b]	0.57	0.03	0.02
BF, cm	1.44[a]	1.24[b]	1.12[b]	1.25[b]	0.107	0.05	0.01
LM area, cm^2	75.7	78.5	80.3	76.6	2.29	0.35	0.22
LM area/45.4 kg	1.66	1.73	1.77	1.69	0.050	0.35	0.22
Marbling[1]	545	559	535	518	26.7	0.58	0.76
Calculated USDA yield grade	3.43[a]	3.14[ab]	2.83[b]	3.12[ab]	0.188	0.04	0.02
Price, $/45.4 kg	196.77	198.40	198.96	197.33	1.15	0.50	0.27
Carcass Value, $	1,453	1,482	1,438	1,414	60.3	0.21	0.35

[1]400 = Small[0], 500 = Modest[0]

[a,b] Means in a rows that lack a common superscript differ ($P \leq 0.10$).

Table 3.3. Meat quality characteristics of LM steaks from beef steers fed Next Enhance® and harvested at the University of Missouri abattoir.

	Treatment					*P*-value	
Item	CON	150	300	600	SEM	Treatment	CON v. NE
Objective Color, d 0							
L*	45.07	45.22	44.62	42.88	0.851	0.13	0.39
a*	26.09	25.49	25.33	25.36	0.983	0.67	0.53
b*	11.31	11.09	10.96	10.66	0.471	0.63	0.42
Objective Color, d 14							
L*	45.28	45.39	46.24	43.33	0.851	0.13	0.76
a*	22.36	21.30	20.75	21.99	0.983	0.67	0.36
b*	10.18	9.73	9.86	9.64	0.471	0.63	0.40
WBSF, N	38.23	38.15	40.29	35.45	4.109	0.87	0.96
Drip Loss, %	1.64	1.40	1.14	1.30	0.288	0.68	0.31
Cook Loss, %	19.95	21.62	19.00	17.40	1.196	0.12	0.66
Moisture, %	71.28	71.34	68.93	71.06	1.374	0.57	0.61
Fat, %	5.58	6.59	6.23	6.01	1.005	0.91	0.56

[a,b] Means in a rows that lack a common superscript differ ($P < 0.05$).

Table 3.4. Consumer sensory evaluation of LM steaks from beef steers fed Next Enhance® and harvested at University of Missouri .[1]

	Treatment					*P*-value	
Item	CON	150	300	600	SEM	Treatment	CON v. NE
Overall Liking	4.58	4.43	3.88	4.18	0.510	0.78	0.49
Liking of							
Tenderness	4.11	4.04	4.21	4.19	0.421	0.99	0.94
Flavor	4.64	4.74	4.40	4.15	0.412	0.59	0.52
Juiciness	5.05	4.95	4.33	4.58	0.502	0.72	0.47
Level of							
Tenderness	4.47	4.15	4.31	4.59	0.450	0.91	0.82
Beef Flavor	4.75	4.51	3.86	4.30	0.408	0.47	0.28
Juiciness	5.46	4.92	4.27	5.06	0.557	0.51	0.29

[1]10 pt. hedonic scale, 1 = like extremely and 10 = dislike extremely

CHAPTER 4

NEXT ENHANCE® 300 FEEDING AFFECTS FERMENTATION CHARACTERISTICS OF RUMEN MICROBIOTA IN CONTINUOUS CULTURE

ABSTRACT

Ionophores and antibiotics are commonly used to promote growth and improve ruminant feed efficiency. However, the search to find alternative methods to promote growth has resulted in increased interest in plant extracts, like essential oils (**EO**). EO are naturally occurring, volatile oils with antimicrobial properties and can be distilled or extracted from most plants. Next Enhance® 300 (**NE**, Novus International Inc.) is a combination of extracts from garlic (diallyl disulfide) and cinnamon (cinnamaldehyde) that have demonstrated the ability to alter rumen fermentation. The objective of this study was to determine the effect of NE on fermentation characteristics of rumen microbiota in continuous culture (**CC**). Dietary treatment (**TRT**) consisted of 0, 15, 30, 60, 120, and 240 mg NE·kg^{-1} DM. Two CC runs were conducted using 24 single-flow CC fermenters. Fermenters were acclimated for four d and then sampled for three d. Effluent was collected daily and fermenters were sampled at 0, 4, 8, and 12 h post feeding. OM digestibility, CP degradation, microbial protein flow, and microbial efficiency all numerically increased in a quadratic manner with NE inclusion, but were not different due to TRT ($P \geq 0.26$). Fermenter pH was reduced ($P \leq 0.04$) in fermenters fed 15 compared to fermenters fed 120 at all measured time points. Fermenters fed 15 had the

greatest butyrate concentrations and acetate:propionate ratio at 0, 4, and 8 h post feeding. Ammonia, peptide, acetate, propionate, and total VFA concentrations did not differ ($P \geq 0.51$) due to TRT at any measured time point. NE inclusion at 15 – 120 mg·kg^{-1} DM increased nutrient digestibility, microbial N flow, and microbial efficiency.

INTRODUCTION

Efficiency in ruminants can be improved by manipulating rumen microbiota. This has historically been achieved by including antibiotics and ionophores in the diet. However, interest in using plant extracts, like essential oils (**EO**), has increased due to their potential to modify fermentation and be alternatives to commonly used antibiotics (Benchaar et al., 2008).

