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An Evaluation of Rumen Modifiers for Lactational Performance and Nutrient Digestibility by Cows

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AN EVALUATION OF RUMEN MODIFIERS FOR LACTATIONAL PERFORMANCE AND NUTRIENT DIGESTIBILITY BY COWS

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree in Biological Science and is acceptable for meeting the thesis requirement 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

Ad lib	Ad libitum
ADICP	Acid Detergent Insoluble Crude Protein
ADIP	Acid Detergent Insoluble Protein
ADY	Active Dried Yeast
AIA	Acid Insoluble Ash
AOAC	Association of Official Analytical Chemists
BHBA	Beta Hydroxybutyric Acid
CH_4	Methane
Co	Cobalt
d	Day
DCAD	Dietary Cation Anion Difference
DHIA	Dairy Herd Improvement Association
DIM	Days in Milk
DM	Dry Matter
DMD	Dry Matter Digestibility
DMI	Dry Matter Intake
DRTF	Dairy Research and Training Facility
EO	Essential Oils
FCM	Fat Corrected Milk
FE	Feed Efficiency
GFB	Gas Fermentation Bottle
h	Hour

HCl	Hydrochloric Acid
hd	Head
IVSD	In Vitro Starch Disappearance
IVTD	In Vitro Total Digestibility
L	Liter
LH	Luteinizing Hormone
ME	Metabolizable Energy
mg	Milligram
MLM	Management Level Milk
mM	milli molar
MUN	Milk Urea Nitrogen
N	Nitrogen
ND	Nutrient Digestibility
NDF	Neutral Detergent Fiber
NDFD	Neutral Detergent Fiber Digestibility
NDIP	Neutral Detergent Insoluble Protein
NE	Net Energy
NEFA	Non-esterified Fatty Acid(s)
NEg	Net Energy of Growth
NE_L	Net Energy of Lactation
NEm	Net Energy of Maintenance
NFC	Non-fiber Carbohydrate
NH_3	Ammonia

NPN	Non Protein Nitrogen
NRC	National Research Council
NRC	National Research Council
OM	Organic Matter
OMD	Organic Matter Digestibility
ppm	Parts per million
psi	Pound per square inch
RCBD	Randomized Complete Block Design
RFQ	Relative Forage Quality
RFV	Relative Feed Value
SCC	Somatic Cell Count
SCFA	Short Chain Fatty Acid
SDSU	South Dakota State University
SIP	Soluble Intake Protein
SP	Soluble Protein
T4C	Time for Cows – Lely Robotic Milking Software
TDN	Total Digestible Nutrients
TMR	Total Mixed Ration
VFA	Volatile Fatty Acids

ABSTRACT

AN EVALUATION OF RUMEN MODIFIERS FOR LACTATIONAL PERFORMANCE AND NUTRIENT DIGESTIBILITY BY COWS

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Three studies were conducted to evaluate a commercial blend of essential oils, active dried yeast product and cobalt lactate on production performance, rumen fermentation and nutrient digestibility by cows by using *in vivo* and *in vitro* methods. The first study evaluated the lactational performance and nutrient digestibility of lactating Holstein dairy cows fed two commercial supplements: 1) a propriety blend of essential oils and Cobalt lactate (EOC) (Stay Strong, Ralco, Inc., Marshall, MN) and 2) active dried yeast (ADY) (Omnigen AF, Prince Agri Products, Inc., Quincy, IL). The 12-week experiment demonstrated no differences in milk production, milk composition, and feed efficiency. However, cows fed EOC were cooler than cows fed ADY. Both commercial products prevented the growth of *Aspergillus fumigatus*. Nutrient digestibility (ND) was higher for cows fed ADY than cows fed EOC. This study demonstrated that while milk production and FE were similar, lactating dairy cows fed EOC were cooler during the hot summer/fall season, whereas ND was significantly higher for the cows fed ADY. In the second *in vitro* study, cobalt carbonate ($CoCO_3$) and cobalt lactate (CoL: CoMax, Ralco, Inc., Marshall, MN) inclusion rates were evaluated for effects on ruminal fermentation and nutrient digestibility when feeding a grass hay as substrate. Treatments included 1) Blank: No feed and no $CoCO_3$; 2) $CoCO_3$ - 0.1 ppm; 3) $CoCO_3$ -3.5 ppm; 4) CoL - 0.11 ppm; 5) CoL - 0.22 ppm; 6) CoL - 0.875 ppm; 7) CoL - 1.75 ppm; and 8) CoL - 3.5 ppm.

Rate of gas production was lower ($P < 0.05$) for 0.1 ppm of $CoCO_3$ compared to 3.5 ppm of $CoCO_3$, and intermediate for 0.11, 0.22, 0.875, and 0.11 ppm of CoL. Total VFA (mmol/L) concentrations were lower ($P < 0.05$) for 0.22 and 3.5 ppm of CoL compared to remaining treatments. Molar percentage of acetate was lower ($P < 0.05$) for 1.75 and 3.5 ppm CoL than remaining treatments. Rumen ammonia concentration was similar ($P > 0.10$) for all treatments. Dry matter digestibility was highest ($P < 0.05$) for 0.11 ppm of CoL and was intermediate for 0.1, 3.5 ppm of $CoCO_3$, and 0.22 ppm of CoL. Digestibility of NDF was higher ($P < 0.05$) for 0.11 ppm of CoL, intermediate for 0.1, 3.5 ppm of $CoCO_3$ and 0.22 ppm of CoL and lower ($P < 0.05$) for 0.875, 1.75, and 3.5 ppm of CoL than other treatments. Results show that lower doses of CoL are more effective for fiber digestion of grass hay than $CoCO_3$. The third *in vitro* study evaluated $CoCO_3$ and CoL on ruminal fermentation and nutrient digestibility evaluating a 60:40 (DM basis) blend of corn silage and alfala baleage as a typical dairy ration substrate. Treatments included 1) Blank: No feed and no treatment, 2) $CoCO_3$ - 3.5 ppm, 3) CoL - 0.11 ppm, 4) CoL - 0.22 ppm, 5) CoL - 0.875 ppm, 6) CoL - 1.75 ppm, and 7) CoL - 3.5 ppm. Rate of gas production was similar ($P > 0.10$) among treatments. Total VFA concentrations (mmol/L) were higher ($P < 0.05$) for 0.11 ppm of CoL, intermediate for 0.22, 0.875, 1.75, and 3.5 ppm of CoL and lowest ($P < 0.05$) for 3.5 ppm of $CoCO_3$. Acetate molar percentage was higher ($P < 0.05$) for 3.5 ppm of $CoCO_3$, intermediate for 0.11, 1.75, and 3.5 ppm of CoL and lowest for 0.22 and 0.875 ppm of CoL. Molar propionate concentration was higher ($P < 0.05$) for all CoL treatments compared to 3.5 ppm $CoCO_3$. Rumen NH_3-N and pH were similar ($P > 0.10$) among the treatments. The DMD was lower ($P < 0.05$) for 3.5 ppm $CoCO_3$, intermediate for 0.11 and 3.5 ppm of

CoL and highest ($P < 0.05$) for 0.11, 0.22, 0.875, and 1.75 ppm of CoL. Results demonstrate that Cobalt Lactate is more effective than $CoCO_3$ for improving fiber digestion with a corn silage and alfalfa baelage based forage.

