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AN EVALUATION OF FEEDING A BLEND OF ESSENTIAL OILS AND COBALT LACTATE TO LACTATING DAIRY COWS

BY

OLIVIA JAYNE KUESTER

A thesis submitted in partial fulfillment of the requirements for the

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AN EVALUATION OF FEEDING A BLEND OF ESSENTIAL OILS AND COBALT LACTATE TO LACTATING DAIRY COWS

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

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ABBREVIATIONS

AA	Amino Acid
ADF	Acid Detergent Fiber
ADP	Adenosine Diphosphate
AIA	Acid Insoluble Ash
ATP	Adenosine Triphosphate
ADICP	Acid Detergent Insoluble Crude Protein
BCS	Body Condition Score
rBST	Recombinant Bovine Somatotropin
CBL	Cobalamin (Vitamin B_{12})
CP	Crude Protein
DHIA	Dairy Herd Improvement Association
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
ECM	Energy Corrected Milk
EO	Essential Oil(s)
EOC	Essential Oils and Cobalt Lactate Blend
FCM	Fat Corrected Milk
FE	Feed Efficiency
GMOs	Genetically Modified Organisms
GN	Gram Negative Bacteria
GP	Gram Positive Bacteria

IVDMD	In Vitro Dry Matter Digestibility
MIC	Minimum Inhibitory Concentration
MUN	Milk Urea Nitrogen
MUT	Methylmalonyl Coenzyme-A Mutase
ND	Nutrient Digestibility
NDF	Neutral Detergent Fiber
NDFD	Neutral Detergent Fiber Digestibility
NDIP	Neutral Detergent Insoluble Protein
NE_L	Net Energy of Lactation
NFC	Non Fiber Carbohydrate
NRC	Nutrient Requirements of Dairy Cattle
RFQ	Relative Forage Quality
SCC	Somatic Cell Count
SP	Soluble Protein
TMR	Total Mixed Ration
T4C	Time for Cows – Lely Robotic Milking Software
VFA	Volatile Fatty Acid

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ABSTRACT

AN EVALUATION OF FEEDING A BLEND OF ESSENTIAL OILS AND COBALT LACTATE TO LACTATING DAIRY COWS

OLIVIA JAYNE KUESTER

2016

The objective of this study was to determine if the inclusion of a blend of essential oils and cobalt lactate (**EOC**) in the diet of lactating dairy cows could result in improved nutrient utilization and lactational performance in addition to assessing the application of EOC to control feed mycotoxins and spoilage. The trial was conducted in two experimental periods (Phase 1 (**P1**) and Phase 2 (**P2**)) on a commercial dairy in southwest Minnesota equipped with two robotic milking units. Phases 1 and 2 were cross over design trials with two pens. Cows were housed in two freestall pens (57 ± 2 cows and 59 ± 3 cows for treatment (EOC) and Control pens, respectively) and were evaluated for cow parity (2.65 ± 1.52 and 2.33 ± 1.20), days in milk (**DIM**) (184 ± 103 and 154 ± 94.2), and milk production (35.4 ± 11.3 kg/d and 36.9 ± 11.3 kg/d) prior to study initiation and assignment to EOC or Control treatments. Each Phase included 14 d for dietary adaptation followed by 9 wk for data collection. Cows were fed a total mixed ration (**TMR**) with no treatment (Control) or 0.23 kg/hd/d of a soy hull carrier containing a proprietary blend of EOC to be fed at a rate of 28 g/hd/d. Daily milk production, management level milk (**MLM**), 3.5% fat corrected milk (**FCM**), and energy corrected milk (**ECM**) were similar ($P > 0.10$) for cows fed EOC and Control. Milk fat percentage and yield (kg) and protein percentage and yield (kg) were similar ($P > 0.10$) for cows fed EOC and Control. Milk fat percentage and yield (kg) measured by the Dairy Herd

Improvement Association (**DHIA**) were significantly greater ($P < 0.05$) for cows fed EOC compared to cows fed Control for Phases 2 and 1 respectively. Both protein and lactose percentage and yield (kg) measured by DHIA were similar ($P > 0.10$) for cows fed EOC and Control. Milk urea nitrogen (**MUN**) was significantly lower ($P < 0.05$) for cows fed EOC than cows fed Control during P1 ($P < 0.01$), but was similar during P2 ($P > 0.10$). Somatic cell counts (**SCC**) were similar ($P > 0.10$) for cows receiving EOC and Control diets. Dry matter intake (**DMI**) was greater ($P < 0.05$) for cows fed EOC compared to cows fed Control during P1 but was similar ($P > 0.10$) during P2. Body weights were greater ($P < 0.01$) for cows fed EOC compared to cows fed Control during P1, however the opposite was true for P2 during which body weights were lower ($P < 0.01$) for cows fed EOC compared to cows fed Control. Body condition scores (**BCS**) were similar ($P > 0.10$) for cows receiving EOC and Control diets during both Phases. Body surface temperatures were similar ($P > 0.10$) between pens during both Phases. When evaluating dry matter (**DM**), crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**), and starch digestibility percentages during each phase, there were no differences between cows fed EOC and cows fed Control ($P > 0.10$), however a numeric advantage for cows fed EOC was observed. Mycotoxins detected in TMR composites from P1 and P2 were similar ($P > 0.10$). The time until a 2°C rise in temperature (aerobic stability of TMR) was numerically greater, but was not significant ($P > 0.10$) for TMR containing EOC compared to untreated TMR. Fecal composites from cows receiving EOC were lower ($P < 0.05$) in normal microbiota compared to cows receiving Control diet. *Streptococcus* sp. were lower ($P < 0.05$) during P1 for cows receiving EOC compared to cows receiving Control diet, but the measurement of

Streptococcus sp. was similar ($P > 0.10$) during P2. Feeding EOC to lactating dairy cows appears to influence milk composition and nutrient digestibility, and may improve the aerobic stability of feed at the bunk.

Keywords: essential oil, cobalt lactate, robotic milker, milk composition, nutrient digestibility

INTRODUCTION

Achieving global food security is an imminent concern as global populations continue to rise. Due to limitations in land and water, gains in milk production must occur due to improved animal productivity and efficient utilization of nutrients. Advances to improve productive efficiency have been met with increasing public scrutiny due to the perception that these technologies are a threat to human and animal health. Many government entities have implemented policies to phase out the indiscriminant use of many xenobiotic agents in animal production. Thus, producers are met with the challenge of increasing animal productivity with fewer technological resources. In response, ruminant microbiologists and nutritionists have been investigating alternative ways to favorably alter ruminal metabolism to improve feed efficiency and animal productivity. Plant extracts such as essential oils and cobalt lactate are two avenues by which producers may continue to achieve a high level of production without the use of "black listed" technologies such as antimicrobial growth promotants. Essential oils are secondary plant metabolites that demonstrate a broad spectrum of antimicrobial properties, particularly against gram positive bacteria. Research has indicated that these compounds can alter milk composition, however a variety of feeding responses has been observed and no optimum dose has been elucidated for dairy cows. Cobalt is an essential trace mineral in ruminant nutrition, as rumen microbes utilize the element to synthesize vitamin B_{12}, a compound that plays a major role in energy metabolism and red blood cell formation. Studies have reported that increasing the supply of dietary cobalt results in an increased amount of vitamin B_{12} synthesis, and appears to influence rumen parameters to the effect of increasing ruminal fiber digestibility and altering volatile fatty acid ratios.

The objective of our study was to determine if an inclusion of a blend of essential oils and cobalt lactate in the diet of lactating dairy cows in a commercial setting could result in improved nutrient utilization and production parameters.

LITERATURE REVIEW

Introduction

With current projections estimating a world population of over 9 billion by the year 2050, achieving global food security has become one of the greatest challenges faced by food production industries (Bauman and Capper, 2011). Dairy products in particular are regarded as the most economical source of limiting essential nutrients, and are included in the dietary recommendations set by many government and public health organizations (Erasmus and Webb, 2013). Due to limitations in land, water, and other resources, 80% of additional food supplies must come about through improved productivity (Godfray et al., 2010). Gains in milk production per cow over the last six decades reflect advances in understanding the biology of dairy cows, and this knowledge has been applied to new technologies which have helped support a high level of milk production and improved productive efficiency (or milk output per unit of resource input) (Bauman and Capper, 2011). Increasingly, these advancements are met with consumer concerns regarding the quality and safety of animal food products (Bauman and Capper, 2011).

Despite assurances from reputable sources, consumers often perceive the use of technology in agriculture (such as genetic modification, antibiotics, and hormone use) as threats to human and animal health (Erasmus and Webb, 2013). These concerns have developed a niche in marketing for absence-free labeling, which include claims regarding

the practice of organic farming and products free of genetically modified organisms (**GMOs**), antibiotics, and recombinant bovine somatotropin (rBST). Thus, in addition to sustainable intensification of production, producers are faced with the challenge of meeting an increased demand for food that is produced with fewer synthetic inputs (Erasmus and Webb, 2013).

The use of antimicrobials (such as ionophores) as growth promotants in food animals has been under intense scrutiny in recent years due to concerns regarding the development of resistance to antibiotics used in animal medicine and the development and spread of cross-resistance to entire classes of antibiotics (Callaway et al., 2003). These compounds are administered in the feed at low doses and are believed to have beneficial effects on productivity by altering ruminal microbiota in a way which changes the proportions of volatile fatty acids (**VFA**) produced during ruminal digestion (Reinhardt, 2013). In 2006, the European Union banned the use of antibiotics (including ionophores, which are regarded as unnatural chemical feed antibiotics) as animal growth promotants (Erasmus and Webb, 2013). In 2007, the American Medical Association passed a resolution to "oppose the use of antimicrobials at non-therapeutic levels in agriculture", and in 2013, the Food and Drug Administration (**FDA**) implemented a policy to phase out the indiscriminate use of antibiotics in cows, pigs, and chickens (Greathead, 2003, Tavernise, 2013)

In response to increased regulations and consumer perceptions, ruminant microbiologists and nutritionists have been exploring ways to favorably alter ruminal metabolism to improve feed efficiency (**FE**) and animal productivity (Benchaar et al., 2008). Plant extracts such as essential oils (**EO**) and the compound cobalt lactate are two

alternatives to xenobiotic feed additives. The objective of this literature review is to support the rational for the inclusion of a blend of these compounds (EOC) in the TMR of dairy cows by evaluating research on the mode of action EO and CL in the rumen environment as well as their impact on production and the overall health status of the animal.

CHAPTER 1:

ESSENTIAL OILS

Characteristics and the Mode of Action of Essential Oils

Essential oils are naturally occurring plant components and are considered safe for human and animal consumption (FDA, 2004, Benchaar et al., 2008). They impart the characteristic essences and fragrances (or *quinta essentia*) to plants. Plants and their extracts have had important roles in human health and wellness since early history, exploited for their flavor, essences, and preservative properties (Benchaar et al., 2008). Many EO have exhibited antifungal, antiviral, bactericidal, and bacteriostatic effects on microorganisms (bacteria, fungi, viruses, and protozoa) at minimum inhibitory concentrations (**MIC**) as low as 0.05 μL mL^{-1} in vitro (Burt, 2004). Essential oils are most commonly acquired through steam distillation or solvent extraction, and can be harvested from multiple parts of plants including the leaves, flowers, stem, seeds, roots, and bark (Greathead, 2003, Benchaar et al., 2008). They are not true oils, but variable mixtures of non-nutrient bioactive plant compounds, or secondary metabolites (Benchaar et al., 2008).

Secondary metabolites differ from primary metabolites (such as carbohydrates, proteins, fats, and nucleic acids) in that their distribution is limited and they are often produced only by particular plants or groups of plants (Greathead, 2003). It is widely believed that only a small percentage of terrestrial species of plants have been investigated for the presence of secondary metabolites (Benchaar et al., 2008). The high diversity of secondary metabolites is thought to promote plant survival, primarily by protecting the plant from pathogens in addition to decreasing the chance of pathogens developing resistance or adaptive responses (Briskin, 2000, Greathead, 2003). The secondary metabolites of EO are principally terpenoids and a variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and N- and S-containing compounds, coumarins, and phenylpropanoids (e.g. lignans, aromatic essential oils, and coumarins) (Benchaar et al., 2008).