EO are secondary metabolites or volatile oils that can be distilled from most plants (Calsamiglia et al., 2007). EO possess antimicrobial properties effective against Gram-positive and Gram-negative bacteria allowing them to alter rumen fermentation (Helander et al., 1998; Dorman and Deans, 2000; Burt, 2004). Several *in vitro* experiments have been conducted to examine EO effects on rumen fermentation (Busquet et al., 2005 b,c; Cardozo et al., 2005; Castillejos et al., 2006). However, results of these studies are varied and inconclusive suggesting that EO effects depend largely on diet, concentration, and active ingredient.

Next Enhance® 300 (**NE**, Novus International Inc.) is a combination of EO extracted from garlic (diallyl disulfide) and cinnamon (cinnamaldehyde; **CIN**) that have been shown to modify rumen fermentation. CIN and garlic oil have been shown to

increase the proportion of propionate, decrease acetate proportion, and reduce ammonia N concentration (Busquet et al., 2004; Cardozo et al., 2005).

This CC fermentation experiment was conducted in conjunction with a live animal study where feeding 150 and 300 $mg \cdot hd^{-1} \cdot d^{-1}$ NE improved performance early in the finishing period and improved carcass measurements in beef steers. Therefore, the objective of this study was to evaluate rumen microbiota fermentation characteristics in continuous culture (**CC**) and determine if NE effects on ruminal fermentation could explain observed growth and carcass improvements.

MATERIALS AND METHODS

Continuous Culture

Two CC fermentation runs were conducted using 24 single-flow CC fermenter polycarbonate vessels (Nalgene, Rochester, NY). Fermenters were randomly assigned to TRT, resulting in four replicate fermenters per TRT. During each CC run fermenters were acclimated for four d followed by sample collection for three d.

Rumen fluid for each run was obtained from a fistulated lactating Holstein cow and a non-lactating Jersey cow housed in free stall facilities at the University of Missouri Foremost Dairy Research Center. Rumen fluid was transported to the Animal Science Research Center at the University of Missouri (approximately 10-15 min travel time), strained through four layers of cheese cloth, and diluted to a 4:1 ratio with buffer (McDougall, 1948).

Fermenters were inoculated and maintained as described by Meng et al. (1999). Inoculum was added to each fermenter to reach the effluent overflow port (approximately 1460 mL). Fermenters were continuously flushed with CO_2 gas, stirred, and submerged in a water bath maintained at 39° C by thermostatically controlled heaters (model 730, Fisher Scientific, Pittsburgh, PA). High capacity buffer solution modified by Slyter (1990) from McDougall's artificial saliva (McDougall, 1948), containing 107.5 mg urea-N/L and 250 mg cysteine-HCl/L was continuously added to fermenters via peristaltic pumps (Masterflex model 75210-10, Cole Parmer Instrument Co., Chicago, IL). Fermenter dilution rate was calibrated to 6% ± 0.2%·h^{-1} for all TRT. Effluent was collected in volumetric cylinders immersed in ice-cooled water.

Diets

Dietary treatments (**TRT**) consisted of 0 (**CON**), **15**, **30**, **60**, **120**, and **240** mg NE·kg^{-1} diet DM (Table 4.1). Corn, soyhulls, and corn gluten feed were ground to pass through a 2 mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA). Fermenters received half the daily ration (50 g) at 0800 h and 2000 h.

Sampling

Prior to feeding (0 h) effluent levels were recorded and approximately one-half of the effluent collected over the previous 24 h was taken as a subsample and stored at 4° C. Subsamples were composited for each fermenter during the collection period and stored until lyophilized. At 0, 4, 8, and 12 h post feeding fermenter pH was recorded. After pH was recorded at 0, 4, and 8 h a 10 mL sample was taken directly from each fermenter and immediately frozen at -20° C. For analysis, samples were composited by hour for each

fermenter. Fermenter contents were collected after the collection period ended and stored at 4° C for later analysis.

Laboratory Analysis

Fermenter samples were blended for 30 seconds to detach microbes from feed particles. Samples were then centrifuged at 1,000 x g for 5 min at 4°C to remove feed particles. Supernatant was re-centrifuged at 22,000 x g for 30 min. The resulting bacteria pellet was collected, lyophilized at -70 to -80°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar and pestle. Effluent subsamples (500 mL) were lyophilized at -70 to -80°C (Genesis, Virtis, Gardiner, NY) and ground using a mortar and pestle.

Diet, effluent, and bacteria samples were analyzed for DM, OM, and CP. Diet and effluent samples were also analyzed for NDF and ADF content. Effluent and bacteria samples were analyzed for purine content using the procedure described by Zinn and Owens (1986) to determine microbial N. Microbial N production was used with OM digested to determine microbial efficiency (**MOEFF**, g microbial N/kg OM truly digested). Ammonia and peptide concentrations (m*M*) were determined colormetrically (DU-65 spectrophotometer; Beckman, Brea, CA). Fermenter VFA and lactate concentrations (m*M*) were determined using gas chromatography (Model 3400, Varian, Palo Alto, CA).

Statistical Analysis

Data were analyzed as a randomized complete block design, where run was treated as block, using the MIXED procedure of SAS 9.3 (SAS Institute, Cary, NC, U.S.A.). The model included run and the run*TRT interaction as random effects and TRT

as a fixed effect. Ammonia, peptide, VFA concentrations, and pH were analyzed as repeated measures in time using compound symmetry as the variance-covariance error structure. LSMeans comparisons were made using Fisher's Least Significance Difference (LSD). Linear and quadratic effects were determined using orthogonal contrast statements.

RESULTS

The time*TRT interaction was not significant ($P > 0.50$) for any variable analyzed. All data are presented as TRT effect over both CC runs.