CHAPTER 1:

LITERATURE REVIEW

Introduction

The rumen is a fermentation vat, which is capable of producing the end products, VFA and microbial protein, as a major source of energy and proteins for the host animal by converting forages and fibrous feeds. The principle is that the more stabilized the rumen the better will be the fermentation, which produces more desired end products. To keep the rumen stabilized, its ecology should be maintained at optimum level. This can be done by keeping rumen microbes (bacteria, protozoa, and fungi) active and functional, which is possible by maintaining optimal pH, ammonia, VFAs, and microbial protein. The balanced situation of these parameters prevents unfavorable conditions of rumen, like acidosis. To maximize animal productivity and increase feed efficiency, nutritionists have continued their efforts to improve fermentation but there is still a challenge in ruminant nutrition to integrate biological constraints in feeding practices to maximize ruminant production by optimizing ruminal fermentation. Therefore, considerable efforts have concentrated on the potential methods to modify rumen fermentation by optimizing rumen functions for increasing animal production. The overall goal of modifying rumen fermentation is to enhance microbial fermentation for producing more end products, i.e. VFAs and microbial protein, which will contribute to improve animal production. Experiments (*in vivo* and *in vitro*) were conducted to evaluate the rumen modifiers viz. essential oils, active dried yeast, and cobalt for the performance and nutrient digestibility of dairy cows. The first study was aimed to evaluate the inclusion of a blend of essential oils and cobalt lactate and active dried yeast products on lactational performance and

nutrient digestibility. Another two studies were conducted *in vitro* to evaluate cobalt carbonate and cobalt lactate on ruminal fermentation and digestibility of grass hay and a blend of corn silage and alfalfa balaege using a gas production measurement system.

The objective of this literature review is to review and summarize the past and current scientific literature of essential oils, cobalt, and yeast as rumen modifiers in the diet of dairy cows and its effectiveness on milk production, feed efficiency, DMI, VFA production, and nutrient digestibility in different feeding conditions.

Introduction: Essential Oils

Essential oils (EO) are aromatic oily liquids, which occur in edible, medicinal, and herbal plants that are commonly extracted by steam distillation or solvent extraction (Kung et. al., 2008 and Benchaar et. al., 2008). They can be extracted from different plant parts of a plant including leaves, buds, flowers, stems, seeds, roots, twigs, fruits, roots and bark and are stored in secretary cells, canals, epidermis or glandular trichomes (Bakkali et. al., 2008; Miguel, 2010). The quality, quantity and composition of EO can vary among the different parts of the same plant (Dorman and Deans, 2000), the maturity of the plant (Delaquis et. al., 2002), and the environment in which the plant was grown (Cosentino et. al., 1999; Masotti et. al., 2003; Angioni et. al., 2006). Most of the commercialized EO are chemo typed by gas chromatography and mass spectrometry in order to obtain EO of constant composition. As they are known for their bactericidal, virucidal and fungicidal, and medicinal properties, they are used in preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthetic remedies (Bakkali et. al., 2008). Additives, antagonistic and synergistic effects have occurred between EO components (Burt, 2004). This suggests that the combinations

of EO of different composition, or specific combinations of EO secondary metabolites may result in additive and/or synergistic effects which may increase the rumen microbial fermentation efficiency. As a result, there are a number of commercial products that are currently available in the market, but limited research has been conducted evaluating these commercial products (Benchaar, 2009).

Mode of Action of EO

Essential oils are chemically mixtures of principally terpenoids, mainly monoterpenes (C10) and sequisterpenes (C15), some diterpenes (C20) may also be present. A variety of low molecular weight aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones and sometimes N- and S- containing compounds, coumarins and homologues of phenylpropanoids are present (Dorman and Deans, 2000). They are very complex natural mixtures which may contain 20-60 components at quite different concentrations. Two or three major components are present at high concentrations compared to other components present in trace amounts (Bakkali et. al., 2008). The specific mode of action of EO is poorly characterized (Helander et al., 1998). The phenolic components of EO are mainly responsible for antibacterial properties (Cosentino et al., 1999). The EO active components are hydrophobic in nature and they dwell in the lipid bilayer of membranes of microbes. These compounds can change the membrane function and permeability of the lipid bilayer by interacting with membrane proteins, or by diffusing into the cytoplasm to alter cell membrane metabolic process (Burton and Erskine, 2003). The loss of membrane stability results in the leakage of ions across the cell membrane resulting in a decrease in membrane ionic gradient. To counter balance these effects the bacteria

diverts large amount of energy into it, which results in slower bacterial growth (Calsamiglia et al., 2007).

Antimicrobial activity of Essential oils

Chao et al. (2000) conducted a study for determining the inhibitory activity of EO on selected bacteria, yeast, molds, and viruses. Of the oils tested, all oils exhibited inhibition activity relative to control. They found that Gram negative bacteria were generally more resistant than Gram positive bacteria to oil treatment. *Pseudomonas aeruginosa* was the most resistant bacteria. Cinnamon bark (*Cinnamomum zeylanicum*) and tea tree (*Melaleuca alternifolia*) oils were found inhibitory effect against all the tested organisms and phage. Coriander oil (*Coriandrum sativum*) highly inhibited Gram positive bacteria and fungi. Lemongrass (*Cybopogin flexuosus*) and Roman chamomile (*Chamaemelum nobile*) oils showed high degree of inhibition against both phage types.

Si et al. (2006) carried out a study of antimicrobial study of essential oils/compounds by determining the inhibition of bacterial growth. About 80% inhibition was found towards *Salmonella typhimurium* DT104 and *Escherichia coli* O157:H7 among the 66 essential oils/compounds tested. Most of them demonstrated high efficacy against *S. typhimurium* DT104, *E. coli* O157:H7, and *E. coli* with K88 pili with little inhibition towards lactobacilli and bifidobacteria. They were found tolerant to the low pH. These oils retained their efficacy against *E. coli* O157:H7 when the bacteria were mixed with pig cecal digesta. In addition, they inhibited *E. coli* and coliform bacteria in the digesta, but had little effect on the total number of lactobacilli and anaerobic bacteria.