Antimicrobial activities of EO have been demonstrated against a wide variety of gram positive (**GP**) and gram negative (**GN**) organisms (Benchaar et al., 2008). The research of Smith-Palmer et al. (1998) determined that out of their EO test articles, the oils of bay, cinnamon, clove, and thyme exhibited the greatest inhibitory effects with bacteriostatic activity at a concentration of 0.075% or less against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enteritidis*, and *Campylobacter jejuni*. However, GN organisms in this study (and others) were slightly less susceptible to EO than GP bacteria (Smith-Palmer et al., 1998, Burt, 2004). Bacteriostatic and bactericidal concentrations were found to be lower (0.02 to 0.075%) for GP bacteria (*S. aureus* and *L. monocytogenes*) and higher (equal to or in excess of 1%) for GN bacteria (*E. coli, S. enteritidis*, and *C. jejuni*) (Smith-Palmer et al., 1998).

The increased resilience of GN bacteria to EO is believed to be related to unique GN outer membrane properties. Gram negative bacteria possess an outer membrane surrounding their cell wall that acts as a permeability barrier which limits the access of hydrophobic compounds, however certain phenolic compounds such as thymol (thyme) and caravacrol (oregano) are capable of inhibiting growth of GN bacteria through cell wall disruption (Benchaar et al., 2008). The mechanism by which most essential oils are thought to exert their antibacterial effects is by disrupting cell wall structures due to their lipophilic character. The chemical components of EO interrupt electron transport, ion gradients, protein translocation, phosphorylation steps, and other enzyme-dependent steps, causing the bacterium to lose chemi-osmotic control (Ultee and Kets, 1999, Cox et al., 2000, Dorman and Deans, 2000).

The spectrum and magnitude of antimicrobial activities of EO are often dependent on a number of factors including their respective compositions, structural configuration of constituent components and functional groups, and possible synergistic interactions between components (Dorman and Deans, 2000). For example, due to the presence of a hydroxyl group, phenolic compounds are thought to be highly effective against a variety of both GP and GN bacteria; however, Delaquis et al. (2002) observed that several cilantro fractions deficient in phenolic compounds exhibited strong antimicrobial activity. While the importance of the hydroxyl group on phenolic compounds has been validated (for example, when carvacrol was compared to its methyl ester), the relative position of the hydroxyl group is believed to influence the components effectiveness (Dorman and Deans, 2000). Additionally, Delaquis et al. (2002) determined that fractions of distilled EO compounds which were rich in long chain (C_6-C_{10}) alcohols and aldehydes were

effective against GP bacteria (antimicrobial properties of alcohols are known to increase with molecular weight).

In some cases, using either crude or fractionated forms of an EO may result in differences in antimicrobial activity. Delaquis et al. (2002) observed that in some cases the antibacterial activity of individual distilled fractions of EO from four plant sources exceeded that of the crude oils. This was most evident for dill EO, which demonstrated weak antimicrobial activity in the crude form which increased when using distilled fractions containing highly modified and enriched main chemical constituents (D-limolene and carvone) (Delaquis et al., 2002). Furthermore, in an attempt to extend the spectrum of antibacterial activity of distilled fractions, researchers in this study combined one fraction with strong effects again GP bacteria and no measurable effect against GN with a fraction effective against all strains. This resulted in additive or synergistic effects against all GP bacteria, one GN bacterium (*Yersinia enterocolitica*), and *Saccharomyces cerevisiae*. Mixtures were antagonistic for the remainder of GN bacteria tested, however the exact reason for this occurrence was not validated and assumed to be related to cell envelope morphologies of GN bacteria (Delaquis et al., 2002).

The increased antimicrobial effects of individual components had also been documented when Mourey and Canillac (1996) evaluated the antibacterial activity of six main components of conifer EO against *L. monocytogenes*. This research determined that all six individual components exhibited higher bacteriostatic activity than whole conifer EO, possibly due to a reduction or dilution of the antimicrobial activity of individual components when using the crude oil (Benchaar et al., 2008). A companion study performed in 2001, however, reported that the MIC of whole spruce oil was better than

that of the individual components, suggesting that the synergistic effects between individual components contained in this particular EO was critical to overall antimicrobial performance (Canillac and Mourey, 2001). Similarly, Lambert et al. (2001) determined that the mixtures of thymol and carvacrol accounted for the majority of the inhibitory activity of oregano EO against *S. aureus* and *Pseudomonas aeruginosa*. When compared to oregano EO itself, it appeared as though other components in the crude oil may have inhibited the independent activity of thymol and carvacrol (Lambert et al., 2001).

Essential Oils and Manipulation of Rumen Metabolism

The efficiency of rumen metabolism can have a major impact on the production efficiency of the animal as well as the output of environment-polluting waste products (including N-rich wastes and methane) (Greathead, 2003). The well-documented antimicrobial activity of EO has prompted researchers to investigate the potential of using certain EO to improve feed efficiency and nutrient utilization by ruminants in the place of production-enhancing ionophore or non-ionophore antibiotics. Due to the range of EO and their component compounds, many have not yet been examined for the purpose of manipulating ruminal fermentation (Benchaar et al., 2008). Oh et al. (1967) and Nagy and Tengerdy (1968) were the first to investigate effects of EO on ruminal microbial fermentation *in vitro*. Both studies observed that EO inhibited gas production, however the degree of inhibition was dependent on the chemical structure of the EO compound added (Nagy and Tengerdy, 1967, Oh et al., 1967). Subsequent laboratory based studies evaluating EO in ruminant nutrition, although short term in nature, have validated that EO and their active components may favorably alter ruminal fermentation by improving

protein metabolism, VFA production, and fiber digestion (Benchaar et al., 2008). Since demonstrating that chemical composition of EO influences ruminal microorganism activity, research today has focused on the potential of EO to improve ruminal N and energy utilization in order to improve animal performance.

Effects on Ruminant Performance

Most studies conducted on the effects of EO on rumen parameters have been done *in vitro*, and of the *in vivo* studies conducted, the range of EO, EO compounds, dose rates, and diets utilized have produced inconsistent results and varied responses (Benchaar et al., 2008). Using 40 multiparous transition cows and early lactation cows as test subjects, Tassoul and Shaver (2009) administered a blend of thymol, eugenol, vanillin, and limonene plant EO at a target rate of 1.2 g per cow per day. Dry matter intake was lower ($P < 0.05$) for EO (22.7 kg/d) compared to control fed cows (24.5 kg/d). While DMI was not greater in EO fed cows, researchers did report an increase in FCM FE (1.98 vs. 1.83 kg/kg of DMI) (Tassoul and Shaver, 2009). Milk yield in this study was also unaffected by cows supplemented with EO, in accordance with previous research performed by Benchaar et al. (2007b) in which there was no effect on milk and 4% FCM yields from the addition of 2.0 g per day of an unspecified mixture of EO to the diet of four early lactation Holstein cows. The work of (Benchaar et al., 2007b) also reported that neither milk concentrations of fat, protein, urea N nor apparent digestibilities of DM, CP, NDF, ADF or starch were effected by EO supplementation.

Spanghero et al. (2009) fed different levels (0, 40, 80, and 120 g per day) of a microencapsulated blend of EO to eight pregnant Holstein heifers within 30 days of parturition. Feeding the EO blend at these increments had no effect on DMI, milk yield,

or nutrient digestibility measures of cows; however, milk protein content (g/kg) tended to be higher in animals fed the intermediate (40 g per cow per day) dose of EO compared to cows fed at other levels of EO (Spanghero et al., 2009). Increased milk fat production (1.63 vs. 1.66 kg/d; $P = 0.02$) was observed by Santos et al. (2010) when feeding an EO complex to two pens of approximately 310 early lactation multiparous cows at a rate of 0.85 g per cow per day compared to cows fed a control diet with no added EO. In the same study, milk yield and whole tract digestion of organic matter were not impacted by EO supplementation, however DMI was numerically lower in EO fed cows (Santos et al., 2010).

Wall et al. (2014) conducted two experiments in which the diets of mid-lactation primiparous and multiparous Holstein cows were supplemented with an encapsulated blend of cinnamaldehyde and eugenol. In the first experiment, 32 primiparous and multiparous cows were assigned to either treatment with the EO blend fed at 0.35 g per day or no EO blend for six weeks with a one week covariate period. In contrast to previous research, the EO blend in this experiment was associated with an increase in DMI in both parity groups, but an increase in milk production was observed only for multiparous cows fed the EO. Feeding the EO blend was also associated with an increase in protein yield and protein percentage ($P < 0.01$) compared to cows which were not fed the EO blend, but resulted in no changes on SCC, milk fat, lactose, or FE. In the second experiment, 48 primiparous and multiparous cows were assigned to treatment with the EO blend at four different rates (0, 0.2, 0.4, and 0.6 g per day) for eight weeks with a one week covariate period. In this experiment, supplementation of the EO blend resulted in a significant parity by treatment interactions for milk yield ($P < 0.001$), DMI ($P < 0.001$),

SCC ($P < 0.001$), and milk components with the exception of protein and lactose percentages ($P < 0.01$). Milk production of multiparous cows decreased at the two highest doses while the milk yield of primiparous cows increased at both low and high doses. The changes in milk yield were accompanied by similar changes in DMI, leading to no change in FE. Milk fat percentage increased in primiparous cows fed at a rate of 0.20 g per day, but decreased milk fat yield was observed for all multiparous cows. The milk protein yield of primiparous cows was not affected by the treatments, but was decreased in multiparous cows fed at the highest rate (Wall et al., 2014).

Summary

Essential oils are secondary plant metabolite compounds which exhibit bactericidal and bacteriostatic effects on microorganisms and are being investigated for their potential to manipulate ruminal fermentation. The results of Spanghero et al. (2009), Santos et al. (2010), and Wall et al. (2014) suggest that EO can alter milk composition, although the exact mechanism behind this effect is unclear. The work of Wall et al. (2014) documented parity by treatment interactions when feeding different levels of a blend of cinnamaldehyde and eugenol EO. Based on data from their second experiment, these authors suggested an inclusion rate of EO in dairy cow diets at 0.35 g per cow per day due to potential negative effects observed at lower and higher doses. Despite the variety of feeding responses and no optimum dose for dairy cows throughout the various stages of lactation, EO products continue to be marketed for dairy cows, driven by an increased interest in replacing growth promotants with naturally occurring compounds.

CHAPTER 2:

COBALT

Importance of Cobalt in the Ruminant Diet

Cobalt (Co) is a trace element that is required in the diets of ruminants for the synthesis of vitamin B_{12} (**CBL**) (Kincaid et al., 2003). The dietary requirement of Co is currently listed at 0.11 mg/kg dietary DM in the 2001 National Research Council (**NRC**) (Nutrient Requirements of Dairy Cattle, 2001). Certain ruminal bacteria utilize Co to produce vitamin B_{12} (or cobalamin, **CBL**) (McDowell, 1992). A member of a group of molecules called corrinoids, CBL consists of a "core ring" structure in which four tetrapyrroles are connected to a single cobalt by a nitrogen atom. Unlike other B-vitamins, *de novo* synthesis of CBL and its analogues appears to be exclusive to a small number of bacteria and archaea (Martens et al., 2002, Stemme et al., 2008). Because of this, ruminants do not require an exogenous source of CBL (McDowell, 1992, Kincaid et al., 2003). It is generally assumed that ruminant requirements for CBL equate with ruminal bacterial requirements for Co, as one atom of Co is incorporated into a CBL molecule (Girard et al., 2009). Early signs of a Co deficiency are reduced intake and depressed growth, however as ruminants become severely Co deficient, unthriftiness, rapid weight loss, pernicious anemia, and fatty degeneration of the liver often occur (Mertz and Underwood, 1986, Akins et al., 2013).

Vitamin B_{12} is essential for the normal formation of red blood cells and serves as a cofactor for two important biological enzymes (Sherwood, 2010, Akins et al., 2013). The two naturally occurring products of CBL synthesis are 5'-deoxyadenosylcobalamin (or coenzyme B_{12}, the cofactor of methylmalonyl coenzyme A mutase) and

methylcobalamin (or MeB_{12}, the cofactor of methionine synthase). Methylmalonyl coenzyme-A mutase (**MUT**) is an enzyme which converts propionate to succinate to enter the Krebs cycle and potentially gluconeogenesis (Akins et al., 2013). The enzyme methionine synthase converts homocysteine to methionine, a process with important implications in both cardiovascular and neurological health (Sherwood, 2010, Akins et al., 2013).

Vitamin B_{12} is not only a cofactor of mammalian enzymes, but is often required for metabolism in microbes, which require the vitamin for the production of propionate, methane, and methionine (Martens et al., 2002). In acetogenic bacteria, MeB_{12} facilitates the synthesis of acetyl-CoA, which undergoes phosphorolysis to acetylphosphate. Acetylphosphate is subsequently converted to acetate through the conversion of adenosine diphosphate (**ADP**) to adenosine triphosphate (**ATP**), an energy transferring molecule used within cells for metabolism. Methane producing archaea require methylcobalamin to facilitate the transfer of methyl groups from methanogenic substrates to the thiol group of coenzyme M. This is a high energy reaction that is coupled with extrusion of sodium ions, eventually leading to a sodium motive force. With the exception of *Escherichia coli*, nearly every known enteric bacterium uses coenzyme B_{12} for anaerobic fermentation of 1,2-propanediol, ethanolamine, and glycerol (Martens et al., 2002). In some bacteria, the products generated from the fermentation of these substrates (propionaldehyde and acetaldehyde) serve as carbon and energy sources following oxidation to propionyl-CoA and acetyl-CoA (Martens et al., 2002).