OM digestibility, CP degradation, microbial N production, and microbial efficiency all increased numerically in a quadratic manner, but did not differ due TRT ($P \geq 0.25$; Table 4.2). Microbial N production and efficiency were increased in all NE TRT compared to the CON. At 0, 4, and 8 h post feeding fermenter pH of 15 differed ($P < 0.05$) from 120.

Fermenters fed 15 had the greatest butyrate concentrations at all measured time points. At 0 h post feeding 15 fermenters had increased butyrate production compared to fermenters fed 240 ($P = 0.05$) and tended to have more than fermenters fed 60 and 120 ($P = 0.06$). At 4 ($P = 0.02$) and 8 h ($P = 0.07$) post feeding fermenters fed 15 had greater butyrate concentrations than CON fermenters.

Acetate:propionate (**AP**) at 0 h post feeding were reduced ($P \leq 0.03$) in CON and 30 and tended to be reduced ($P \leq 0.06$) for 60 and 120 when compared to fermenters fed 15. Fermenters fed 30 also tended ($P < 0.10$) to have decreased AP compared to fermenters fed 240. At 4 h post feeding, CON AP was less ($P < 0.02$) and 60 tended to be

less ($P < 0.08$) than fermenters fed 15. 8 h AP was reduced ($P < 0.05$) in CON and tended to be reduced for ($P \leq 0.10$) fed 30 and 60 when compared to 15.

Total VFA, acetate, ammonia, and peptide concentrations at 0, 4, and 8 h post feeding did not differ ($P > 0.15$) among TRT.

DISCUSSION

NE inclusion at 15 – 120 mg·kg^{-1} DM numerically increased OM and CP degradation by rumen microflora in a CC environment. As expected, the numerical increase in digestibility caused NE fermenters to have numerically greater microbial N flow. However, MOEFF was not statistically different, but this would be expected as dilution rate was held constant across all TRT. Minimal differences in pH, ammonia, and peptide concentrations were observed suggesting NE did not alter species dominating the fermentation environment.

NE influence on N degradation and OM digestibility are similar to observations by Yang et al. (2007) where feeding garlic oil at 5 g·hd^{-1}·d^{-1} increased OM and N rumen digestibility in cannulated dairy cows. However, our findings are contradictory to other research using *in vitro* fermentation methods where garlic oil and diallyl disulfide included in the diet at 31.2 or 312 mg/L resulted in no change in protein degradation compared to the control (Busquet et al., 2005b). The inconsistency may be due to the differences in EO dose (the low dose in the referenced study [31.2 mg/L] was nearly four times greater than the highest dose used in this study [8.2 mg/L]). The higher dose may result in increased antimicrobial activity that exceeds the favorable response threshold, decrease fermentation activity, and becomes detrimental to rumen fermentation.

Although pH of 15 differed from 120 at all measured time points and 60 at 12 h post feeding, the average difference was approximately 0.20 and was not interpreted to be biologically relevant.

Total VFA production did not differ ($P > 0.15$) at any measured time point. This observation was supported by previous research where garlic oil and CIN fed separately had no effect on total VFA concentration, (Busquet et al., 2005b; Cardozo et al., 2005).

Excluding 30 at 0 h post feeding, all NE fermenters had greater AP at 0, 4, and 8 h post feeding than CON fermenters. This is partially due to CON fermenters having numerically greater propionate levels at all measured time points when compared to fermenters fed NE. Data from previous research where EO decreased propionate proportions reported similar increases in AP ratio (Cardozo et al., 2005; Fraser et al., 2007).

Due to increased N degradation, we expected NE to cause increased ammonia and peptide concentrations in a similar manner. However, while concentrations increased numerically, no statistical differences were observed. NE did not affect ammonia, peptide, acetate, and propionate production which contradicts previous research using EO (Evans and Martin, 2000; Cardozo et al., 2004, 2005; Busquet et al., 2005a,b,c; Busquet et al., 2006; Fraser et al., 2007). The lack of TRT effect is likely due to the decreased experimental power. A power test was conducted on all dependent variables and results showed all variables tested had a power less than 0.66, with all but three being less than 0.35. Typical acceptable power values are 0.80 or above, indicating this experiment lacked experimental power and as a result differences between TRT may not have been

detected. The decreased power is likely due to the limited number of fermenters used to complete the experiment and the large variance between runs.

CONCLUSION

If ruminants were in a production scenario, we hypothesize NE could improve animal performance via increased microbial N production and efficiency. In a companion growth study steers fed 150 – 300 $mg \cdot hd^{-1} \cdot d^{-1}$, levels similar to those eliciting a response in this experiment, had improved ADG early in the feeding period. We concluded increased microbial N flow and MOEFF could contribute to improved calf performance early in the feeding period during lean tissue growth.

Thus, we determined NE inclusion at 15 – 120 $mg \cdot kg^{-1}$ alters rumen fermentation by increasing nutrient digestibility, microbial N flow from the rumen, and MOEFF. However, data from this experiment should be interpreted with caution as the test had low power and short term *in vitro* studies may not represent rumen microbiota adaption to EO dietary inclusion. The next step would be to determine the mechanism by which NE results in greater fermentation activity.

Table 4.1. Ingredient and nutrient composition of diet (DM basis) fed to continuous culture fermenters.

Item	%
Ingredient	
Corn	60.3
DDG	24.3
Soyhulls	8.0
Corn Gluten Feed	4.0
Lime	2.3
Vitamins & Minerals	1.1
Rumensin 90	0.03
Analyzed Composition	
DM	88.2
OM	93.5
CP	12.7
NDF	24.7
ADF	9.1

Table 4.2. Nutrient digestibility, microbial N production and efficiency, pH, and N fraction concentrations of continuous culture fermenters fed Next Enhance® at 0, 4, and 8 h post feeding.