Burt, (2004) reviewed the antibacterial properties of EO and their potential use in foods. *In vitro* studies using EO have demonstrated antibacterial activity against *Listeria monocytogens, Salmonella typhimurium, Escherichia coli O157:H7, Shigella dysenteria, Bacillus cereus* and *Staphylococcus aureus*. Gram negative organisms are slightly less susceptible than gram positive bacteria. As EO comprise a large number of components, their mode of action involves several targets in the bacterial cell. The hydrophobicity of EO enables them to penetrate in the lipids of the cell membrane and mitochondria, making them permeable and leaking the cell contents. Low oxygen levels, low pH, and low temperature improve the action of EO.

Hammer et al. (1999) evaluated the antimicrobial activity of EO and other plant extracts. They investigated 52 plant oils and extracts for their activity against *Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella puemoniae, Pseudomonas aerogenosa, Salmonella enterica, Serratia marcescens* and *Staphylococcus aureus* using an agar dilution method. Lemon grass, oregano, and bay inhibited all microbes.

Baskaran et al. (2009) investigated the antimicrobial effects of plant-derived antimicrobials *trans*-cinnamaldehyde, eugenol, carvacrol, and thymol on the major bacterial mastitis pathogens *Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus aureus,* and *Escherichia coli* on milk. All the plant-derived molecules exhibited antimicrobial activity against the mastitis pathogens tested, but among all, the *trans*-cinnamaldehyde was found the most effective in killing the pathogens. From this study, they concluded that *trans*-cinnamaldehyde has the potential to be evaluated as an alternative or adjunct to antibiotics as an intra-mammary infusion to treat bovine mastitis.

Essential oils and production efficiency

Kung et al, (2008) conducted a lactation trial on 30 Holstein lactating cows by feeding a blend of EO as a feed additive. The EO was mixed directly into their TMR to provide 1.2 g/cow per d for a 9-week experimental period. The results showed that the cows fed EO ate 1.9 kg more dry matter per day and produced 2.7 kg more 3.5% FCM/d than did cows fed the control diet. The milk fat and protein percentages, SCC, MUN concentrations were not affected by the treatment. Feed efficiency, change in BW, and change in BCS were also similar between the treatments. They also performed in vitro fermentations by using the moderate and high doses of EO and reported no effect on the concentration of total VFA and pH.

Wall et al. (2014) conducted two experiments using lactating dairy cows to determine the effect of a plant extract additive, an encapsulated blend of cinnamaldehye and eugenol (CE) on milk production performance across a range of doses. In experiment 1, 32 Holstein primiparous and multiparous dairy cows in mid-lactation were assigned to no additive or supplementation with CE (350 mg/d; n = 16 animals/treatment) for 6 wk. In experiment 2, 48 Holstein primiparous and multiparous dairy cows were assigned to no additive or supplementation with CE (200, 400, or 600 mg/d; n = 12 animals/treatment) for 8 wk. In experiment 1, there was increase in DMI in both parity groups, but an increase in milk production was observed only with the multiparous cows. In experiment 2, the milk yield of multiparous cows decreased at the 2 highest doses, but it was increased at the low and high doses of CE of primiparous cows. These responses were accompanied by similar changes in DMI, so there was no effect of CE on feed efficiency. They observed no effect of CE on SCC and milk composition.

Tassoul and Shaver, (2009) performed a feeding trail to determine the effects of specific mixture of EO supplemented in the diet of periparturient and early lactation dairy cows. 40 multiparous Holstein cows were randomly assigned to either control or EO (1.2 g/cow/d) supplemented TMR. Feeding the treatment diet started 3 wk before the calving date and continued through 15 wk of lactation. There were no differences between treatments for prepartum DMI, but it was 1.8 kg/d less for EO than C across the 15 wk lactation trial. There were no effects of treatment on plasma concentrations of glucose, NEFAs, BHBA, and Urea-N on samples collected in different dates. Actual or FCM yields, BW and BCS were not affected by the treatments. Feed Efficiency tended to be higher for EO than control and was greater during 8 to 14 wk of lactation. Diet with EO reduced DMI in early lactation with no effect on milk yield.

Spanghero, et al., (2009) fed a commercial blend of micro-capsulated EO to 8 high yielding primiparous pregnant cows 30 days before their parturition to determine the impacts on milk yield and composition. Different doses of EO (0, 0.32, 0.64 and 0.96 g/d) were used as treatment. The EO treatments had no effect on DMI, digestibility, milk and milk components. The protein content of milk ($P = 0.06$) and milk energy concentration ($P = 0.05$) tended to be higher when fed intermediate EO level.

A study was carried out on mid-lactating Holstein dairy cows by Yang, et al., (2007) to evaluate the effects of feeding EO from garlic and juniper berry or monensin on feed intake, ruminal fermentation, site and extent of digestion, microbial protein synthesis, milk production and immune status. Four cows fitted with ruminal and duodenal cannulas were used in 4 x 4 Latin square designs with 21 d period and 4 treatments: control, monensin, garlic, and juniper berry fed with ad lib TMR. There were no effects of

treatment on DMI, total tract digestibility of DM, organic matter, fiber, and starch. Similarly, there was no treatment observed for milk production, ruminal microbial protein synthesis, ruminal pH, and ruminal VFA and NH_3-N concentration.

Castillejos et al. (2006) carried out *in vitro* studies to determine the effects of EO active compounds on rumen microbial protein synthesis. In the first experiment, 4 levels (5, 50, 500, and 5,000 mg/L) of 5 EO active compounds eugenol, guaiacol, limonene, thymol, and vanillin were used. The total VFA concentration was decreased and the pH was increased with the highest doses all treatments. All other treatments had minor effects or changes occurred only after total VFA concentration decreased. In second experiment, they studied the effects of thymol and eugenol (monensin as positive control) on rumen microbial fermentation by using 8 dual flow continuous culture fermenters in 3 replicated periods with 6 d of adaptation and 3 d of sampling. Most of the EO compounds demonstrated their antimicrobial activity by decreasing total VFA concentration at higher doses. But eugenol in batch fermentation and 5 mg/L of thymol in continuous culture modified the VFA profile without decreasing total VFA concentration, and Eugenol in batch fermentation decreased NH_3 concentration.

Vendramini et al., (2016) evaluated the effects of a blend of EO, chitosan or monensin on nutrient intake and digestibility, nitrogen utilization, microbial protein synthesis, ruminal fermentation, blood profile, milk yield, and milk composition on twenty-four multiparous mid to late lactating Holstein cows. Treatments did not influence nutrient intake, milk yield and composition, microbial protein synthesis and efficiency. Cows fed all types additives had similar nutrient digestibilities compared to the control. Animals fed Chitosan had higher DM and CP digestibility than those fed EO mixtures.