Additionally, certain microbial species have a requirement for vitamin B_{12} as a growth factor (Stemme et al., 2008). One of the four classes of ribonucleotide reductases,

which play an important role in DNA synthesis, are adenosylcobalamin-dependent reductases. These class II reductases are primarily found in microorganisms (Martens et al., 2002). If there is a deficiency of ruminal vitamin B_{12}, protein synthesis of certain microbial species may be reduced (Stemme et al., 2008).

Due to the increased production demands of dairy cows, it is likely that their B-vitamin requirements surpass what is supplied from ruminal synthesis alone (Santschi et al., 2005). The amount of vitamin B_{12} synthesized by rumen microbial organisms is dependent on a number of factors, of which the dietary Co concentration is believed to be the most important (Stemme et al., 2008). The following section will present research which has investigated the response of dietary Co supplementation at or above NRC recommendations on the ruminal digestion and production performance of dairy cows to further substantiate feeding an EOC blend to lactating dairy cows.

Efficiency of Cobalt Utilization & Cobalt Supplementation Considerations

Although it is required in ruminant diets, the efficiency of CBL production is low (Kincaid et al., 2003, Girard et al., 2009). Girard et al. (2009) administered Co in the form of Co_2Co_3 at a rate of 0.76 mg of Co/kg of DM to 4 multiparous lactating Holstein cows. Approximately 129 mg of corrinoids were produced daily by ruminal microbiota, 50 mg of which was CBL (in other words, ruminal synthesis of CBL represented 38% of the total amount of corrinoids produced in the rumen). Based on an estimation of the molecular weight of Co represented in CBL, approximately 5.6 mg of Co was incorporated into the total amount of corrinoids reaching the duodenal cannula. Given an average daily Co intake of 50 mg (cobalt in the TMR and supplemental cobalt carbonate), 11 percent of the average daily intake of Co was used for apparent ruminal synthesis of

total corrinoids. Of this, only four percent was used for CBL synthesis. The low efficiency of Co utilization may be improved by increasing the ruminal solubility of Co, using a source such as Co-lactate.

Girard et al. (2009) advised against increasing dietary Co supply, as this may increase the production of biologically inactive CBL analogues in the rumen at the expense of active forms of the vitamin. However, it is likely that the relative production of CBL to CBL analogues is affected by the diet. Diets high in roughages promote greater CBL production whereas diets containing higher amounts of concentrates lower the ratio of CBL to various analogues (Sutton and Elliot, 1972). Reduced ruminal synthesis of CBL as a result of feeding a forage to concentrate ratio in the diet was confirmed by Walker and Elliot (1972). Increasing dietary Co has been correlated with an increase in total ruminal anaerobic bacteria with an increase in lactic acid production (Young, 1979). Additionally, Co toxicity may reduce feed intake and weight gain, cause emaciation, increased liver Co, and hyperchromia (Nutrient Requirements of Dairy Cattle, 2001). Nevertheless, Co fed above the dietary recommendations set by the NRC has produced several notable results.

Effect of Dietary Supplementation of Cobalt on Fiber Digestibility

Feeding supplementary Co in diets containing up to 10 mg/kg Co using *in vitro* procedures has been reported to increase cellulose digestibility (Allen, 1986). Lopez-Guisa and Satter (1992) added Co in excess of NRC dietary recommendations to the diet of heifers (at 0.25 to 0.35 mg/kg) and reported an increased rate of DM disappearance from Dacron bags placed in the rumen for alfalfa hay, corn cobs, and corn crop residue silage. Pretz and Casper (2015) determined that feeding 50 mg/kg cobalt-lactate (a

highly soluble source of Co in the rumen) to late lactation dairy cows appeared to alter ruminal fermentation. Ruminal ammonia concentrations were lower ($P = 0.03$) in cows fed supplemented Co, presumably due to increased microbial protein yield. Additionally, the ruminal percentage of acetate was higher ($P = 0.04$) for Co supplemented cows compared to cows fed the control. While there was no difference between the two treatments when evaluating DM, CP, NDF, ADF, and starch digestibility percentages, a numeric advantage existed in fiber digestion (NDF and ADF) for cows fed a Co supplemented ration (Pretz and Casper, 2015). Milk production, milk components, FCM and ECM did not differ ($P > 0.05$) between Co supplemented and unsupplemented animals (Pretz and Casper, 2015).

Effect of Dietary Supplementation of Cobalt on CBL Production

Smith and Marston (1970) reported that the efficiency of production of CBL increases from approximately 3 to 4 percent to 13 percent when Co intake was low in sheep (1 mg Co/kg DM). Contrary to this finding, a substantial amount of research has determined that increasing Co supplementation in ruminant diets can increase CBL production efficiency. Stemme et al. (2008) examined the effects of an elevated dietary Co supply by feeding an unsupplemented ration (0.17 mg cobalt/kg DM) and a ration supplemented with Co (0.29 mg cobalt/kg DM) in sequence to five lactating dairy cows. Numerically, the recovery rate of Co was higher in the unsupplemented ration (93.4%) than the Co supplemented ration (85.8%). Based on an estimation of the molecular weight of Co represented in CBL, approximately 7.1% of the Co at the duodenum was bound by CBL in unsupplemented cows, whereas approximately 9.5% of Co was utilized for CBL production in Co supplemented animals. Significantly higher amounts of CBL

were determined to reach the duodenum when the ration was supplemented with Co compared to the unsupplemented diet. It was hypothesized that an elevated supply of CBL may increase microbial protein synthesis, however no significant differences were detected between unsupplemented and supplemented treatments. Finally, there were no significant differences in ruminal pH, ammonia concentration, or short chain fatty acid concentrations between unsupplemented and cobalt supplemented animals.

Increasing CBL production by increasing in dietary Co supplementation has been also been documented in several other studies. Ruminal synthesis of CBL increased nearly 20-fold in sheep when dietary Co was increased from 0.1 to 0.5 mg/kg (Mills, 1981), and Kawashima et al. (1997) reported an increase of CBL in the serum and liver of sheep when dietary Co was fed at 40 mg/kg. Tiffany et al. (2006) noted an increased synthesis of CBL when the Co concentration in the diet increased from 0.2 to 1.0 mg/kg.

Effect of Dietary Supplementation of Cobalt on Production Parameters

Kincaid and Socha (2007a) investigated the effects of dietary Co supplementation during late gestation and early lactation on serum and liver Co concentrations, CBL concentrations in serum and milk, and overall milk yield. Serum and liver CBL concentrations typically drop during early lactation when the secretion of CBL into milk becomes a drain on maternal reserves (Elliot et al., 1965). Three different concentrations of Co (0.15, 0.89, and 1.71 ppm) were administered to dry cows, which averaged 55 days prepartum. Feeding the low, medium, and high Co treatments continued post-partum through 120 DIM at dietary concentrations of 0.19, 0.57, and 0.93 mg/kg of Co.

Although serum CBL concentrations were not affected by Co supplementation, milk CBL concentrations tended ($P = 0.11$ and $P = 0.16$, respectively) to increase as

dietary Co supplementation increased from low to high concentrations. Because of the work by Judson et al. (1997), which suggested that CBL concentration in milk may be a better indicator of the effectiveness of Co supplementation than plasma CBL concentrations, Kincaid and Socha (2007) concluded that dietary Co supplementation likely increased ruminal synthesis of CBL. Additionally, Co concentrations in milk were increased by Co supplementation, indicating intestinal Co absorption was greater in cows receiving Co supplementation. The liver is the main storage site for Co and typically contains the highest Co concentration of body tissues (Underwood and Suttle, 1999), however liver Co concentration was not affected by either Co intake or the day of sampling (-55 and 120 d postpartum). Finally, there was no effect of Co supplementation ($P > 0.15$) on milk composition or yields of milk, milk components, ECM, FCM, or FE.

Akins et al. (2013) investigated the effects of Co supplementation (inorganic and organic forms) above NRC (2001) requirements on CBL status and milk production by lactating primiparous and multiparous cows. Forty-five multiparous and 45 primiparous Holstein cows were assigned to one of five treatments: 1) no dietary Co supplementation (Control), 2) 25 mg/d supplementary Co carbonate (CoCarb), 3) 25 mg/d supplementary Co glucoheptonate (LCoGH), 4) 75 mg/d supplementary Co glucoheptonate (HCoGH), and 5) control diet plus weekly intramuscular injections of CBL (10 mg). Dietary concentrations of Co were higher than intended in the control diet, resulting in a concentration nine times above (1.1 mg Co/kg DM) the NRC recommendation. Body weight, BCS, energy balance and DMI of lactating cows were unaffected among treatments ($P > 0.10$). There was no significant effects due to treatment ($P = 0.15$) or a treatment by time interaction ($P = 0.15$) on milk yield, however there was a tendency (P

= 0.07) for a significant contrast between CoCarb and LCoGH for milk yield (39.7 vs. 42.9 kg/d respectively). Contrasts between Control and the mean of LCoGH and HCoGH for all lactation measures were non-significant ($P > 0.10$), indicating that Co concentrations in the Co diet (1.1 mg Co/kg DM) were more than adequate. The authors believe that the lack of lactation performance response of cows to Co supplementation compared to Control fed cows was due to the Co concentration being nine times higher than NRC (2001) requirements. Kincaid et al. (2003) determined increased milk and FCM yield when multiparous cows were fed 1.26 mg Co/kg DM compared to cows fed diets with 0.37 or 0.68 mg Co/kg DM. Fat corrected milk yield, ECM, fat content, protein content, lactose content, and log SCC were not affected by treatment or its interactions with parity or time ($P > 0.10$).

Summary

Vitamin B_{12} plays an important role in maintaining key metabolic enzymes and may influence bacterial growth. In mammals, vitamin B_{12} synthesis is not possible. Ruminants are dependent on rumen microbial synthesis of the vitamin, which is complex and dependent on a number of factors including the composition of the diet, dry matter intake, and the concentration of Co supplied in the ration. Studies report that an increased supply of dietary Co results in increased amounts of vitamin B_{12} synthesis, however it does not seem to have an effect on milk yield when fed above dietary recommendations. Supplementing diets with Co does appear to influence rumen parameters, such as increasing ruminal fiber digestibility and altering VFA ratios.

Study Rationale

A review of literature on EO and Co indicates that while there is room to continue research of these compounds independently, there has been a demonstrable amount of evidence supporting the feeding of a combination of these compounds in the diet of lactating dairy cows. We hypothesize that including a proprietary blend of EO and Co-lactate in the diets of dairy cows will result in improved nutrient utilization and production parameters. This project was designed to 1) evaluate the response of feeding a proprietary EOC blend on the lactational performance, nutrient digestibility, and fecal pathogen profile of lactating dairy cows and 2) determine whether any preservative effects were imparted to the TMR due to treatment with the EOC.

MATERIALS AND METHODS

Animals and Diets

The trial was conducted in two experimental periods (P1 and P2) on a commercial dairy in southwest Minnesota equipped with two Lely Astronaut A4 robotic milking units (Gorter's Clay & Dairy Equipment of MN, Inc., Pipestone, MN). Phases 1 and 2 were cross over design trials with two pens and two experimental periods. Phase 1 was conducted in late 2014 and P2 in early 2015. Cows were housed in two freestall pens (57 ± 2 cows and 59 ± 3 cows for EOC and Control pens, respectively) and were evaluated for cow parity (2.65 ± 1.52 and 2.33 ± 1.20), DIM (184 ± 103 and 154 ± 94.2), and milk production (35.4 ± 11.3 kg/d and 36.9 ± 11.3 kg/d) prior to study initiation and assignment to EOC or Control treatments. Each Phase included 14 d for dietary adaptation followed by 9 wk for data collection.