	Treatment							*P*-value	
Item	CON	15	30	60	120	240	SEM	Treatment	CON v. NE
OM digestibility, %	54.5	54.8	58.0	57.7	56.0	54.1	2.12	0.69	0.51
CP degradation ,%	23.9	34.0	36.4	38.7	29.7	24.1	8.87	0.26	0.14
Micro N $g \cdot d^{-1}$	0.30	0.36	0.34	0.35	0.33	0.32	0.08	0.87	0.40
MOEFF[1]	11.7	13.6	12.5	13.2	11.9	12.6	3.42	0.95	0.57
pH									
0 h	6.34^{ab}	6.27^{b}	6.42^{ab}	6.42^{ab}	6.48^{a}	6.37^{ab}	0.220	0.07	0.53
4 h	6.19^{ab}	6.12^{b}	6.23^{ab}	6.29^{ab}	6.35^{a}	6.22^{ab}	0.220	0.07	0.55
8 h	6.23^{ab}	6.16^{b}	6.30^{ab}	6.30^{ab}	6.37^{a}	6.25^{ab}	0.220	0.07	0.54
12 h	6.36^{ab}	6.27^{b}	6.41^{ab}	6.45^{ab}	6.50^{a}	6.38^{ab}	0.220	0.07	0.59
Ammonia, m*M*									
0 h	2.4	3.2	2.8	3.1	3.2	2.6	1.26	0.62	0.31
4 h	1.5	2.3	1.9	2.2	1.9	1.7	1.26	0.62	0.34
8 h	1.3	1.8	1.5	2.0	1.7	1.7	1.26	0.62	0.40
Peptide, m*M*									
0 h	6.3	7.6	6.7	7.0	6.1	6.7	1.18	0.65	0.49
4 h	9.2	10.1	9.7	9.6	9.0	9.7	1.18	0.65	0.55
8 h	8.1	8.6	8.0	8.4	8.1	8.2	1.18	0.65	0.78

[1] MOEFF = Microbial efficiency (g microbial N/kg OM truly digested)

[abc] Means within a row that lack a common superscript differ ($P \leq 0.10$).

Table 4.3. VFA concentrations of continuous culture fermenters fed Next Enhance® at 0, 4, and 8 h post feeding.

	Treatment							*P*-value	
Item	CON	15	30	60	120	240	SEM	Treatment	CON v. NE
VFA, mol/100 mol									
Acetate									
0 h	40.56	42.98	36.99	38.23	38.65	40.37	2.947	0.51	0.74
4 h	49.57	47.41	44.13	43.99	44.55	47.36	3.108	0.51	0.23
8 h	44.87	48.91	43.30	43.63	44.35	47.76	2.947	0.51	0.83
Propionate									
0 h	26.57	22.85	26.11	24.77	25.13	23.12	8.088	0.66	0.44
4 h	32.26	24.51	27.32	26.72	26.40	26.20	8.117	0.66	0.05
8 h	28.29	25.46	27.86	26.55	25.98	26.47	8.088	0.66	0.51
Butyrate									
0 h	12.39^{ab}	14.75^{a}	12.50^{ab}	11.41^{b}	11.31^{b}	11.16^{b}	5.781	0.10	0.90
4 h	11.43^{b}	15.63^{a}	13.62^{ab}	12.28^{ab}	12.74^{ab}	12.37^{ab}	5.796	0.10	0.16
8 h	12.87^{b}	16.14^{a}	13.44^{ab}	12.26^{ab}	12.41^{ab}	12.43^{ab}	5.781	0.10	0.72
Total VFA, m*M*									
0 h	84.31	85.82	81.01	78.94	79.45	78.84	7.933	0.80	0.63
4 h	102.32	94.85	90.70	87.71	88.01	90.33	8.163	0.80	0.10
8 h	90.73	95.64	90.01	86.93	86.77	91.01	7.933	0.80	0.93
Acetate:Propionate									
0 h	1.64^{bc}	2.05^{a}	1.60^{c}	1.70^{bc}	1.70^{bc}	1.90^{b}	0.504	0.05	0.27
4 h	1.64^{b}	2.11^{a}	1.87^{ab}	1.79^{b}	1.91^{ab}	1.97^{ab}	0.507	0.05	0.05
8 h	1.76^{b}	2.14^{a}	1.83^{b}	1.84^{b}	1.90^{ab}	1.99^{ab}	0.504	0.05	0.20

^{abc} Means within a row that lack a common superscript differ ($P \leq 0.10$).

CHAPTER 5

EFFECTS OF NEXT ENHANCE® ON DIGESTIBILITY AND FERMENTATION CHARACTERISTICS OF RUMEN MICROBIOTA

ABSTRACT

Ionophores and antibiotics are commonly used to promote growth and improve ruminant feed efficiency. However, the search to find alternative methods to promote growth has resulted in increased interest in plant extracts, like essential oils (**EO**). EO are naturally occurring, volatile oils that have antimicrobial properties and can be distilled or extracted from most plants. Next Enhance® 400 (**NE**, Novus International Inc.) is a combination of extracts from garlic (diallyl disulfide) and cinnamon (cinnamaldehyde) that have demonstrated the ability to alter rumen fermentation. The experiment was designed as a 5 x 5 Latin square using 5 ruminally and duodenally cannulated Hereford steers with 5 treatments (**TRT**): 0 (**CON**), **7.5**, **15**, **27.5**, and **30** mg NE·kg^{-1} diet DM, and five 17 d experimental periods. DM and nutrient intakes were quadratically decreased ($P = 0.03$) by NE. True rumen OM digestibility was decreased ($P < 0.03$) in calves fed 7.5 when compared to calves fed 27.5. Rumen NDF digestibility decreased linearly ($P = 0.04$) due to NE inclusion. N digestibility in the rumen responded inversely and was linearly increased ($P < 0.01$) by NE. NDF total tract digestibility tended ($P = 0.09$) to decrease linearly, but OM and N digestibility were not affected ($P > 0.17$) by NE