Cows fed monensin had lower acetate to propionate ratio than control or EO mixtures. There were no effects of treatments on blood profiles.

Benchaar et al., (2006) carried out study on effects of EO and monensin premix on digestion, ruminal fermentation, milk yield and composition by using four ruminally cannulated lactating Holstein cows used in a 4 X 4 Latin square design with 2 X 2 factorial design. There was no effect of treatment on DMI, apparent digestibility of DM, OM, NDF, and starch. Apparent digestibility of CP was higher for cows fed monensin. Ruminal pH increased with addition of EO. Ruminal NH_3-N concentration was lower for cows fed the monensin supplemented diet compared to cows fed no monensin. No effect of EO and monensin was observed on total VFA concentrations and molar proportions of individual VFAs. Milk production and 4% FCM were similar among the treatments. Milk fat content was lower for cows fed monensin than for without it.

Essential oils for heat stressed animals

Animal productivity and efficiency are greatly influenced by environmental factors. The thermal neutral zone of dairy cattle is about 5 to 20 ^{0}C, but varies among animals (NRC, 2001). The temperature below and above the thermal neutral range is detrimental as it alters the intake and metabolic activity. Economic losses are due to decreased performance (feed intake, growth, milk), increased mortality and decreased reproduction. In the USA, a total loss of $ 897 million has been estimated annually from the dairy sector alone by heat stress (St-Pierre et. al., 2003). The reduction in the revenue associated with heat stress is not only a result of decreased milk yield, but also includes impaired reproduction, increased cost on health care and reduced milk quality (Rhoads et al., 2009). Furthermore, heat stress can suppress anterior pituitary release of LH without

having measurable influence on ovarian steroid hormone secretion thereby depresses the seasonal fertility (Wise et al., 1988). High producing cows are more susceptible to heat stress than low producing due to high metabolic heat increment and extra heat accommodated by physiological adaptations (Kadzere et. al., 2002). Maintaining cow performance in hot climate require improved cooling capability, continued advances in nutritional formulation, and the need for genetic advancement which includes selection of heat tolerance or the identification of genetic traits which enhance heat tolerance (West, 2003). Many feeding technologies have been tried for heat stressed cows. Some of the studies conducted on use of EO for heat stressed cows are reviewed as follows.

Reza-Yazdi et al. (2014) conducted a study to determine the effect of dietary EO compounds, which contained cinnamaldehyde, eugenol, peppermint, coriander, cumin, lemongrass, and an organic carrier on feed intake, milk composition, and rumen fermentation of dairy cows during heat exposure. They conducted a 28 d study on 32 Holstein cows assigned to one of two treatment groups: a control and EO fed. DM intake was measured daily and milk yield was measured weekly. They found that DMI and milk yield was decreased ($P < 0.01$) in control fed cows compared to EO fed cows. Also, there was decrease in the molar proportion of propionate ($P < 0.05$) and an increase ($P < 0.05$) in the acetate to propionate ratio. They concluded that EO supplementation can be useful to alleviate the decrease in DMI and milk production during heat stress in lactating dairy cows.

Serbester et al. (2012) conducted a study to determine the effect of an EO combination which contained cinnamaldehyde and diallyl disulfide on performance, milk composition, blood parameters and pregnancy rate of early lactating dairy cows during

heat exposure. They used 25 Holstein cows fed one of two treatments: Control and EO supplement fed. They found that EO supplementation had no effect ($P > 0.05$) on performance, milk composition and pregnancy rate, but they found increased ($P < 0.05$) insulin concentration and tended to decrease ($P = 0.074$) serum total cholesterol concentration, and increased ($P = 0.097$) NEFA concentration.

Essential oils for methane mitigation

As EO has strong antibacterial properties, they inhibited the energy metabolism of *Streptococcus bovis* and *Selenomonas ruminantium* (Evans and Martin, 2000) and the growth of rumen Archaea, *Methanobrevibacter smithii* (McIntosh et al. 2003). So, there has been a major attempt to decrease CH_4 emission from ruminants by EO supplementation. Many review studies have been conducted for the potential use of EO for mitigation CH_4 (Boadi et al., 2004; Benchaar and Greathead, 2011). Using natural products as feed additives, like EO, to manipulate enteric fermentation and potentiality reduce CH_4 emission from livestock production has an increasing interest (Wallace et al., 2002). The potentiality of EO for modulating ruminal function on a long term basis has not been evaluated. To know the most effective level of EO inclusion in the diet, as well as, possible adaptation of ruminal microbes to this feed additive is another important factor to elucidate (Boadi et al., 2004).

Castro-Montoya et al. (2015), looked the effects of a blend of EO on CH_4 emission *in vivo* and *in vitro*. Four lactating and four beef heifers were supplemented 0.2 g/d of EO during eight weeks. First two weeks were control with no EO supplementation. They found that the EO blend was effective at reducing the daily CH_4 emission in dairy cattle and emissions relative to BW in beef cattle, however, these effects were not observed *in*

vitro regardless of the techniques used to replicate the *in vivo* results. They concluded that this could be due to differences in the mode of action of EO between *in vitro* and *in vivo*, which merits the attention of future research.

Patra and Yu, (2012) conducted an *in vitro* experiment to evaluate the effects of EO on CH_4 production and fermentation. They used five different EO, namely clove oil, eucalyptus, garlic oil, origanum oil, and peppermint oil at 3 different doses (0.25, 0.50, and 1.0 g/L) for their effects on CH_4 production, fermentation, and select groups of ruminal microbes including total bacteria, cellulolytic bacteria, archaea, and protozoa. All of the EO significantly reduced CH_4 production with increasing doses. However, apparent degradability of DM and NDF linearly decreased with increasing doses by all EO, except garlic oil. All EO differed in altering molar proportions of acetate, propionate and butyrate. All EO decreased the abundance of archaea, protozoa and major cellulolytic bacteria (*Fibrobacter succinogenes, Ruminococcus flavefaciens,* and *R. albus*) linearly with increasing EO doses. From this experiment, they concluded that a single EO may not be effective and practical in mitigating methane emission from ruminants, unless used at low doses in combinations with other anti-methanogenic compounds.

Benchaar and Greathead, (2011) have reviewed the opportunities of EO to mitigate enteric methane emissions from ruminants. They have summarized that the EO derived from thyme, oregano, cinnamon, garlic, horse radish, rhubarb, and frangula have decreased CH_4 production *in vitro* depending on doses. There was inhibition of CH_4 production at high doses of EO (>300mg/L), but with these high doses there was decrease in total VFA concentration and feed digestion. Though many of the EO concentrations used *in vitro* affected the rumen fermentation, they have found deleterious effects on

efficiency of rumen, palatability and causing toxicity *in vivo*. So there is a need for in vivo investigation to determine the efficacy of these compounds to inhibit methanogenesis successfully. The challenge is to identify EO that selectively inhibit rumen methanogenesis at practical feeding rates that do not have deleterious effects on feed digestion and animal productivity.