Cows were fed a TMR (Table 1) 1x/d consisting of 60% forage (58% corn silage, 12% alfalfa hay, and 30% alfalfa haylage from the 2013 crop season) and 40% grain on a DM basis during P1. During P2, cows were fed a TMR (Table 1) 1x/d consisting of 65% forage (55% corn silage, 3% grass hay, 14% alfalfa hay, and 28% alfalfa haylage) and 35% grain on a DM basis. Forage and TMR samples were collected monthly throughout the trial by Hubbard Feeds (Worthington, MN) and were submitted to Dairyland Laboratories, Inc. (Arcadia, WI) for DM and nutrient analysis. The final nutrient composition of the TMR for each Phase (Table 2) was based on three representative samples taken throughout the experimental period. A salt lick drum was placed between pens to prevent animals from reaching out and consuming feed distributed to the adjacent pen throughout the trial. Cows also received concentrate (Table 3) which was dispensed according to the current level of milk production during a given milking session. Robots would not attempt to milk cows or dispense concentrate to cows which had met their quotient for milking during a 24 h period. Prior to study initiation, cows to be fed EOC averaged 2.72 ± 0.11 milking/d and cows fed C averaged 2.80 ± 0.13 milkings/d. Pens receiving the EOC treatment received an additional 0.5 lb/head/d of a soy hull carrier that included 28 g/head/d of EOC.

For both P1 and P2, EOC was added to the feed wagon after the Control diet had been dispensed to the appropriate pen and was integrated into the TMR for 5 min before being distributed to the EOC pen. The diet was balanced using MilkCheck v. 2.11 (Hubbard Feeds Inc., Worthington, MN). Feed was distributed once daily between 0900 h and 1200 h and refusals were collected once per 7 d period for each pen. Cows had

unlimited access to fresh water during the study. All other bedding, cow monitoring, and manure scraping was performed by farm personnel.

Sample Collection

Milk production was recorded electronically (Lely Time for Cows (**T4C**) milking software, Gorter's Clay & Dairy Equipment of MN, Inc., Pipestone, MN) at each individual milking and was downloaded remotely to a private computer system at approximately 1000 h daily throughout the trial (Alfred Dairy Science Building, South Dakota State University, Brookings, SD). Data files collected during each milking also included body weights, lactation days, cow parity, percentages of milk fat and milk protein, and the total amount of concentrate consumed during a 24 h period. Milk samples were collected using a Lely Shuttle milk sampling unit once every 14 d and were analyzed by DHIA (Zumbrota, MN) for fat, protein, lactose, MUN (Fourier Transformed Infrared Technology) and SCC (flow cytometry using ethidium bromide for white blood cell staining, Fossomatic 5000, Foss Electric, Denmark). Milk samples were tempered in a water bath prior to analysis.

Body condition scores were determined weekly by two individuals on a scale of 1 to 5, with one being emaciated and 5 as obese (Wildman et al., 1982). Body surface temperatures of each cow was recorded at this time using a thermal imaging camera (Fluke Ti25 Infrared Camera, Fluke Inc., Everett, WA) targeted at the chest directly posterior to the elbow joint. Due to the freestall layout, temperatures were recorded within 10 feet or less of the animal.

Two representative fecal samples were collected weekly from each pen based on a composite from 15 cows. Equipment (bucket, scoop, and ladle) was cleaned thoroughly

in hot water between pens. One sample was submitted weekly to Rural Technologies Inc. (Brookings, SD) to be evaluated for normal microbiota and the presence of the following fecal pathogens: *Bacillus*, *Streptococcus*, coliform, *Proteus*, and *Campylobacter* species. The second sample was stored for further analysis at -20°C.

One quart of TMR and half a quart of concentrate samples were collected weekly from each pen and robot and were composited once every 4 wk. One quart of the composite was submitted to Rural Technologies Inc. (Brookings, SD) to be screened for *Aspergillus fumigatus*. At Rural Technologies Inc., a subsample of this composite was also submitted to Shadow Government Statistics (SGS, Brookings, SD) to be screened for the following mycotoxins: zearalenone, T2, ochratoxin, fumonisin, vomitoxin, and aflatoxin. All concentrate composites and the second TMR composite for each pen were stored for further analysis at -20°C.

Feed aerobic stability monitoring was set up weekly by filling two 20 gallon buckets with fresh TMR from EOC and Control bunks and inserting a SmartButton temperature data logger (ACR Systems Inc., Surrey, B.C. Canada) into the center of the feed in the bucket. The SmartButton was programmed to log temperatures at half hour intervals beginning at 1630 h on day one until 1200 h on day 7. The temperature logger was extracted the following week and buckets were emptied and washed thoroughly in hot water before resetting for the next 7 d. In attempt to control seasonal temperature variation during the trial, buckets were left in a corridor adjacent to the milk parlor where temperature was to be maintained at approximately 21°C throughout the trial. To monitor ambient room temperature, a third button was left adjacent to the buckets and was programmed to run at the same time intervals and duration as EOC and Control

buttons. Each week, data from the loggers was transferred from the SmartButton to TrendReader for SmartButton (Version TR3 3.02.000.12407-10, ACR Systems Inc., Surrey, B.C. Canada) using an ACR Systems connection driver (ACR SmartButton Adapter Connection Driver, ACR Systems Inc., Surrey, B.C. Canada). On day 7, data was extracted from TrendReader for SmartButton onto an Excel spreadsheet (Microsoft Office Professional Plus 2013), where time until a 2°C rise was determined, i.e. when aerobic stability was reached (Kleinschmit and Kung, 2006).

Laboratory Analysis

During the trial, DM and nutrient analyses of feeds and forages were determined by Dairyland Laboratories, Inc. (Arcadia, WI). Dry matter was determined following Method 2.1.4 of the National Forage Testing Association Reference (NFTA, Omaha, NB) by drying samples for 3 h in a 105°C oven. Samples were analyzed for nutrients using the following methods: CP (AOAC 990.03; 1998), NDF (AOAC 2002.04; 2005: modification included use of Sea Sand for filter aid and Whatman GF/C filter paper for residue collection), ADF (AOAC 973.18, 1996: modification included use of Sea Sand for filter aid as needed), acid detergent insoluble crude protein (**ADICP**) and neutral detergent insoluble protein (**NDIP**), (where residues were collected following ADF methods by use of Whatman 541 (nitrogen-free) paper or Whatman GF/C filter paper before analysis for nitrogen by CP (AOAC 990.03; 1998)), lignin (AOAC 973.18; 1996: modification included use of Sea Sand for filter aid as needed, use of Whatman GF/C filter paper to collect residue, and holding crucibles in beakers to cover fiber with 72% sulfuric acid for full time required), ash (AOAC 942.05; 1996), pH (Orion Research, Inc., 1977) soluble protein (**SP**) (AOCS Method, Ba 10-65), starch (Starch Analysis in Animal

Feed: Method workshop from 30th Annual MW AOAC Meeting and Exposition with the following modification: glucose analysis completed on WSI 2700 Select Biochemistry analyzer instead of using GOPOD), crude fat (AOAC 920.39), neutral detergent fiber digestibility (**NDFD**) (Goering and Van Soest, 1970), ammonia nitrogen (NH_3-N) through distillation from Recommended Methods of Manure Analysis (modification using Kjelsorb reagent), lactic acid and acetic acid (Muck and Dickerson, 1988), non-fiber carbohydrate (**NFC**) (NRC, 2001), net energy of lactation ($\mathbf{NE_L}$) (NRC, 2001), and relative forage quality (**RFQ**) (Rohweder et al., 1978). Metals (Fe, Cu, Zn, and Mn) were analyzed using AOAC method 953.01. All other minerals (Ca, P, Mg, Na, and K) were determined by adding 5 mL of HCl to 0.5 g of sample to dryness, followed by 10 mL of Aquaregia for 10 min on a hot plate, then diluted to 100 mL of water and analyzed by inductively-coupled plasma (ICP). Sulfur was determined using ICP-OES and microwave digestion (Mullins et al., 2007). Chloride was extracted in water, filtered, and measured using a Corning 926 Chloride Analyzer.

At the end of the trial, TMR and concentrate composite samples were next-day shipped to Analab (Fulton, IL) along with frozen fecal samples for nutritional digestibility analysis using acid insoluble ash (**AIA**) as an internal marker for determining nutrient digestibility coefficients as described by Van Keulen and Young (1977). Samples were analyzed using the following AOAC (1998) methods: DM (935.29), CP (990.03), NDF (2002.04), ADF (973.18), ADICP (973.18 and 976.06), NDICP (2002.04 without sulfite and 976.06), lignin (973.18), ash (942.05), Ca (985.01), P (985.01), Mg (985.01), Na (985.01), Cl (915.01), S (923.01), Fe (985.01), Cu (985.01), Zn (985.01), K (985.01) and Mn (985.01). The remaining parameters were measured using the following

methods: starch (Glucose Reagent Set, AMRESCO, Solon, OH and ALPKEM Corporation, 1990), soluble protein (**SP**) (Krishnamoorthy et al.), oil (Damon, 1966), *in vitro* dry matter digestibility (**IVDMD**) (24 h ruminal and 24 h enzymatic digestion using the Kansas State Buffer (Marten and Barnes, 1980)), neutral detergent fiber digestibility (**NDFD**) (Van Soest et al., 1991, incubated for 30 h using the Kansas State Buffer (Marten and Barnes, 1980)), NH_3-N (United State Environmental Protection Agency, 1993, method 351.2 and International Organization for Standardization, 2013, method 11732), NFC (National Research Council, 2001), and net energy of lactation (**NE_L**) (National Research Council, 2001). Nutritional digestibility of a nutrient was calculated using the following equation:

$$ND\ (\%) = 100 - (100 \times \left(\frac{\%\ AIA\ in\ feed}{\%\ AIA\ in\ feces} \times \frac{Nutrient\ in\ feces, \%}{Nutrient\ in\ feed, \%}\right))$$

Fecal samples were analyzed for fecal pathogens (Rural Technologies Inc., Brookings, SD) by plating samples on non-selective media (blood agar plate) to estimate normal microbiota and other pathogens within the sample. To inoculate a sample, a sterile swab was used to initiate the first streak. Following inoculation, a disposable inoculating loop was used to streak for isolation using a quadrant method. Following incubation at 37 ± 2°C for 48 h (with the exception of *Campylobacter*, which was incubated in an anaerobic chamber at 41 ± 2°C for 48 h), the amount of bacteria present was described in the following manner: few (1-10 colonies), small (growth from initial streak only; 11-50 colonies), moderate (growth in second quadrant; 50-100 colonies), or large amounts (growth in third or greater quadrants; more than 100 colonies). Initial observations were recorded at 24 h and a final observation at 48 h.

Coliform species were determined using a MacConkey agar plate. A positive *Escherichia coli* sample was indicated by the presence of pink colonies able to grow and ferment lactose. These colonies were verified as *E. coli* using an indole test. The number of phenotypes present was based on different amounts of colony morphology. If two colonies appeared different in color or appearance in any way, but were both confirmed as *E. coli*, then it was assumed that two different phenotypes were present. To determine *Streptococcus* and *Campylobacter* species, the same methods described above were employed for isolation, however selective media specific for isolating each bacteria was utilized. *Proteus* and *Bacillus* are common contaminating species, but were also described during the analysis.

Total mixed ration composites were submitted throughout the trial (Rural Technologies Inc., Brookings, SD) to check for the presence *A. fumigatus*. Samples were mixed and 50 g was weighed out and added to 450 mL of peptone diluent. This mixture was then mixed in a stomacher for two minutes before being serially diluted down to 10^{-4}. Following the dilution step, 100 µL of each of the dilutions were plated onto potato dextrose agar. Plates were incubated at 30°C prior to interpretation. A TMR subsample was submitted to SGS (Brookings, SD), where USDA-GIPSA validated enzyme linked immunosorbent assay (ELISA) testing methods were used to detect and quantify the following mycotoxins: zearalenone, T-2, ochratoxin, fumonisin, vomitoxin, and alfatoxin.