inclusion. Liquid passage rate was quadratically increased ($P = 0.05$) by NE. Average pH and pH at all measured time points were unaffected by NE inclusion ($P > 0.15$). Total VFA ($P = 0.06$) and acetate production ($P = 0.05$) decreased quadratically in response to NE. TRT effects on microbial N production, microbial efficiency (g microbial N/kg OM truly digested), acetate:propionate, and ammonia, peptide, propionate, and butyrate concentrations were not significantly ($P > 0.15$) different among TRT. Due to incorrect duodenal cannula placement and subsequent sampling challenges we do not believe the values observed for rumen digestibility, microbial N production, and MOEFF are correct. However, since these issues were consistent across animal, period, and TRT relative differences observed among TRT should be true. NE inclusion at 15 – 30 $mg \cdot kg^{-1}$ increased N degradation and decreased NDF digestibility in the rumen, while all NE levels increased microbial N production.

INTRODUCTION

Antibiotics and ionophores are commonly used to promote growth and efficiency in feedlot animals. However, interest in using plant extracts, like essential oils (EO), has increased due to their potential to modify rumen fermentation and serve as an alternative to the antibiotics commonly used today (Benchaar et al., 2008a). EO are secondary metabolites or volatile oils that can be distilled from many plants (Calsamiglia et al., 2007). EO possess antimicrobial properties that are effective against both Gram-negative and Gram-positive bacteria suggesting they can inhibit rumen bacterial growth and modify fermentation (Helander et al., 1998; Dorman and Deans, 2000; Burt, 2004).

Several studies have been conducted using dairy cattle or *in vitro* fermentation methods to determine EO effects on rumen microbial fermentation and animal performance (Busquet et al., 2005 b,c; Cardozo et al., 2005; Castillejos et al., 2006; Yang et al., 2007). However, limited information exists on the fermentation characteristics of cattle fed high starch, feedlot type diets when EO are included.

Next Enhance® 400 (**NE**, Novus International, Inc.) is a combination of EO extracted from garlic (diallyl disulfide) and cinnamon (cinnamaldehyde ; **CIN**), both of which have been shown to modify rumen fermentation. NE is similar to Next Enhance® 300 that was used in previous studies except CIN and diallyl disulfide concentrations are increased from 150 $mg \cdot lb^{-1}$ to 200 $mg \cdot lb^{-1}$.

Busquet et al. (2004) reported CIN increased the proportion of propionate and reduced ammonia N concentration while garlic oil (**GAR**) increased propionate and butyrate proportions and decreased acetate production compared to the control. CIN is thought to work by increasing bacteria cell membrane permeability through protein denaturation and causing cell constituents to coagulate (Juven et al., 1994; Gustafson and Bowen, 1997). Garlic oil may function by inhibiting production of cholesterol and other isoprenoids responsible for membrane stability (Gebhart and Beck, 1996; Busquet et al., 2005b) or by its ability to interact with sulfhydryl groups found in other active compounds (Reuter et al., 1996; Ross et al., 2001; Busquet et al., 2005b). Burt (2004) proposed combining EO with different modes of action may result in synergistic effects that could further improve rumen fermentation, suggesting that feeding CIN and garlic oil in combination could further improve animal performance. Therefore, this study's

objective was to evaluate rumen fermentation characteristics *in vivo* and determine if NE effects on fermentation and digestion could explain observed growth improvements.

MATERIALS AND METHODS

All surgical and experimental procedures were approved by the University of Missouri Animal Care and Use Committee.

Experimental Design

Five Hereford steers (548 ± 25.6 kg) were surgically fitted with rumen fistulas and duodenal T-type cannulas and utilized in 5x5 Latin square design. For each period steers were randomly assigned to treatment (**TRT**). Dietary TRT included: 0 (**CON**), **7.5**, **15**, **27.5**, and **30** $mg \cdot kg^{-1}$ diet DM NE. Each experimental period lasted 17 d with 14 d for acclimation to TRT and 3 d for sampling. Steers were housed in a bedded concrete pen, completely covered by roof at the University of Missouri Beef Research and Teaching Farm. Steers were allowed free movement except during feeding and sampling. During the 72 h sampling period, steers were restricted to individual stanchions, bedded with rubber mats, and given unlimited access to fresh water.

Diets & Markers

Steers were weighed prior to project initiation and the diet (Table 5.1) was formulated to meet or exceed NRC (2000) nutrient requirements. All ingredients, excluding hay, were mixed at the University of Missouri Feed Mill, bagged, and stored in a feed room protected from weather. Steers were offered 1.8% of their initial BW on a DM basis daily and daily feed offering was held constant for project duration. Diets were

prepared twice daily, fed at approximately 0700 and 1900, and feed refusals were recorded.

During each morning feeding, a titanium bolus (10 g) was inserted in the rumen to determine digesta flow. On d 15 after 0 h sample collection and prior to feeding, cobalt-EDTA solution (200 mL) was introduced into the rumen to allow liquid passage rate determination (Gaylean, 1987). Steers were fed immediately following 0 h sample collection and marker introduction.