Cobellis et al. (2016) evaluated different EO to mitigate CH_4 and NH_3 production by rumen microbiota *in vitro*. They used oregano, rosemary, Ceylon cinnamon, cinnamon leaves, cinnamon bark, dill seeds, and eucalyptus individually (1.125 ml/L culture) and in three-way EO combinations (at total 0.8 ml/L, equal ratio). All EO and their combinations decreased production of total gas ($P < 0.001$), CH_4 ($P < 0.001$), and NH_3 (except eucalyptus), however, they (except Ceylon cinnamon-dill seeds-eucalyptus EO combination) also decreased DMD ($P < 0.001$). They concluded that combination of EO from Ceylon cinnamon, dill seeds, and eucalyptus might be practical at lower concentration to mitigate CH_4 emission and nitrogen excretion from ruminants without adverse effect on feed digestion or fermentation.

Joch et al. (2016) investigated the effects of the active compounds EO on rumen fermentation characteristics and CH_4 production in vitro. They found up to an 86% of reduction in CH_4 compared to control ($P < 0.05$). Among different active compounds of EO they used, only limonene, 1,4-cineole, bornyl acetate, and α-pinene did not inhibit VFA production, and only bornyl acetate produced less methane per mole of VFA compared with control ($P < 0.05$). They used monensin as positive control in another subsequent trial. Positive effects of bornyl acetate on CH_4 and VFA production were more pronounced than the effects of monensin. From this trial they came to the

conclusion that the ability of bornyl acetate to decrease CH_4 production may help to improve the efficiency of energy use in the rumen.

Agrawal et al., (2009) studied the effect of inclusion of peppermint (*Mentha piperita*) oil (at 0, 0.33, 1.0, and 2.0 µl/ml of incubation medium) on gas and CH_4 production, fermentation of feed and microbial profile *in vitro.* The buffalo rumen fluid was used as inoculum. They found CH_4 emission was reduced by 19.9%, 46.0%, and 75.6% at 0.33, 1.0, and 2.0 µl/ml concentrations respectively. The concentration of total VFA was reduced by inclusion of peppermint oil at higher levels at 1.0 and 2.0 µl with no effect at 0.33 µl. At 0.33 µl level, the population density of total bacteria was similar to that of control but, fungi, *Ruminococcus flavefaciens,* and methanogens increased by 4, 6 and 2-folds respectively. They concluded that the higher the doses of peppermint oil for the rumen microbes, but the lower level could be further explored in in vivo experiments as methanogenesis is reduced by rumen modifiers.

OmniGen-AF Introduction

OmniGen-AF (produced by Prince Agri Products, Inc., Quincy, IL) is a commercial nutritional supplement, which contains a proprietary blend of active dried yeast (*Saccharomyces cerevisiae*), enzymes (*Trichoderma longibrachiatum*), vitamin B-complex, sodium aluminosilicate, calcium carbonate and diatomaceous earth. This product has been reported to help support normal immune function, heat and other stressful conditions, and improves health and performance of dry, pre-fresh and lactating dairy cows. There is no peer reviewed research reviews found on the use of OmniGen-AF.

Mode of Action

This product has been shown to enhance health and performance by increasing L-selection mRNA and interleukin-8 receptor mRNA expression (Wang et. al. 2004). Both the L-selection mRNA and interleukin-8 receptors increase the ability of milk somatic cells to kill mastitis causing pathogens. L-selectin is a molecule essential for neutrophil adhesion, and increased expression may aid in the innate immune response to the infection (Barkley & Waldron, 2013). Studies have documented that glucocorticoids-mediated stress and immunosuppression bring about a reduction in innate function by causing release of the extracellular pool of neutrophil L-selectin (Tempelman et. al., 2002; Weber et al. 2001). This then reduces the ability of neutrophils to search for pathogens and enhances the chances of infection. In dairy cattle, during parturition, there is marked reduction in L-selectin due to stress of peak of glucocorticoids (Burton and Erskine, 2003). Wang et al., 2004 carried out a study on sixty sheep by feeding OmniGen-AF if it increases innate immune function when the animals are immunocompromised. The five treatments were: 1) control, 2) immunosuppressed with dexamethasone injection, 3) immunosuppressed with OmniGen-AF, 4) immunosuppressed with a pathogen (Aspergillus fumigatus), and 5) immunosuppressed with pathogen challenge and OmniGen-AF. The sheep were assessed for L-selectin and interleukin-1β after a 28 d experimental period. They found there was reduced innate immune function ($P < 0.05$) i.e. reduced neutrophil L-selectin and IL-1β concentrations. There was increased ($P < 0.05$) L-selectin, but no effect ($P > 0.05$) on IL-1β with the OmniGen-AF fed non pathogen challenged sheep. There was no effect ($P > 0.05$) on L-selectin of IL-1β on pathogen challenged sheep. By this experiment, they found that

OmniGen-AF restored normal level of neutrophil L-selectin and boosted the level of IL-1β in pathogen challenged sheep. Another research trial conducted by Nickerson et al. (2013) on eighty Holstein heifers fed 4 g of OmniGen-AF/45.35 kg of BW/d. There was significantly greater ($P < 0.05$) mRNA expression of L-selectin in OmniGen-AF fed heifers than in control during 7, 12 and 14 months' period of trial. Chapman et al. (2010) also found increase in the amount of L-selectin in OmniGen fed cows in 30 DIM than cows fed control.

OmniGen-AF and production efficiency

Chapman et al. (2010) evaluated the milk production, herd health and immune competency for OmniGen-AF fed cows during the dry and early lactation phases. Cows (n=262) were fed OmniGen-AF supplemented diet on 250 cows were fed a control diet during dry off (-60 d from calving) and early lactation (30 DIM). OmniGen-AF fed cows had 200 kg of total milk more than cows fed control when calculated over a 305 days.

Another study reported by Chapman et al., (2005) evaluated the effects of OmniGen-AF in milk production and lactation persistence during a 60-day trial period in a commercial dairy setting. Holstein cows (n = 670) were randomly assigned to either a control (n = 342) or OmniGen fed (n=328) TMR. They fed 56 g/hd/d for first 30 d of trial and 28 g/hd/d for the second 30 d trial period. They reported that the addition of OmniGen-AF to the TMR increased ($P < 0.05$) milk production from 33.4 to 34.1 kg/hd/d during the 60 d feeding period. There was a decline in production of 2.42 kg/hd/d over the 60 d feeding trial for control fed cows, whereas cows declined only 1.08 kg/hd/d for OmniGen-AF fed. They reported that the benefit was more pronounced among the multiparous cows.