Data Handling and Statistical Analysis

Within a phase, only data collected from animals which completed the 11 weeks were considered in the final statistical analysis. Data collected from the T4C software was reduced to weekly observations per cow. The first two weeks of data

collection occurred during the dietary adaptation period and were omitted from the data analysis. Lactation limits were set as follows: if lactation ≥ 2, then lactation = 2. A cut off for SCC was defined according to the California Mastitis Test (CMT), where a SCC higher than the CMT score of 1 (1,200,000 cells/mL) was excluded from data analysis. Management level milk was calculated as: ((29.15 x Milk Factor) + (12.3 x Fat Factor x Test-day Fat percent) + (6.56 x Prot Factor x Test-day Prot percent)) x Test-day Milk pounds/100 (Steuernagel, 2008). Energy corrected milk was calculated as follows: 0.327 x milk yield + 12.95 x fat yield + 7.2 x protein yield (Orth, 1992). Fat corrected milk was calculated as: 0.4 x milk yield + 15 x milk fat yield (Orth, 1992). To compensate for variation in production parameters that arose due to variations in DIM and lactation, ECM, FCM, and kg of fat, protein, and lactose (from both T4C and DHIA) were calculated based on MLM production calculations. Dry matter intake was calculated by pen in the following manner: the total weight of feed mixed and weight of feed left over after feeding the Control pen was recorded. The weight of feed dispensed to the EOC pen was subtracted from the total weight to calculate weight of feed distributed to the Control pen. After receiving the weight of refusals, the total amount of feed distributed to each pen during the time since the last weigh back was calculated. The weight of refusals was subtracted from this number to calculate feed consumed per pen during the time period. This value was divided by the number of cows that were in each pen during that time period, which provided the kg consumed per cow within the time period. This number was divided by the number of days in the time period to calculate the kg per cow per day. The DMI was then calculated based on the DM content of the TMR. To calculate individual DMI, each cow in the pen was assigned the value that was calculated

as the average DMI for that pen during the time period, and the individual DMI of concentrate during this period was added to this number. Because DMI could not be corrected given DIM and lactation (as milk production was using MLM), FE was calculated based on the true 24 h production level (day production) and kg of DMI.

All data was subjected to least squares analysis of variance (ANOVA) for a single cross over design using the PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) as a repeated measure ANOVA. Means for the remaining data were calculated from the data gathered during the collection time period of each phase. The statistical model used for data collected from T4C, DHIA, and BCS was $Y_{ijkl} = \mu + T_i + P_j + W_k + D_l + (T \times P)$ where Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment, P_j = phase, W_k = week, D_l = DIM, and (T x P) = treatment by phase. The probability of the interaction of T x W was not significant and was eliminated from the model ($P > 0.90$). Lactation was not significant and was eliminated from the model ($P > 0.60$). All other data (fecal pathogens, SmartButton data, and mycotoxins) utilized the same model described above, but excluded DIM.

Fecal pathogens were subjected to least squares ANOVA for a single cross over design using the PROC GENMOD procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) as a repeated measure ANOVA. The following numerical values were assigned to the fecal pathogen descriptive results: 1 = not detected, 2 = trace/rare/few, 3 = small, or small amount of 1 phenotype *E. coli*, 3.5 = small, or small amount of 2 phenotypes *E. coli*, 4 = moderate, or moderate amount of 1 phenotype *E. coli*, 4.5 = moderate, or moderate amount of 2 phenotypes *E. coli*, 5 = large, or large amount of 1 phenotype *E. coli*, 5.5 = large, or large amount of 2 phenotypes *E. coli*. For all data,

significant differences were declared when $P < 0.05$ and trends were declared at $0.05 \leq P < 0.10$.

RESULTS and DISCUSSION

Nutrient Composition of the Diet

The ingredient and nutrient composition of diets are presented in Tables 1 and 2, respectively (the nutrient composition was of the TMR only and does not include the concentrate). The diets for both P1 and P2 (Table 1) contained similar proportions of forages, however grass hay was not included in the diet during P1. Although grass hay was provided during P2, the TMR nutrient analysis for this trial (Table 2) resulted in a similar nutrient composition between P1 and P2. The NRC (2001) recommends lactating, large breed dairy cows at 90 DIM or greater producing 35 kg/d of milk with 3.5% milk fat and 3.0% milk protein to consume a diet with 15.1% CP, 1.47 Mcal/kg NE_L, 25 to 33% NDF, 17 to 21% ADF, and 36 to 44% NFC. For P1 and P2 respectively, the diet was 15.5% and 15.9% CP, 1.70 and 1.69 Mcal/kg NE_L, 28.9% and 32.7% NDF, 20.1% and 20.4% ADF, and 41.6% and 40.6% NFC on a DM basis (Table 2). The TMR met or exceeded recommended nutrient guidelines (NRC, 2001).

Milk Production, Composition, and Quality

Daily milk production, MLM, 3.5% FCM, and ECM were similar ($P > 0.10$) for cows fed both treatments (Table 4). Milk yields from this study were consistent with findings from Benchaar et al. (2007b), Tassoul and Shaver (2009), Santos et al. (2010), Spanghero et al. (2009), and Tager and Krause (2011), in which milk yields of cows fed various amounts and blends of EO (ranging from 0.5 to 120 g/d) were not different from milk yields of cows fed control diets. Although the present study did not investigate a

treatment by parity interaction, Wall et al. (2014) determined a significant parity by treatment interaction for milk yield ($P < 0.001$) when cows were fed an encapsulated blend of cinnamaldehyde and eugenol compared to cows fed the control diet without EO. This interaction was characterized by an increase in milk yield of multiparous cows at a moderate dose (350 mg/d), and an increased milk yield of primiparous cows at the lowest (200 mg/d) and highest (600 mg/d) doses. Such results indicate that the optimal dose of an EO may depend on the parity of the cow, however the mechanism underlying dose responsiveness of EO, as well as the effects of parity on the optimum dose, is not clear (Wall et al., 2014). In summary, little published evidence is available which demonstrates increased production due to EO supplementation (Tager and Krause, 2011). Results from the present study are also consistent with the findings of Pretz and Casper (2015) , Kincaid and Socha (2007b), and Akins et al. (2013) in which additional Co supplementation did not impact milk production.

During both Phases, Lely T4C milk fat and protein percentage and yield (kg) was similar ($P > 0.10$) for cows fed both diets, although during P1 milk fat was six percentage points higher ($P > 0.10$) for cows fed EOC compared to cows fed Control (Table 4). Milk fat percentage reported by DHIA was similar ($P > 0.10$) during P1, but was greater ($P < 0.05$) during P2 for cows fed EOC compared to cows fed Control (Table 5). Milk fat yield (kg) was greater ($P < 0.05$) during P1, but was similar ($P > 0.10$) during P2 for cows fed EOC compared to cows fed Control (Table 5). Protein and lactose percentages and yields reported by DHIA were similar ($P > 0.10$) for cows fed both diets (Table 5).

Robotic milking units require routine calibration to ensure consistent accuracy of daily measures of milk fat and protein percentages. Because proper calibration of robots

throughout the trial could not be validated by standard reference methods and standard operating procedures on the farm was to only calibrate once per month, we propose that the milk component data reported by DHIA was a more accurate reflection of milk quality and components during this trial.

Benchaar et al. (2007b) and Tager and Krause (2011) determined no effect of EO supplementation on milk percentages and yields of fat, protein, and lactose compared to cows fed a control diet. Spanghero et al. (2009) measured a tendency ($P = 0.06$) for higher protein content (g/kg) when feeding a microencapsulated blend of EO at a rate of 40 g/d than cows fed a control diet. During their first experiment, Wall et al. (2014) demonstrated an increase in both protein percentage and yield ($P < 0.01$) when cows were fed EO compared to control fed cows. Santos et al. (2010) observed increased milk fat yield (kg/d) ($P < 0.05$) when feeding 0.85 g/head/day of an EO complex to early lactation cows, and Wall et al. (2014) detected significant parity by treatment interactions associated with milk fat percentages, which increased in primiparous cows fed at a rate of 0.20 g/d compared to cows receiving no EO. Eugenol, a popular component of EO mixtures which have been fed to dairy cattle, was found to sharply suppress propionate concentrations *in vitro* and sharply increase the acetate to propionate ratio (Benchaar et al., 2007a). Although no VFA measures were taken during this trial, this theory would support the increased DHIA milk fat percentage and yield observed during both Phases, as acetate is a precursor for milk fat synthesis. Alternatively, this effect could be the result of a shift in energy partitioning which alters milk component synthesis (Santos et al., 2010, Wall et al., 2014). Whereas EO has been documented to influence milk components according to the literature, the present review of literature did not report an

effect of Co supplementation on milk composition (Kincaid and Socha, 2007b, Akins et al., 2013, Pretz and Casper, 2015).

Milk urea nitrogen was significantly lower for cows fed EOC than for cows fed Control for P1 ($P < 0.01$), however MUN was similar, though numerically lower, during P2 for cows fed both diets ($P > 0.10$) (Table 5). Borchers (1965) and Broderick and Balthrop (1979) demonstrated a reduction in rumen ammonia N and an increase of amino acids (**AA**) when supplementing thymol to ruminal fluid (1 g/L) *in vitro*, suggesting an inhibition of AA deamination by ruminal bacteria (Benchaar et al., 2008). The decreased MUN levels in this study may reflect decreased ruminal AA deamination, leading to a reduction in passive ammonia transfer to the blood stream. This would reduce the rate of conversion of toxic ammonia to urea in the liver (a highly endergonic process), and consequently MUN levels. Because no blood urea nitrogen (**BUN**) and rumen ammonia levels were measured during this trial, this theory cannot be validated; however, Pretz and Casper (2015) determined that feeding Co decreased ruminal ammonia concentrations, which could indicate an increase in ruminal microbial protein synthesis.

Somatic cell counts were similar ($P > 0.10$) for cows fed both diets (Table 5). Although feeding the EOC did not appear to affect SCC in the present study, *in vitro* work by Baskaran et al. (2009) demonstrated that trans-cinnamaldehyde, eugenol, carvacrol, and thymol reduce bacterial pathogens in milk at minimum bactericidal concentrations (0.4-0.45%). These results could indicate that certain combinations of EO may be used as an alternative to (or could be used adjunct to) antibiotics as a intramammary infusion to treat bovine mastitis (Baskaran et al., 2009). In accordance with observations from the current study, Wall et al. (2014), Benchaar et al. (2007b),

Santos et al. (2010) and Spanghero et al. (2009) reported no effect of EO supplementation on SCC using various plant extracts. The effect of feeding EO on improving SCC may be impacted by baseline SCC, or the "risk" of mammary infection present during the experiment (Wall et al., 2014).

Dry Matter Intake and Feed Efficiency

Dry matter intake was greater ($P < 0.05$) for cows fed EOC compared to cows fed Control during P1 (Table 6). Because DMI was not accompanied by a significant increase in milk production, a numerically similar ($P > 0.10$) FE resulted (Table 6). During P2, DMI was similar ($P > 0.10$) for cows fed both diets (Table 6). Feed efficiency increased ($P < 0.05$) during P2 compared to P1 for cows fed both diets, however FE was similar ($P > 0.01$) between cows fed EOC and Control in both P1 and P2 (Table 6). How a combination of EO and Co may influence DMI and FE is unclear, but improvements by feeding EO may be related to apparent improvements in rumen function and increased ruminal fiber digestibility (Tekippe et al., 2013, Wall et al., 2014). Essential oils may have a greater influence on FE when used for a longer duration, however this would require a full-lactation experiment (Wall et al., 2014). The majority of the literature reviewed for EO and Co reported either decreases or no differences in DMI when feeding either supplemented or unsupplemented EO or Co diets to lactating cows (Kincaid and Socha, 2007b, Spanghero et al., 2009, Tassoul and Shaver, 2009, Akins et al., 2013, Pretz and Casper, 2015), however Kincaid et al. (2003) observed that the DMI of lactating cows was affected ($P < 0.05$) by parity, week, and the interaction of treatment x parity x week of the study. Milk production efficiency was not affected by treatments during this study (Kincaid et al., 2003).

Body Weight, and Body Condition Score, and Body Surface Temperature

Body weights were greater ($P < 0.01$) for cows fed EOC compared to cows fed Control during P1, however the opposite was true during P2, in that body weights were lower ($P < 0.01$) for cows fed EOC compared to cows fed Control (Table 6). During P1, the initial average body weight was 695 ± 79 kg for EOC fed cows and 664 ± 79 kg for Control fed cows. During P2, the cows receiving EOC (which had been fed Control during P1) initially weighed 636 ± 76 kg. The cows receiving Control (which had been fed EOC during P1) initially weighed 684 ± 77 kg. Thus, one pen on average had greater body weights than the other pen throughout the trial, which ultimately led to the observed results. Body condition scores were significantly different ($P < 0.01$) between Phases, but within each Phase BCS were similar ($P > 0.10$) for cows fed both diets (Table 5). Kincaid and Socha (2007b), Kincaid et al. (2003), Akins et al. (2013), and Pretz and Casper (2015) reported no differences in body weights or BCS due to dietary Co supplementation. Tassoul and Shaver (2009) reported no effects on body weight and BCS of cows fed a diet containing EO compared to cows fed a control diet, however a primary finding of Santos et al. (2010) was that there was an apparent energetic shift away from body condition gain to milk fat output as a result of feeding an EO complex (Santos et al., 2010).