Sample Collection

Random feed samples were collected, dried in a 55°C drying oven, and ground to pass through 2mm screen in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) for proximate analysis.

During the 3 d sampling period, sampling occurred at 0, 2, 4, 8, 12, 18, 24, 30, 36, 48, and 72 h after marker introduction to the rumen. At each time point whole rumen contents (**WRC**; approximately 450 mL) were collected in a 16 oz. cup and rumen fluid pH was determined. After pH was determined, WRC were strained through four layers of cheesecloth, the fluid portion was poured into 3, 15 mL centrifuge tubes, and the solids fraction was placed back in the sample cup. Both liquid and solid fractions were immediately frozen for later analysis. An additional 450 mL of WRC were collected at 0, 12, 24, 36, 48, and 72 h and frozen to be later thawed and composited by steer within period. At each sampling point, duodenal digesta (approximately 200) was collected into a whirl-pack bag by attaching the bag to a piece of PVC pipe that was placed in the open duodenal cannula and diverted digesta flow into the sample bag. Samples were

immediately frozen until analysis upon completion of the experiment. Fecal grab samples were collected at 12, 24, and 36 h and immediately frozen.

Laboratory Analysis

WRC were thawed to room temperature, composited by steer within period, mixed with approximately 500 mL DI water, and blended using a commercial blender for approximately 30 seconds to detach bacteria from feed particles (Fu et al., 2000). Blended contents were strained through four layers of cheesecloth and differential centrifugation was used to isolate rumen bacteria. Bacterial samples were then lyophilized at -70 to -80°C (Genesis, Virtis, Gardiner, NY) and ground using a mortar and pestle. Duodenal samples were combined by steer within period, a 750 mL subsample was lyophilized at -70 to -80°C (Genesis, Virtis, Gardiner, NY), and ground using a mortar pestle. Fecal samples were dried at 55°C, ground to pass through a 2 mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA), and composited by steer within period. Feed, rumen bacteria, duodenal, and fecal samples were analyzed for DM, OM, and N content. Feed, duodenal, and fecal samples were also analyzed for NDF and ADF content. Rumen bacteria and duodenal purine contents were determined as described by Zinn and Owens (1986). Composited duodenal and fecal samples were digested using the Kjeldahl method. Sample titanium content was determined colormetrically and used to calculate digesta flow from the rumen and fecal output (DU-65 spectrophotometer; Beckman, Brea, CA).

One 15 mL centrifuge tube of strained rumen fluid was thawed and centrifuged at 10,000 x g for 20 minutes. Three mL subsamples were taken from each sample time point

and composited by steer within period for rumen VFA, ammonia, and peptide analysis. Ammonia and peptide concentrations (m*M*) were determined colormetrically (DU-65 spectrophotometer; Beckman, Brea, CA). VFA and lactate concentrations (m*M*) were determined using gas chromatography (Model 3400, Varian, Palo Alto, CA). A second 15 mL tube was thawed and centrifuged at 10,000 x g for 20 minutes; supernatant was collected from each time point, and analyzed for cobalt concentration using atomic absorption spectroscopy with air-plus-acetylene flame according to Hart and Polan (1984). Cobalt concentrations were regressed to the natural logarithm after dosing time and the slope was described as ruminal passage rate.

Statistical Analysis

Data were analyzed as a 5 x 5 Latin square using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The model included animal and period as random effects and TRT as a fixed effect. pH was analyzed as repeated measures in time using compound symmetry as the variance-covariance error structure. LSMeans comparisons were made using Fisher's Least Significance Difference (LSD). Linear and quadratic effects and CON v. NE differences were determined using the CONTRAST function of SAS.

RESULTS

DM, OM, NDF, and N intake (Table 5.2) decreased quadratically ($P \leq 0.03$) due to NE inclusion.

True rumen OM digestibility was decreased ($P < 0.03$) in 7.5 when compared to 27.5. Rumen NDF digestibility decreased linearly ($P = 0.04$) due to NE inclusion. N digestibility in the rumen was linearly increased ($P < 0.01$) by NE.

NDF total tract digestibility tended ($P = 0.09$) to decrease linearly, but OM and N digestibility were not affected ($P > 0.17$) by NE inclusion.

Liquid passage rate was quadratically increased ($P = 0.05$) by NE. TRT effect on microbial N production, microbial efficiency (**MOEFF**, g microbial N/kg OM truly digested), and ammonia and peptide concentrations were not significant ($P > 0.15$).

Average pH (Figure 5.1) and pH at all measured time points were unaffected by NE inclusion ($P > 0.15$).

Total VFA ($P = 0.06$) and acetate production ($P = 0.05$) decreased quadratically in response to NE. Propionate and butyrate production and acetate:propionate (**AP**) were unaffected ($P > 0.15$) by NE dietary inclusion.

DISCUSSION

The reason DM and nutrient intake decreased quadratically due to NE is unclear. It contradicts several previous studies where EO had no effect on intake (Benchaar et al., 2006; Yang et al., 2007; Chaves et al., 2008a,b; Meyer et al., 2009; Chaves et al., 2011; Klevenhusen et al., 2011). It also conflicts Yang et al. (2010b) findings where beef steer DMI was quadratically increased by CIN inclusion at 0, 400, 800, or 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$, suggesting intake may be stimulated when EO are included at low levels (Yang et al., 2010b).

Due to incorrect duodenal cannula placement and subsequent sampling challenges we do not believe the absolute values observed for rumen digestibility, microbial N production, and MOEFF are correct. However, since these issues were consistent across animal, period, and TRT relative differences observed among TRT should be true.