Omnigen and heat stressed animal

Hall et al., (2014) evaluated OmniGen-AF in 30 lactating heat stressed Holstein cows in a commercial dairy and a controlled environmental chamber. The control group received a basal TMR with no supplement and the treatment group received OmniGen-AF mixed into the basal TMR for 45 d. After the commercial dairy phase was complete, 12 cows with 6 from each group were kept in the environmentally controlled rooms for 21 d. The cows were subjected to 7 days of thermal neutral, 10 days of heat stress and 4 days of thermal neutral as recovery days. During heat stress, the temperature humidity index was greater than 68 for 16 h/d, whereas in thermal neutral, it was less than 68. They reported reduced thermal stress in OmniGen-AF fed cows as measured by rectal temperatures and respiration rates. Feed intake was higher for OmniGen-AF fed cow during heat stress with a lower cortisol spike on the first day of heat stress. Decreased milk yield was with both treatments, but cows fed OmniGen-AF had lower SCC than the cows fed the control during the recovery period.

***Saccharomyces cerevisae* for ruminants**

Direct fed microbial are live or viable naturally occurring organisms supplemented to animals generally during periods of stress or lower DMI. These products are fed continuously as an attempt to enhance production performance, alter ruminal fermentation, or improve nutrient utilization (NRC, 2001). *Saccharomyces cerevisiae* fermentation product is one of the most widely used ruminal fermentation modifiers for improving animal performance.

Active dried yeast (*Saccharomyces cerevisiae*) is one of the components in OmniGen-AF. The inclusion of yeast has demonstrated inconsistent results by various researchers. This product has shown to increase DMI (Dann et al., 2000; Williams et al., 1991; Stella et al., 2007; Desnoyers et al., 2009; Robinson and Garrett, 1999), increased milk production (Bitencourt et al., 2011; Ramsing et al., 2009; Harisson et al., 1988; Zaworski et al., 2014; Desnoyers et al., 2009; Bruno et al., 2009; Robinson and Garrett, 1999), no effect on milk production (Kung et al., 1997; DeVries and Chevaux, 2014; Malekkhahi et al., 2016; Salvati), no effect on DMI (Kung et al., 1997; Malekkhahi et al., 2016), and increased feed efficiency (Schingoethe et al., 2004). This variation is due to the difference in the inclusion rate, type of yeast used, level of DMI, use of other additional feed additives, animal age, physiological stage, health, and stress.

Vitamin-B_{12} for ruminants

Vitamin B_{12} is required for ruminants for efficient energy metabolism, which is required to maximize productivity. Although B_{12} is synthesized by rumen microbes, sometimes there is a reduction or prevention of vitamin B_{12} synthesis when the rumen function is disturbed by abrupt ration changes in the diet or by other stressful conditions. When the diet is deficient in cobalt, the absorption of vitamin B_{12} can be impaired. Ruminal microbes can produce all of the vitamin B12 required by the cow if provided adequate available cobalt in diet (NRC, 2001).

Several studies have shown that Vitamin B_{12} is required as a growth factor for some ruminal microbes (Tanner and Wolfe, 1988; Strobel, 1992) and is utilized to produce propionate. Vitamin B_{12} is very important for propionate metabolism, which is a major precursor of glucose for ruminants. When there is severe deficiency of vitamin $B_{12,}$

a serious impairment in the animal's ability to utilize propionate occurs (Elliot, 1980). In ruminal microbes, the function of vitamin B_{12} is as a cofactor for the enzyme methylmalonyl-CoA mutase, which catalyzes the conversion of succinyl CoA to methylmalonyl-CoA during the formation of glucose (Nagaraja et al. 1997). It is also a cofactor for another enzyme, tetrahydrofolate methyl transferase, which catalyzes the transfer of methyl groups from 5-methyltetrahydrofolate to homocysteine to form methionine and tetrahydrofolate (NRC, 2001).

Trichoderma longibrachiatum for ruminants

Dietary fiber is an important energy source for ruminants. The ruminal digestion of fiber is a slower process and can affect the animal performance and increase the cost of production if it is not efficient. Rumen microorganisms produce enzymes that catalyze the breakdown of the structural carbohydrates, like cellulose and hemicellulose, but due to a complex network formed by these carbohydrates and lignin, this reduce their digestibility and inhibits the efficient utilization of feed (Giraldo et al. 2008). The use of exogenous enzymes is one of the emerging technologies in ruminants for improving forages utilization to increase fiber digestion. Various researchers have demonstrated that using exogenous enzymes in the diet of dairy cows has the potential to improve fiber digestion (Yang et al. 1999; Morgavi et al. 2000; Yang et al. 2002; Rode et al. 1999). Fiber digesting rumen microbes were increased by the use of exogenous enzymes *in vitro* (Varel et al., 1993) and *in vivo* (Yang et al. 1999). However, the effect of enzymes is influenced by various factors like type and dose of enzymes, animal diet, enzymes application method, and the level of productivity of animal (Beauchemin et al. 2003). When the enzymes are sprayed onto the feed, they may be partially protected from

ruminal degradation by binding with substrates that may cause conformational changes, which protects them from ruminal proteases (Kung et al., 2002). Feeds treated with fibrolytic enzymes just before feeding have enhanced animal performance (Schingoethe et al. 1999). However, to ensure consistency of responses and to develop more effective enzyme products, more studies are needed. Identifying key enzyme activities involved in these positive responses in the specific temperature and pH environment of rumen is another important consideration (Beauchemin et al., 2004).

Trichoderma longibrachiatum is an anaerobic fungus and is a common source of enzymes used in the feed industry. Morgavi et al., (2000) found synergistic effects between ruminal enzymes and *T. longibrachiatum* preparations containing different proportions of xylanase and cellulose activities. They found increased hydrolytic potentiality of *T. longibrachiatum* within the rumen environment improved feed digestion. Yang et al. (1999) showed higher digestion of organic matter and NDF, and higher ruminal digestibility of CP with increased milk production for enzymes fed cows than the control. The enzymes contained primarily cellulase and xylanase. In another study reported Yang et al. (2002) demonstrated that supplementation of commercial blend of enzymes containing relatively high xylanase and cellulase extracted from *T. longibrachiatum* did not affect total VFA concentrations, but increased the proportions of acetate and reduced propionate as a result of increased digestion of NDF and ADF. Similarly, Rode et al. (1999) investigated the effects of exogenous enzymes on DM intake, milk production, and digestibility for twenty multiparous lactating Holstein cows. They used an enzyme mixture containing xylanase and cellulase activities. They reported no differences in DMI, but total digestibility of nutrients (DM, NDF, ADF and CP) were

increased by enzyme treatment and milk yield tended to increase ($P < 0.10$). They concluded that supplementation of exogenous fibrolytic enzymes have the potential to enhance milk yield and nutrient digestibility of early lactation cows without affecting feed intake.