Body surface temperatures were collected weekly on individual cows in order to assess differences in body temperature between cows fed EOC or Control, however surface temperatures were similar ($P > 0.10$) between pens during both Phases (Table 6). A difference in body temperature may reflect a change in the efficiency of nutrient utilization, a decrease in heat stress, or an increase in dissipation of body heat. However,

if feeding EO is increasing the acetate to propionate ratio in the rumen, a slight (but perhaps undetectable) increase in body temperatures may occur due to the heat of fermentation associated with acetate production. Tympanic thermometers may be a more sensitive way of determining slight differences in body temperatures between cows fed different diets if temperature monitoring were conducted in the future.

Nutrient Digestibility

Much of the current research regarding EO in the diets of ruminants is based upon the potential to manipulate ruminal fermentation as a means to improve feed efficiency and nutrient utilization (Benchaar et al., 2008). In addition, feeding additional dietary Co in diets has been reported to increase *in vitro* cellulose digestibility (Allen, 1986). Crude protein digestibility tended ($0.05 \leq P < 0.10$) to be higher during P1 than P2, and DMD was greater ($P < 0.05$) during P1 than P2 (Table 7). When evaluating DM, CP, NDF, ADF, and starch digestibility percentages during each phase, there were no differences among cows fed EOC and Control ($P > 0.10$), however a numeric advantage across all nutrient digestibilities were observed (Table 7). Pretz and Casper (2015) reported numerically greater fiber digestibility when cows were fed additional Co, which was reported to have caused the increase ($P < 0.05$) in ruminal percentages of acetate. Additionally, lower rumen ammonia concentrations ($P < 0.05$) were reported and were hypothesized to be associated with increased microbial protein synthesis, though this direct measurement was not taken. An increased rate of DM disappearance for hay and silage *in situ* was documented by Lopez-Guisa and Satter (1992) when adding Co in excess of NRC recommendations to the diet of heifers. Divalent cations, such as cobalt, are believed to serve as a bridge between bacteria and plant cell walls, which tend to be

negatively charged (Lopez-Guisa and Satter, 1992). The work of Benchaar et al. (2007b) and Spanghero et al. (2009) did not report significant differences in apparent organic matter digestibilities between cows fed EO and unsupplemented (control fed) cows, however several other studies have reported increases in ruminal true OM and N digestibility as well as total-tract ADF, NDF, and starch digestibility (Benchaar et al., 2006, Yang et al., 2007, Tekippe et al., 2013). Tekippe et al. (2013) postulated that the lack of response in animal production to increased dietary NDF digestibility during their study was not surprising, and was potentially due to the small magnitude of the digestibility effect and the apparent oversupply of dietary energy.

Application of EOC to Control Mycotoxins and Feed Spoilage

Mycotoxins are secondary metabolites produced by fungi that contaminate feed ingredients and crops (SGS, 2015). Although the formation of mycotoxins are dependent on weather and environmental conditions, when they are present they tend to become concentrated during the processing of crops, presenting a potential hazard to human and animal health (Soliman and Badeaa, 2002). Soliman and Badeaa (2002) determined that the oils of six different medicinal plants were able to completely inhibit all tested fungi, however the extent of inhibition of fungal growth and mycotoxin production was dependent on the concentration of the EO used (Soliman and Badeaa, 2002). During P2, there were greater ($P < 0.05$) amounts of zearalenone and T-2 ($P > 0.10$) than were present during P1. Additionally, ochratoxins were detected in the TMR during P2 and numerically less of these mycotoxins were produced from sample composites of TMR that had been treated with EOC compared to no treatment, although this difference was not significant ($P > 0.10$). During P1, zearalenone, T-2, and vomitoxin were detected in

the TMR composites, however the amounts detected were similar among both diets ($P > 0.10$) (Table 8). Nevertheless, metabolites produced by plants remain a promising alternative to the indiscriminate use of synthetic antifungals, which has led to the development of resistant fungal strains (da Cruz Cabral et al., 2013). This requires higher dietary concentrations of antifungals to achieve the same effect, which has increased the amount of toxic residues in food products. 9

The TMR containing EOC was numerically greater in aerobic stability, however this observation was not significant ($P > 0.10$) (Figure 1). It is worth noting that it took significantly longer for spoiling to occur during P1 than P2 ($P > 0.01$), indicating that the temperature of TMR in buckets was influenced by the seasonal temperatures despite being kept in a climate controlled room.

Impact of Feeding EOC on Intestinal Pathogens

Fecal composites from cows fed EOC were lower ($P < 0.05$) in normal microbiota compared to cows fed the Control. *Streptococcus* sp. were lower ($P < 0.05$) during P1 for cows fed EOC compared to cows fed the Control, but the amounts of *Streptococcus* sp. was similar ($P > 0.10$) during P2. No *Proteus* or *Campylobacter* sp. were detected throughout the trial and the amounts of coliform sp. were similar ($P > 0.10$) in both P1 and P2 for cows fed both diets (Table 9). The components of EO have been demonstrated to inhibit several common pathogens, including *E. coli* O157:H7, *S. aureus*, *L. monocytogenes*, and *Salmonella* sp., however, such a broad spectrum of antimicrobial activity impedes feeding EO to target specific pathogens within the ruminant digestive tract (Benchaar et al., 2008). Essential oils may also combat parasites which commonly reside in the intestine, such as *Cryptosporidium*, coccidia, or nematodes, although this has

not been tested previously (Benchaar et al., 2008). Overall, the degree to which EO inhibits microbial growth in the lower gastrointestinal tract is likely to be dependent on the ability of the antimicrobial components to remain active after passage through the rumen (Benchaar et al., 2008). To our knowledge, research is lacking on the extent to which EO escape the rumen and flow to the lower digestive tract.

CONCLUSIONS

When feeding EO, a variety of beneficial feeding responses have been observed, but the optimum dosage has not been elucidated for lactating dairy cows. Studies have reported that increasing the dietary supply of cobalt results in an increased synthesis of vitamin B_{12}, which appears to influence ruminal parameters by increasing ruminal fiber digestibility and altering VFA ratios. In the present study, feeding EOC to lactating dairy cows influences milk composition and nutrient digestibility, while potentially improving the aerobic stability of feed in the bunk. This study was limited by being unable to collect physiological samples and to monitor rumen VFA profiles. Because we were not able to ascertain carry over effects, in the future it would be worthwhile to compare Control fed cows from P1 to EOC fed cows during P2, as these units were the same pen (and therefore, same animals) from P1 to P2. In the future, examining rumen EO stability would be worthwhile, as well as elucidating the optimal dose of EO on rumen fermentation, fiber digestibility, metabolism, and immunity (Wall et al., 2014). Additionally, work comparing EO to diets where ionophores have been removed would be beneficial.

Table 1. Ingredient composition of the total mixed ration (**TMR**) during Phase 1 and 2.

	% of DM	
Ingredient	Phase 1	Phase 2
Corn silage	35.3	35.7
Grass hay	--	2.13
Alfalfa hay	7.48	9.26
Alfalfa haylage	18.2	18.2
High moisture ear corn	8.49	7.40
Molasses	1.81	1.82
Corn/cottonseed mix	14.0	11.8
EOC[1]	0.001	0.001
Energy Booster[2]	0.76	0.76
Protein mix[3]	13.8	13.0

[1] EOC = essential oil and cobalt lactate blend. This was included only in the diet of the treatment pen during each phase and was fed at a target rate of 28g/hd/d.
[2] Milk Specialties Global, Eden Prairie, MN.
[3] The protein mix contained the following ingredients on a percent DM basis: mineral mix (New Vision Feed, Mankato, MN) (2.87), canola meal (1.92), distillers grains (1.92), 48% CP soybean meal (1.92), SoyPlus (Landus Cooperative) (1.74), bone meal (0.77), blood meal (0.77), liquid fat (0.79), sodium bicarbonate (0.60), corn gluten meal (0.39), corn starch (0.29), urea (0.21), Omnigen (Phibro Animal Health Corporation, Teaneck, NJ) (0.16), yeast culture concentrate (0.11), DCAD plus (0.10), magnesium oxide (0.09), Bio-Mos (Alltech, Lexington, KY) (0.04), Vitamin E, 20,000 IU/kg (0.03), Rumensin (Elanco Animal Health, Greenfield, IL) (0.01), Vitamin AD (<0.01).

Table 2. Calculated nutrient composition of the total mixed ration (**TMR**) in Phases 1 and 2. All values are % of dry matter (**DM**) unless noted otherwise.

	TMR[1]	
Measurement	Phase 1	Phase 2
DM	51.1	47.0
CP	15.5	15.9
UIP[2]	37.5	36.4
SIP[3]	35.0	35.5
CP-NPN[4]	0.32	0.29
NDF	28.9	32.7
ADF	20.1	20.4
ADIP	0.48	0.48
NDIP	1.40	1.40
NFC	41.6	40.6
Starch	26.5	24.1
NE_L (Mcal/kg)	1.70	1.69
Fat	5.32	5.28
Oil	3.85	3.7
IVDMD	80.4	80.2
NDFD, %NDF (30h)	53.1	49.7
IVTD[5]	85.1	84.9
Lignin	3.95	3.56
Ash	7.83	8.25
AIA	0.84	1.27
Ca	1.04	1.01
P	0.41	0.41
Mg	0.36	0.35
K	1.48	1.58
Na	0.60	0.57
Cl	0.42	0.42
S	0.21	0.21
Salt	0.44	0.42
Fe, ppm	270	406
Cu, ppm	20.5	21
Zn, ppm	89.3	107
Mn, ppm	78.2	92.4

[1] The TMR had a forage-to-concentrate ratio of 60:40 (dry matter basis) with the forage ratio containing 58% corn silage, 12% alfalfa hay, and 30% alfalfa haylage during Phase 1. During Phase 2, the TMR had a forage-to-concentrate ratio of 65:35 (dry matter basis) with the forage ratio containing 55% corn silage, 3% grass hay, 14% alfalfa hay, and 28% alfalfa haylage.
[2] UIP = Undegradable intake protein.
[3] SIP = Soluble intake protein.
[4] NPN = Non protein nitrogen.
[5] IVTD = In vitro total digestibility.

Table 3. Nutrient composition (% of dry matter (**DM**) unless otherwise noted) of corn silage, alfalfa haylage, alfalfa hay, grass hay, and robot concentrate fed during Phases 1 (**P1**) and 2 (**P2**).

	Feed Ingredient							
	Corn silage		Alfalfa haylage		Alfalfa hay	Grass Hay	Pellet	
Nutrient	P1	P2	P1	P2	P1 & P2	P2	P1	P2
DM, %	33.8	34.7	36.1	39.8	83.6	84.0	87.3	88.1
RFV[1]	--	--	135	168	146	116	--	--
TDN[2]	71.2	73.2	53.7	60.0	53.5	62.2	--	--
CP	7.05	6.95	22.0	22.8	22.5	14.9	27.4	21.9
SIP[3], % CP	68.1	61.4	66.6	68.9	40.2	39.0	--	27.9
Ammonia CP	0.69	0.52	1.8	0.96	--	--	--	--
NDF	39.7	36.0	40.9	35.4	40.7	49.9	19.1	26.6
ADF	25.7	22.9	37.8	32.12	33.0	34.2	10.9	16.2
ADIP[4]	0.58	0.60	1.92	1.40	1.60	--	--	--
NDIP	1.32	1.30	4.20	3.32	4.74	--	--	--
NFC	46.1	50.3	24.9	32.2	22.1	23.8	--	--
Sugar	1.72	2.50	1.79	1.61	6.21	8.27	--	--
Starch	29.3	33.2	--	--	--	--	22.0	20.1
NE_L (Mcal/kg)	1.62	1.67	1.20	1.35	1.31	1.41	--	--
Fat	3.40	3.24	3.58	4.10	2.31	--	--	--
NDFD, %NDF (30h)	54.3	52.1	42.0	42.2	--	--	--	--
IVSD[5] (7 h)	88.6	86.6	--	--	--	--	--	--
Lignin	4.19	3.05	8.55	7.94	8.24	--	--	--
Ash	4.45	4.18	11.0	10.8	13.5	--	--	--
AIA	--	--	--	--	--	--	0.22	7.81
Ca	0.24	0.23	1.39	1.46	1.52	0.56	0.38	0.42
P	0.24	0.25	0.38	0.40	0.36	0.34	0.85	0.81
Mg	0.17	0.17	0.20	0.30	0.31	0.30	0.43	0.41
K	1.18	1.06	3.13	2.89	3.12	1.91	1.49	1.88
Na	--	--	--	--	--	--	0.07	0.07

Cl	--	--	--	--	--	--	0.12	0.15
S	0.09	0.09	0.22	0.27	0.27	0.20	0.34	0.31
Salt	--	--	--	--	--	--	--	--
Fe, ppm	--	--	--	--	--	--	248	237
Cu, ppm	--	--	--	--	--	--	24	15.8
Zn, ppm	--	--	--	--	--	--	104	118
Mn, ppm	--	--	--	--	--	--	159	80.3
pH, 0-14	3.77	3.76	4.56	4.40	--	--	--	--
Lactic acid	4.69	4.34	6.30	6.25	--	--	--	--
Acetic acid	1.05	0.69	1.78	0.49	--	--	--	--
Propionic acid	<0.01	<0.01	<0.01	<0.01	--	--	--	--
Butyric acid	--	--	<0.25	<0.25	--	--	--	--

[1] RFV = Relative feed value.
[2] TDN = Total digestible nutrients.
[3] SIP = Soluble intake protein.
[4] ADIP = Acid detergent insoluble protein.
[5] IVSD = In vitro starch disappearance.