The reason true rumen OM digestibility was decreased in 7.5 compared to 27.5 is unclear and contradicts the findings of Yang et al. (2010a) where true rumen OM digestibility was increased by low doses and decreased with high inclusion rates. Yang et al. (2007) also reported increased rumen OM digestibility when garlic oil (**GAR**) and juniper berry extracts were included in dairy cow diets.

Due to their antimicrobial properties, EO inclusion can result in decreased fermentation activity when included at higher doses and may explain why rumen NDF digestibility as decreased when 30 was fed. Yang et al. (2010a) reported rumen NDF digestibility linearly decreased in cannulated heifers fed low fiber diets with CIN inclusion at 0, 400, 800, and 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$, with 1,600 causing a greater than 12% reduction in digestibility. NDF digestibility was also decreased *in vitro* by GAR and diallyl disulfide inclusion (Busquet et al., 2005b).

NE influence on rumen N degradation is similar to results from a companion continuous culture study where Next Enhance 300® linearly increased N degradation when included at 0-60 $mg \cdot kg^{-1}$. Yang et al. (2007) also reported GAR and juniper berry extract increased N degradation in dairy cows fed high fiber diets. However, these results contradict other studies where low fiber diets were fed and CIN had no effect (Benchaar et al., 2008a) or linearly decreased rumen N digestibility (Yang et al., 2010a). GAR and

diallyl disulfide fed at 312 $mg \cdot L^{-1}$ also decreased protein degradation *in vitro*, while low levels had no effect (Busquet et al., 2005b). These inconsistencies maybe due to differences in dose, as levels fed in the current experiment were less than those used in the other mentioned studies and inclusion did not becomes detrimental to N digestibility.

Previous research shows OM, NDF, and N total tract digestibility are largely unaffected by CIN and GAR inclusion (Yang et al., 2007; Benchaar et al., 2008a,b; Yang et al., 2010a; Klevenhusen et al., 2011). Similarly, OM and N total tract digestibility in the current study were not affected by NE inclusion. However, NDF total tract digestibility tended to decrease linearly due to decreased rumen NDF digestibility. This is consistent with Yang et al. (2010) finding that NDF total tract digestibility decreased linearly in heifers fed 0, 400, 800, and 1,600 $mg \cdot hd^{-1} \cdot d^{-1}$ CIN and was reduced by over 10% in heifers fed 1,600.

Microbial N production was numerically improved by NE, but due to observed differences in digestibility and passage rate, MOEFF did not differ between TRT. Yang et al. (2010a) reported similar results in beef heifers where microbial N production was increased but MOEFF was not influenced by CIN inclusion. MOEFF was also not affected by GAR or diallyl disulfide (Busquet et al., 2005b) or blended EO (Castillejos et al., 2007) inclusion *in vitro*.

The reason total VFA and acetate production decreased quadratically is unclear as this does not follow observed trends in the nutrient digestibility; feeding 15 resulted in the greatest rumen NDF digestibility but the lowest acetate production. These findings also contradict much available research where total VFA production was not affected by

garlic or cinnamon extract inclusion (Cardozo et al., 2004; Yang et al., 2007; Chaves et al., 2008b; Klevenhusen et al., 2011). Other studies reported garlic and cinnamon extracts decreased total VFA (Busquet et al., 2005b; Chaves et al., 2011) and acetate production (Busquet et al., 2005a,b,c; Cardozo et al., 2005; Busquet et al., 2006). However, this does not explain the quadratic response observed in the current study.

Minimal observed differences in pH, ammonia, and peptide concentrations suggest NE did not alter species dominating the fermentation environment or rumen microbiota may become adapted to EO inclusion during longer duration experiments.

CONCLUSION

We determined NE inclusion at 15 – 30 $mg \cdot kg^{-1}$ increased N degradation and decreased NDF digestibility in the rumen, while all NE levels increased microbial N production. However, before final conclusions can be drawn about NE effects on rumen fermentation and digestion this experiment should be repeated using animals with correctly placed duodenal cannulas to see if discrepancies in digestibility data can be resolved.

Table 5.1. Ingredients and nutrient composition of diet (DM basis) fed to cannulated steers.

Item	Amount
Ingredient, %	
Ground Corn	70.5
DDGs	15.1
Alfalfa Hay	8.0
AminoPlus	3.5
Vitamins & Minerals	2.9
Analyzed Composition	
DM, %	90.5
OM, %	94.5
CP, %	15.4
NDF, %	17.8
ADF, %	30.6

Table 5.2. Intake, nutrient digestibility, microbial N production and efficiency, and N fraction concentrations in cannulated beef steers fed Next Enhance®.