Cobalt and ruminant nutrition

Cobalt is an essential trace mineral in the diets of ruminants. The ruminal microbes can produce the entire required amount of vitamin B_{12} when adequate cobalt is available in the diet (NRC, 2001). Campbell et al. (1998) carried out a study on lactating Holstein to evaluate the effect of Co on reproduction and milk yield that were receiving bovine somatotropin. They used 60 cows and blocked them by breed, lactation number and incidence of retained fetal membranes. Two diets were used as treatment from parturition to 154 DIM with the first diet being a control and the second diet being the control supplemented with 26 mg of Co as Co glucoheptonate. Days to first service, days open, and days from first service to conception, services per conception, milk yield, milk components, and SCC were similar for control and supplemented cows. The cows supplemented with trace minerals demonstrated fewer days to first estrus.

Kincaid and Socha, (2006) conducted a study on 36 multiparous cows to determine the effects of dietary Co supplementation (0.15, 0.89, or 1.71 mg/kg of Co on DM basis during late gestation and 0.19, 0.57, or 0.93 mg/kg of Co on DM basis during early lactation). The treatment tended to increase ($P < 0.10$) the concentration of vitamin B_{12} in colostrum and milk compared to cows fed lower amount of Co. There was difference in concentrations of Co in the liver or serum, but increased Co concentration in milk and no effect on DMI or milk yield and components. They concluded that serum

concentrations of vitamin B_{12} are reduced in the early dry period, and added dietary Co may increase ruminal vitamin B_{12} synthesis, as observed an increase in vitamin B_{12} concentration in colostrum and milk of the cows given dietary Co.

Nasser and Ismail, (2011) conducted research on effect of Co supplementation on gas production measurements, estimated energy values and microbial protein in vitro in 3 Egyptian rams by using 0, 0.35, 0.70 and 1.0 mg/kg DM of feed. Cumulative gas production was recorded at 3, 6, 12, 24, 48, 72, and 96 h of incubation. At 24 h, they reported the highest gas production volume for the sample with Co 0.70 mg/kg DM followed by the samples with Co 0.35 and 1.0 mg/kg DM than the sample with no Co. They found significant difference in ME and NE, VFA, DMD, OMD, SCFA and microbial protein with the samples containing Co. They concluded that the Co addition improved ruminal fermentation.

Three studies were done by Kincaid et al. (2003) to evaluate the effect of dietary Co on Co metabolism and performance of dairy cows. In the first study, they examined the cow age and dietary Co on Co concentration in liver and blood, in the second study, they used 21 d pre-partum until 120 d postpartum cows and in the third study, they used Co in the starter diet. They reported that the concentration of Co in serum, blood, and liver was not affected by dietary Co supplementation. Age of the animal affected the liver Co concentration with highest concentrations in younger than in older cows. The decline of liver Co with age, may be due to a combination of low intestinal absorption, endogenous losses, and Co secretion into milk.

Akins et al. (2013) evaluated the lactational performance and metabolism of 45 primiparous and multiparous cows fed different levels and sources of Co or given weekly

vitamin B_{12} injections. The cows at 60 d prepartum were blocked by expected calving date and randomly assigned to 1 of 5 treatments in a RCBD. The 5 treatments were CON: no supplemental dietary Co, CoCarb: 25 mg/d of supplemental dietary Co from CoCO3, LCoGH: 25 mg/d of supplemental dietary Co from Co glucoheptonate, HCoGH: 75 mg/d of supplemental dietary Co from Co glucoheptonate, and IB12: CON diet plus weekly 10 mg I/M of vitamin B_{12} injections. Co concentrations in the lactating diets were 1.0, 1.9, 2.3, and 5.1 mg/kg of DM for CON/IB12, CoCarb, LCoGH, and HCoGH, respectively. They found that DMI, BW, and BCS were similar among treatments. The LCoGH treatment tended ($P < 0.10$) to have greater milk yields than CoCarb, and CON had similar milk yields to the mean of LCoGH and HCoGH. Co supplementation or vitamin B_{12} injections did not influence energy metabolism via the plasma or liver. However, vitamin B_{12} injections increased plasma, liver, and milk vitamins B_{12} contents. Dietary Co addition did not affect plasma vitamin B_{12} concentrations, but increased milk vitamin B_{12} concentrations throughout the lactation. In conclusion, Co supplementation (organic or inorganic) or vitamin B_{12} injections improved the measures of vitamin B_{12} status, but not lactational performance compared with cows fed a control, which could be due to Co being above the requirements in the control diet.

Tiffany et al. (2006) conducted an experiment to determine the effects of dietary concentrations of Co on vitamin B_{12} production and fermentation of mixed ruminal microbes grown in continuous culture fermenters. In the experiment, four fermenters were fed 14 g of DM/d and the DM consisted of a corn and cottonseed hull-based diet with Co supplemented as $CoCO_3$. Treatment used were 1) control: containing 0.05 mg of Co/kg of DM, 2) 0.05 mg of supplemental Co/kg of DM, 3) 0.10 mg of supplemental

Co/kg of DM, and 4) 1.0 mg of supplemental Co/kg of DM. Fermenters were sampled over a 3-d sampling period after a 3-d adjustment period. This was repeated 2 additional times for a total of 3 runs. The results demonstrated that ruminal fluid vitamin B_{12} were affected by Co supplementation ($P<0.01$). At d 3 of sampling, cultures fed the basal diet supplemented with 0.10 mg of Co/kg DM had greater vitamin B_{12} concentrations than those supplemented with 0.05 mg of Co/kg of DM. Increasing supplementation of Co from 0.10 to 1.0 mg/kg of DM increased ruminal fluid vitamin B_{12} concentrations. Molar proportions of acetate, propionate, and isobutyrate, and acetate propionate ratio were not affected by the addition of supplemental Co to the basal diet. However, molar proportions of butyrate, valerate, and isovalerate increased in response to supplemental Co.

Stemme et al., (2008) studied the effects of an elevated dietary Co supply to dairy cows on rumen fermentation parameters and microbial vitamin B_{12} synthesis in the rumen. For this five lactating cows were fed a diet supplemented with native Co (0.17 mg Co/kg DM) or a diet with Co sulfate (0.29 mg Co/kg DM). The ruminal pH, NH_3 concentration and molar proportions of SCFA were not significantly influenced by the high Co supplementation. There were no differences in microbial protein flow; however, the cobalamin flow to the duodenum was significantly higher in the Co supplemented cows.