Table 4. Milk production and milk component composition for cows during Phase 1 and 2 fed Control or essential oil and cobalt blend (**EOC**).[1]

	Phase 1		Phase 2			*P* <		
Measurement	Control	EOC	Control	EOC	SEM	Treatment	Phase	TRT*Phase
Milk, kg/d	41.4	40.8	44.0	41.4	1.27	NS	NS	NS
MLM[2], kg	39.1	39.1	40.7	42.2	1.17	NS	*	NS
ECM[3], kg	37.8	37.9	39.0	40.5	1.11	NS	NS	NS
FCM[4], kg	35.0	35.3	36.2	37.5	1.04	NS	NS	NS
Fat, %	3.28	3.34	3.28	3.26	0.05	NS	NS	NS
Protein, %	2.96*	2.91	2.91*	2.94	0.02	NS	NS	†
Fat, kg	1.29	1.31	1.33	1.38	0.04	NS	NS	NS
Protein, kg	1.16	1.14	1.18	1.23	0.07	NS	NS	NS

*Main effect means differ, $P \leq 0.05$.

** Main effect means differ, $P < 0.01$.

† $0.05 < P \leq 0.10$.

NS = Non-significant, $P > 0.10$.

[1] Energy corrected milk (**ECM**), fat corrected milk (**FCM**), fat (kg), and protein (kg) are based on management level milk (**MLM**) calculations.

[2] Management level milk = ((29.15 x Milk Factor) + (12.3 x Fat Factor x Test-day Fat percent) + (6.56 x Prot Factor x Test-day Prot percent)) x Test-day Milk kg/100.

[3] Energy corrected milk = (0.327 x kg of milk) + (12.95 x kg of milk fat) + (7.2 x kg of milk protein).

[4] Fat corrected milk = (0.4 x kg of milk) + (15 x kg of milk fat).

Table 5. Dairy Herd Improvement Association (**DHIA**) data on milk production and milk components for cows during Phase 1 and 2 fed Control or essential oil and cobalt blend (**EOC**).[1]

	Phase 1		Phase 2			*P* <		
Measurement	Control	EOC	Control	EOC	SEM	Treatment	Phase	TRT*Phase
Fat, %	3.36[ab]	3.54[a]	2.95[c]	3.21[b]	0.09	*	**	NS
Protein, %	3.02	3.05	2.94	3.01	0.04	NS	**	NS
Lactose, %	4.98	4.90	4.92	4.92	0.03	NS	NS	NS
Fat, kg	1.27[b]	1.44[a]	1.09[c]	1.23[bc]	0.20	**	**	NS
Protein, kg	1.12	1.21	1.10	1.13	0.04	NS	NS	NS
Lactose, kg	2.05	2.03	1.84	1.85	0.11	NS	**	NS
SCC[2], cells/mL	158	210	168	132	27.8	NS	NS	NS
MUN[3], mg/dL	16.5[a]	14.9[b]	17.1[a]	16.9[a]	0.30	**	**	*

[a-c] Means within a row with different superscripts differ ($P < 0.05$).
*Main effect means differ, $P \leq 0.05$.
** Main effect means differ, $P < 0.01$.
NS = Non-significant, $P > 0.10$.
[1] Fat (kg), protein (kg), and lactose (kg) are based on management level milk (**MLM**) calculations.
[2] Somatic cell count, x1000.
[3] Milk urea nitrogen.

Table 6. Dry mater intake (**DMI**), feed efficiency, body condition score (BCS), body weight, and body temperature for cows during Phase 1 and Phase 2 for cows fed Control or essential oil and cobalt blend (**EOC**).[1]

	Phase 1		Phase 2			*P* <		
Measurement	Control	EOC	Control	EOC	SEM	Treatment	Phase	TRT*Phase
Total DMI, kg	25.5[b]	26.3[a]	24.3[c]	24.3[c]	0.38	**	**	**
Feed efficiency[2]	1.54	1.50	1.68	1.74	0.04	NS	NS	NS
BCS[3]	3.03	3.05	3.19	3.26	0.03	NS	**	NS
Body weight, kg	616[c]	657[ab]	676[a]	627[bc]	42.0	NS	NS	**
Body temperature, °C	29.5	28.7	26.7	25.1	2.30	NS	*	NS

[a-c] Means within a row with different superscripts differ, $P < 0.05$.

*Main effect means differ, $P \leq 0.05$.

** Main effect means differ, $P < 0.01$.

NS = Non-significant, $P > 0.10$.

[1] Total DMI includes total mixed ration (TMR) and concentrate DMI.

[2] Kilograms of milk produced per kilogram of DM consumed.

[3] Body condition score, 1-5.

Table 7. Nutrient digestibility by cows for Phase 1 and 2 for cows fed Control or essential oil and cobalt blend (**EOC**) as determined by using acid insoluble ash (**AIA**) as an internal marker.

	Phase 1		Phase 2			*P* <		
Measurement	Control	EOC	Control	EOC	SEM	Treatment	Phase	TRT*Phase
Dry matter, %	72.5	74.7	69.3	71.0	1.61	NS	*	NS
Crude protein, %	73.7	76.3	70.2	71.6	2.17	NS	†	NS
NDF[1], %	50.6	54.4	46.7	49.8	2.90	NS	NS	NS
ADF[2], %	50.1	54.3	49.8	52.2	2.80	NS	NS	NS

*Main effect means differ, $P \leq 0.05$.
† $0.05 < P \leq 0.10$.
NS = Non-significant, $P > 0.10$.
[1] Neutral detergent fiber.
[2] Acid detergent fiber.

Table 8. Effects of treatment[1] on measures of mycotoxins in the total mixed ration for Phase 1 and Phase 2. Units are in ppm unless specified otherwise.

	Phase 1		Phase 2			*P* <		
Measurement	Control	EOC	Control	EOC	SEM	Treatment	Phase	TRT*Phase
Zearalenone	62.0	66.4	81.8	73.8	4.14	NS	*	NS
T2	51.7	58.8	56.2	52.0	4.44	NS	NS	NS
Ochratoxin	0	0	2.09	1.45	1.27	NS	NS	NS
Fumonisin, ppb	0	0	0	0	--	--	--	--
Vomitoxin, ppb	0.63	0.65	0.75	0.77	0.07	--	--	--
Aflatoxin	0	0	0	0	--	--	--	--

*Main effect means differ ($P \leq 0.05$).

NS = Non-significant, $P > 0.10$.

[1] Control = no supplementation of EOC; EOC = 28 g/hd/d of EOC.

Table 9. Measurements of normal microbiota, yeast, mold, and fecal pathogens for Phase 1 and 2 for cows fed Control or essential oil and cobalt blend (**EOC**).[1]

	Phase 1 (N=13)		Phase 2 (N=11)			*P* <		
Measurement	Control	EOC	Control	EOC	SEM	Treatment	Phase	TRT*Phase
Normal microbiota	4.09^{a}	3.36^{bc}	3.61^{ab}	3.15^{c}	0.19	*	NS	NS
Bacillus sp.	1.64^{ab}	1.18^{ab}	0.44^{b}	1.91^{a}	0.39	*	NS	*
Streptococcus sp.	0.64^{b}	$5.12x10^{-17c}$	2.98^{a}	3.26^{a}	0.31	NS	**	NS
Coliform sp.	3.95	4.00	3.95	3.67	0.17	NS	NS	NS
Proteus sp.	ND	ND	ND	ND	--	--	--	--
Campylobacter sp.	ND	ND	ND	ND	--	--	--	--

[a-c] Means within a row with different superscripts differ ($P < 0.05$).

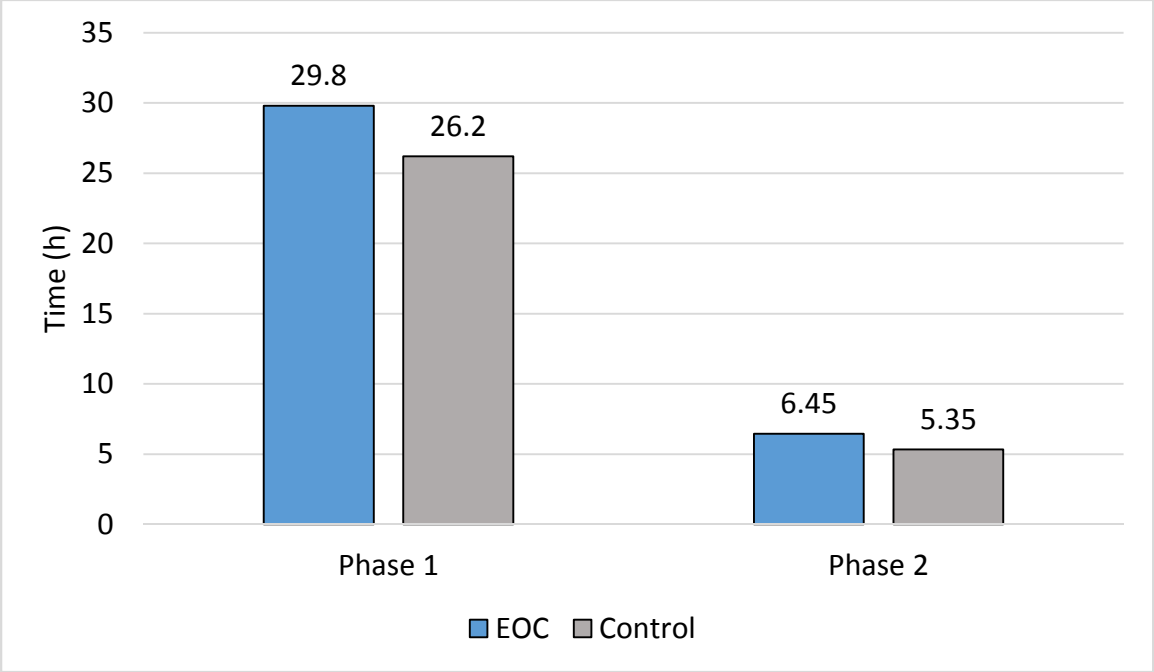
*Main effect means differ, $P \leq 0.05$.

** Main effect means differ, $P < 0.01$.

NS = Non-significant, $P > 0.10$.

[1] Numerical values assigned to fecal pathogen results are as follows: 1 = not detected; 2 = trace/rare/few; 3 = small/small amount 1 phenotype *E. coli*; 3.5 = small/small amount 2 phenotype *E. coli*; 4 = moderate/moderate amount 1 phenotype *E. coli*; 4.5 = moderate/moderate amount 2 phenotype *E. coli*; 5 = large/large amount 1 phenotype *E. coli*; 5.5 = large/large amount 2 phenotype *E. coli*.

Figure 1. Total mixed ration aerobic stability (time until 2°C rise).



LITERATURE CITED

Akins, M. S., S. J. Bertics, M. T. Socha, and R. D. Shaver. 2013. Effects of cobalt supplementation and vitamin B12 injections on lactation performance and metabolism of Holstein dairy cows. J. Dairy Sci. 96:1755-1768.

Allen, M. 1986. Effects of cobalt supplementation on carbohydrate and nitrogen utilization by ruminal bacteria in continuous culture. M.S. Thesis. Univ. Minnesota, St. Paul.

Baskaran, S. A., G. W. Kazmer, L. Hinckley, S. M. Andrew, and K. Venkitanarayanan. 2009. Antibacterial effect of plant-derived antimicrobials on major bacterial mastitis pathogens in vitro. J. Dairy Sci. 92(4):1423-1429.

Bauman, D. E. and J. L. Capper. 2011. Sustainability and dairy production: challenges and opportunities. Proc. Cornell Nutr. Conf. Feed Manuf.:136-153.

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

Benchaar, C., A. V. Chaves, G. R. Fraser, Y. Wang, K. A. Beauchemin, and T. A. McAllister. 2007a. Effects of essential oils and their components on in vitro rumen microbial fermentation Erratum: 2008 June, v. 88, no. 2, p. 341. Canadian journal of animal science (3).