	Treatment						*P*-value	
Item	CON	7.5	15	27.5	30	SEM	Linear	Quadratic
Intake, kg/d								
DM	9.77[ab]	9.61[b]	9.61[b]	9.82[a]	9.90[a]	0.292	0.08	0.03
OM	9.29[a]	9.08[b]	9.10[b]	9.25[ab]	9.31[a]	0.275	0.40	0.03
NDF	1.71[b]	1.72[b]	1.81[a]	1.69[b]	1.72[b]	0.052	0.62	0.01
N	1.55[a]	1.46[c]	1.48[bc]	1.49[b]	1.53[a]	0.045	0.95	<0.001
Digestibility, %								
Rumen								
OM (true)	73.2[ab]	64.6[b]	72.4[ab]	79.5[a]	76.4[ab]	5.40	0.17	0.55
NDF	40.7[a]	30.6[ab]	43.5[a]	32.2[ab]	22.2[b]	5.91	0.04	0.23
N	81.3[b]	67.3[b]	87.3[b]	95.3[b]	129.6[a]	11.86	<0.01	0.07
Total								
OM	77.0	76.9	77.3	81.9	78.2	2.40	0.36	0.76
NDF	87.2	86.8	86.7	84.9	85.5	0.99	0.09	0.95
N	71.5	71.2	72.5	74.9	72.7	3.15	0.50	0.79
Liquid Passage Rate, %/h	3.22[b]	3.55[ab]	4.23[a]	3.59[ab]	3.44[b]	0.312	0.58	0.05
Microbial N, g/d	347.0	363.5	352.1	376.3	397.3	55.01	0.36	0.75
MOEFF[1]	51.0	50.4	54.9	44.9	60.5	5.99	0.33	0.27
Ammonia N, m*M*	19.7	18.1	20.6	20.3	18.2	2.40	0.89	0.59
Peptide, m*M*	19.9	20.9	19.6	18.6	19.2	1.17	0.16	0.91
VFA, m*M*								
Acetate	76.8	76.5	65.7	67.4	75.5	4.07	0.33	0.05
Propionate	47.6	45.8	42.4	42.6	47.9	7.79	0.89	0.42
Butyrate	17.9	21.1	18.6	16.3	18.3	2.43	0.58	0.78
Total VFA	157.5	156.5	137.9	138.5	155.5	8.58	0.35	0.06
Acetate:Propionate	1.80	2.06	1.77	1.81	1.76	0.408	0.77	0.83

[ab] Means within a row that lack a common superscript differ ($P \leq 0.10$).

[1] MOEFF = Microbial efficiency (g microbial N/kg OM truly digested)

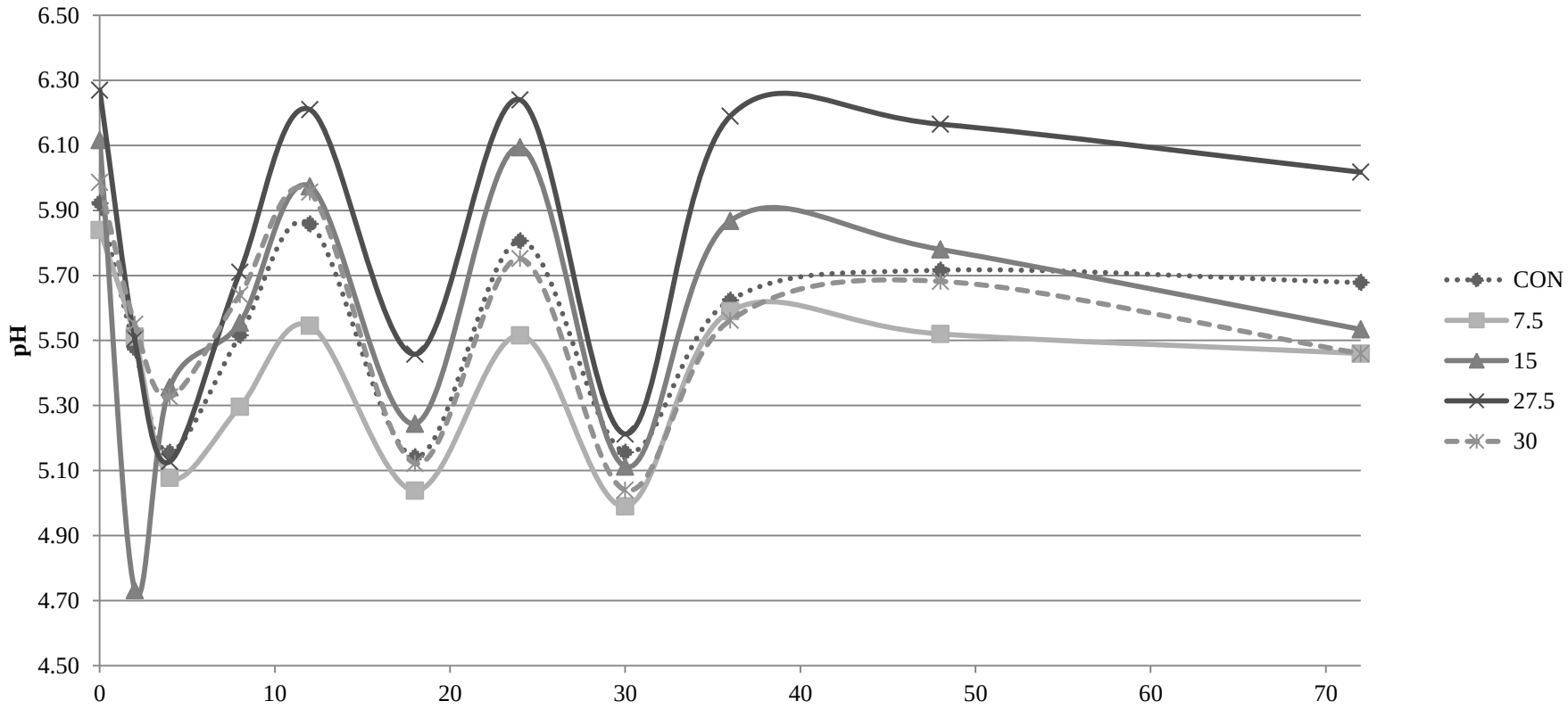

Figure 5.1 Rumen pH of cannulated beef steers fed Next Enhance® measured between just prior to (0 h) and 72 h after marker introduction.

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