Benchaar, C., H. V. Petit, R. Berthiaume, D. R. Ouellet, J. Chiquette, and P. Y. Chouinard. 2007b. Effects of Essential Oils on Digestion, Ruminal Fermentation,

Rumen Microbial Populations, Milk Production, and Milk Composition in Dairy Cows Fed Alfalfa Silage or Corn Silage1. J. Dairy Sci. 90(2):886-897.

Benchaar, C., H. V. Petit, R. Berthiaume, T. D. Whyte, and P. Y. Chouinard. 2006. Effects of Addition of Essential Oils and Monensin Premix on Digestion, Ruminal Fermentation, Milk Production, and Milk Composition in Dairy Cows. J. Dairy Sci. 89(11):4352-4364.

Borchers, R. 1965. Proteolytic Activity of Rumen Fluid In Vitro1. J. Anim. Sci. 24(4).

Briskin, D. P. 2000. Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. Plant Physiol. 124(2):507-514.

Broderick, G. A. and J. E. Balthrop. 1979. Chemical Inhibition of Amino Acid Deamination by Ruminal Microbes in Vitro1 ,2. J. Anim. Sci. 49(4).

Burt, S. 2004. Review: Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. 94:223-253.

Callaway, T. R., T. S. Edrington, J. L. Rychlik, K. J. Genovese, T. L. Poole, Y. S. Jung, K. M. Bischoff, R. C. Anderson, and D. J. Nisbet. 2003. Ionophores: Their Use as Ruminant Growth Promotants and Impact on Food Safety. Curr. Issues Intestinal Microbiol.

Canillac, N. and A. Mourey. 2001. Antibacterial activity of the essential oil of Picea excelsa on Listeria, Staphylococcus aureus and coliform bacteria. Food Microbiol. 18(3):261-268.

Cox, S. D., C. M. Mann, J. L. Markham, H. C. Bell, J. E. Gustafson, J. R. Warmington, and S. G. Wyllie. 2000. The mode of antimicrobial action of the essential oil of Melaleuca alternifolia (tea tree oil). J. Appl. Microbiol. 88(1):170-175.

da Cruz Cabral, L., V. Fernández Pinto, and A. Patriarca. 2013. Application of plant derived compounds to control fungal spoilage and mycotoxin production in foods. Int. J. Food Microbiol. 166(1):1-14.

Damon, C. 1966. Hawk's physiological chemistry. 14th ed. McGraw-Hill, New York, NY.

Delaquis, P. J., K. Stanich, B. Girard, and G. Mazza. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. Int. J. Food Microbiol. 74:101-109.

Dorman, H. J. D. and S. G. Deans. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J. Appl. Microbiol. 88(2):308.

Elliot, J. M., D. E. Hogue, and H. F. Tyrrell. 1965. Blood Vitamin B12 Status of the Dairy Cow in Late Pregnancy and Early Lactation1. J. Dairy Sci. 48(10):1335-1338.

Erasmus, L. J. and E. C. Webb. 2013. The effect of production system and management practices on the environmental impact, quality and safety of milk and dairy products. S. Afr. J. Anim. Sci. 43(3):425-434.

FDA. 2004. Food and Drug Administration of the US. 21 CFR 184 Online.(Available at: http://www.cfsan.fda.gov/eafus.html).

Girard, C. L., D. E. Santschi, S. P. Stabler, and R. H. Allen. 2009. Apparent ruminal synthesis and intestinal disappearance of vitamin B12 and its analogs in dairy cows. J. Dairy Sci. 92(9):4524-4529.

Godfray, H. C. J., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas, and C. Toulmin. 2010. Food Security: The

Challenge of Feeding 9 Billion People. Page 812. American Association for the Advancement of Science.

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analyses. [electronic resource] : (apparatus, reagents, procedures, and some applications). Agriculture handbook: no. 379. Washington, D.C. : Agricultural Research Service, U.S. Dept. of Agriculture, 1970.

Greathead, H. 2003. Plants and plant extracts for improving animal productivity. Proc Nutr Soc 62(2):279-290.

Judson, G. J., J. D. McFarlane, A. Mitsioulis, and P. Zviedrans. 1997. Vitamin B12 responses to cobalt pellets in beef cows. Aust. Vet. J. 75(9):660-662.

Kawashima, T., P. R. Henry, C. B. Ammerman, R. C. Littell, and J. Price. 1997. Bioavailability of cobalt sources for ruminants. 2. Estimation of the relative value of reagent grade and feed grade cobalt sources from tissue cobalt accumulation and vitamin B12 concentrations. Nutrition research (New York, N.Y.) (USA) (6):957.

Kincaid, R. L., L. E. Lefebvre, J. D. Cronrath, M. T. Socha, and A. B. Johnson. 2003. Effect of Dietary Cobalt Supplementation on Cobalt Metabolism and Performance of Dairy Cattle. J. Dairy Sci. 86(4):1405-1414.

Kincaid, R. L. and M. T. Socha. 2007a. Article: Effect of Cobalt Supplementation During Late Gestation and Early Lactation on Milk and Serum Measures1. J. Dairy Sci. 90:1880-1886.

Kincaid, R. L. and M. T. Socha. 2007b. Effect of Cobalt Supplementation During Late Gestation and Early Lactation on Milk and Serum Measures1. J. Dairy Sci. 90(4):1880-1886.

Kleinschmit, D. H. and L. Kung, Jr. 2006. A Meta-Analysis of the Effects of *Lactobacillus buchneri* on the Fermentation and Aerobic Stability of Corn and Grass and Small-Grain Silages. J. Dairy Sci. 89(10):4005-4013.

Krishnamoorthy, U., T. V. Muscato, C. J. Sniffen, and P. J. Van Soest. Nitrogen Fractions in Selected Feedstuffs. J. Dairy Sci. 65(2):217-225.

Lambert, R. J. W., P. N. Skandamis, P. J. Coote, and G. J. E. Nychas. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J. Appl. Microbiol. 91(3):453-462.

Lopez-Guisa, J. M. and L. D. Satter. 1992. Effect of copper and cobalt addition on digestion and growth in heifers fed diets containing alfalfa silage or corn crop residues. Journal of dairy science (USA) (1):247.

Marten, G. C. and R. F. Barnes. 1980. Prediction of Energy Digestibility of Forages with In Vitro Rumen Fermentation and Fungal Enzyme Systems, in Standardization of analytical methodology for feeds: Proceedings of a workshop held in Ottawa, Canada. 12-14 March 1979. Ottawa, Ont. IDRC.

Martens, J.-H., H. Barg, M. Warren, and D. Jahn. 2002. Microbial production of vitamin B12. Appl. Microbiol. Biotechnol. 58(3):275-285.

McDowell, L. R. 1992. Minerals in animal and human nutrition. Animal feeding and nutrition. San Diego : Academic Press, c1992.

Mertz, W. and E. J. Underwood. 1986. Trace elements in human and animal nutrition. 5th ed. San Diego, CA. Aacademic Press, 1986-1987.

Mills, C. F. 1981. Cobalt deficiency and cobalt requirements of ruminants. W. Haresign, Ed. Recent Advances in Animal Nutrition:129-140.

Muck, R. E. and J. T. Dickerson. 1988. Storage Temperature Effects on Proteolysis in Alfalfa Silage. Trans. ASAE 31(4):1005-1009.

Nagy, J. G. and R. P. Tengerdy. 1967. Antibacterial Action of Essential Oils of Artemisia as an Ecological Factor: I. Antibacterial Action of the Volatile Oils of Artemisia tridentata and Artemisia nova on Aerobic Bacteria. Applied Microbiology 15(4):819-821.

Nutrient Requirements of Dairy Cattle, S. R. E. 2001. National Academy Press.

Oh, H. K., T. Sakai, M. B. Jones, and W. M. Longhurst. 1967. Effect of Various Essential Oils Isolated from Douglas Fir Needles upon Sheep and Deer Rumen Microbial Activity. Applied Microbiology 15(4):777-784.

Orion Research, Inc. 1977. Analytical Methods Guide. Cambridge, MA. 33 p.

Orth, R. 1992. Sample day and lactation report. DHIA 200 Fact Sheet A-2. Mid-States Dairy Records Processing Center, Ames, IA.

Pretz, J. and D. P. Casper. 2015. Cobalt-lactate inclusion in a high forage total mixed ration fed to late lactation dairy cows. PhD Dissertation. South Dakota State Univ.

Reinhardt, C. D. 2013. Antimicrobial Feed Additives. The Merck Veterinary Manual.

Rohweder, D. A., R. F. Barnes, and N. Jorgensen. 1978. Proposed Hay Grading Standards Based on Laboratory Analyses for Evaluating Quality1. J. Anim. Sci. 47(3):747-759.

Santos, M. B., P. H. Robinson, P. Williams, and R. Losa. 2010. Effects of addition of an essential oil complex to the diet of lactating dairy cows on whole tract digestion of nutrients and productive performance. Anim. Feed Sci. Technol. 157:64-71.

SGS. 2015. Analytical Services: Mycotoxin Testing.

Sherwood, L. 2010. Human Physiology: From Cells to Systems (7e). Brooks/Cole, Cengage Learning.

Smith-Palmer, A., J. Stewart, and L. Fyfe. 1998. Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. Lett. Appl. Microbiol. 26(2):118-122.

Smith, R. M. and L. H. R. Marston. 1970. Production, absorption, distribution and excretion of vitamin B12 in sheep. British Journal of Nutrition 24(04):857-877.

Soliman, K. M. and R. I. Badeaa. 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food & Chemical Toxicology 40(11):1669.

Spanghero, M., P. H. Robinson, C. Zanfi, and E. Fabbro. 2009. Effect of increasing doses of a microencapsulated blend of essential oils on performance of lactating primiparous dairy cows. Anim. Feed Sci. Technol. 153(1–2):153-157.

Stemme, K., P. Lebzien, G. Flachowsky, and H. Scholz. 2008. The influence of an increased cobalt supply on ruminal parameters and microbial vitamin B12

synthesis in the rumen of dairy cows. Archives of Animal Nutrition 62(3):207-218.

Sutton, A. L. and J. M. Elliot. 1972. Effect of ratio of roughage to concentrate and level of feed intake on ovine ruminal vitamin B_{12} production. J. Nutr. 102:1341-1346.

Tager, L. R. and K. M. Krause. 2011. Effects of essential oils on rumen fermentation, milk production, and feeding behavior in lactating dairy cows. J. Dairy Sci. (5).

Tassoul, M. D. and R. D. Shaver. 2009. Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows. J. Dairy Sci. 92(4):1734-1740.

Tavernise, S. 2013. F.D.A. Restricts Antibiotics Use for Livestock. The New York Times.

Tekippe, J. A., R. Tacoma, A. N. Hristov, C. Lee, J. Oh, K. S. Heyler, T. W. Cassidy, G. A. Varga, and D. Bravo. 2013. Effect of essential oils on ruminal fermentation and lactation performance of dairy cows. J. Dairy Sci. (12).

Tiffany, M. E., V. Fellner, and J. W. Spears. 2006. Influence of cobalt concentration on vitamin B12 production and fermentation of mixed ruminal microorganisms grown in continuous culture flow-through fermentors. J. Anim. Sci. 84(3):635-640.

Ultee, A. and E. P. W. Kets. 1999. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. Applied & Environmental Microbiology 65(10):4606.

Underwood, E. J. and N. F. Suttle. 1999. Cobalt. Pages 251-282. The Mineral Nutrition of Livestock. CABI Publishing, New York, NY.

Van Keulen, J. and B. A. Young. 1977. Evaluation of Acid-Insoluble Ash as a Natural Marker in Ruminant Digestibility Studies1 ,2. J. Anim. Sci. 44(2):282-287.

Walker, C. K. and J. M. Elliot. 1972. Lactational trends in vitamin B12 status on conventional and restricted-roughage rations. Pages 474-479.

Wall, E. H., P. H. Doane, S. S. Donkin, and D. Bravo. 2014. The effects of supplementation with a blend of cinnamaldehyde and eugenol on feed intake and milk production of dairy cows. J. Dairy Sci. 97:5709-5717.

Wildman, E. E., G. M. Jones, P. E. Wagner, H. F. Troutt, Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics Milk production. Journal of Dairy Science (USA) (3):495.

Yang, W. Z., C. Benchaar, B. N. Ametaj, A. V. Chaves, M. L. He, and T. A. McAllister. 2007. Effects of Garlic and Juniper Berry Essential Oils on Ruminal Fermentation and on the Site and Extent of Digestion in Lactating Cows1. J. Dairy Sci. 90(12):5671-5681.

Young, R. 1979. Cobalt in biology and biochemistry. London: Academic Press.