Cobalt for fiber digestion

The divalent cations, like Co, may act as bridges between the microbes and cell walls of plants, both of which tend to be negatively charged. Thus, the supplementation of dietary Co may improve fiber digestion in the rumen by increasing bacterial activity (Hussein et al., 1994). Hussein et al. (1994) conducted *in vitro* digestibility experiments

to evaluate the effects of Cobalt glucoheptonate as a source of Co on fiber digestion of forages and by-products. In experiment 1, Co Supplementation (0, 5, and 10 ppm of five substrates (leaf and stem fractions of alfalfa and orchard grass hay and ground corn) was evaluated. In experiment 2, four concentrations of Co (0, 10, 20, and 30 ppm) were added to five substrates (alfalfa hay, orchard grass hay, corn cobs, recycled newsprint treated with HCl, and cellulose casings). Both were incubated for 24 or 48 h with rumen fluid from steers. There were no interactions among the treatments for digestibilities of DM, OM, or NDF in both experiment or for VFA in experiment. In vitro digestibilities of DM, OM, and NDF were higher for inoculum from steers fed alfalfa versus concentrate in experiment 1. Whereas, digestibilities of DM, OM and NDF were highest for alfalfa hay and lowest for newsprint treated with HCl. They found that Co concentrations that were above the minimum requirements did not improve digestion of DM, OM, or fiber in contrast to the findings of Lopez-Guisa and Satter, (1992).

Cu and Co were added to the diets containing alfalfa silage and corn crop residues of heifers in higher doses than recommended by NRC to determine the effect of these minerals on digestion and growth (Lopez-Guisa and Satter, 1992). The rate of DM disappearance from dacron bags placed in the rumen in experiment 1 was increased by the additional dietary Cu and Co for alfalfa hay (14.6 vs. 8.4%/h) and corn cobs (5.8 vs. 3.1%/h), but it did not affect the DM disappearance rate for corn residue silage (3.6 vs. 3.4%/h). In experiment 2, DM disappearance rate of corn crop residue silage (6.2 vs. 3.4%) was increased, but there was no influence on the rate of alfalfa hay (8.6 vs. 7.6%/h). From these results they concluded that the addition of Cu and Co improves the DM digestibility of low quality forages.

An experiment was carried out in horses to determine the effect of dietary Co concentrations on fiber digestion in horses (LeCompte, 2015). The experimental groups were fed 0 mg, 2.8 mg, 8.4 mg, or 14.0 mg of Co. A linear trend for an increase in DM and NDF digestibility ($P = 0.06$) with no effect of Co supplementation on the digestibility of NDF, ADF, or lignin was reported.

The evaluation of supplementation of a cobalt-lactate product on fiber digestibility and milk production parameters by feeding 70% forage diet to 24 late lactation cows was done by Pretz and Casper (2015). They used two treatments: 1) control diet containing 12.5 mg/cow/d of $CoCO_3$ and 2) test diet with same basal diet by supplying additional 50 mg/cow/d of Co by a 1% Co-lactate product. There was no effect on production parameters by feeding Co-lactate, but it decreased rumen ammonia concentrations and increased ruminal molar concentrations of acetate and increased fiber digestion numerically. Pretz et. al., (2015) submitted the manure samples from the cows of same trial for stereo microscopy and scanning electron microscopy evaluation. They demonstrated that manure samples from cows fed Co-lactate were more transparent than from cows fed control diet. There were small visible improvements in fiber digestibility observed as appearance of hollow pits in the fiber particles.

Conclusion

A literature review on inclusion of the individual compounds and their commercial blends viz. EO, cobalt lactate, active dried yeast, exogenous enzymes, and cobalt carbonate in the diets of cows shows positive response for improving ruminal fermentation and lactational performance, but still this is an area for improvement. These experiments (*in vivo* and *in vitro*) were hypothesized that EO, cobalt lactate, active dried yeast, exogenous enzymes, and cobalt carbonate will improve the performance and nutrient digestibility of dairy cows.

CHAPTER 2:

EVALUATION OF COMMERCIAL PRODUCTS ON THE MARKET TO IMPROVE GUT HEALTH AND DIGESTIBILITY BY LACTATING DAIRY COWS

ABSTRACT

Different technologies are available on the market as feed additives to improve milk production and health of lactating dairy cows. The study objective was to evaluate the lactational performance and nutrient digestibility by lactating Holstein dairy cows fed two commercial products. The commercial products were: 1) EOC: product based on a propriety blend of essential oils and Cobalt lactate and 2) ADY: active dried yeast product. The robotic dairy barn was split into two pens (Robot 101, n = 40 and Robot 102, n = 38) each having a Lely Astronaut A4 robotic milker. Treatments were added into a soyhull carrier and mixed into the Total Mixed Ration (TMR). Both treatment groups received the same basal diet. The feed was supplied once daily and the orts were recorded weekly for each pen. The cows also received a pelleted concentrate in the milking robot. Each day, cow performance data was downloaded using Lely Time for Cows (T4C) robotic milking software. Weekly TMR samples were collected for Aspergillus and nutrient digestibility (ND). Fecal samples were collected weekly from a composite of 15 cows per treatment and analyzed for ND and bacterial presence. The TMR aerobic stability was measured weekly using a temperature recording button. Body condition scoring (BCS) was conducted weekly. Body surface temperature was measured weekly using a thermal imaging camera. The TMR amounts fed and pellet intake were recorded daily for calculating dry matter intake (DMI) and feed efficiency (FE). Milk samples were collected and analyzed every 2 weeks by Dairy Herd Improvement Association

(DHIA) for fat, protein, lactose, MUN and SCC. Milk production (36.1 and 37.7 kg/d for EOC and ADY, respectively) and management level milk (37.8 and 39.3 kg/d) were similar ($P > 0.25$) for cows fed both products. Cows fed EOC had lower ($P < 0.01$) DMI (21.8 and 22.7 kg/d), but feed efficiency (1.65 and 1.66 kg/kg; FE) were similar ($P > 0.10$) for cows fed both products. Milk fat was similar ($P < 0.41$) for cows fed EOC (3.46%) than cows fed ADY (3.30%). Cows fed EOC were significantly ($P < 0.01$) cooler (33.0 and 33.6 ^{0}C) than cows fed ADY. Both commercial products prevented the growth of *Aspergillus fumigatus* with all samples being below than the detection limit. Fecal presence of *Escherichia coli* was lower (3.0 and 3.3; $P < 0.02$) and yeast (1.2 and 1.9) tended to be lower ($P < 0.10$) for cows fed EOC than cows fed ADY. Nutrient digestibility (ND) for dry matter (DM) ($P < 0.05$), neutral detergent fiber (NDF) ($P < 0.04$), hemicellulose ($P < 0.02$), calcium ($P < 0.02$), and phosphorus ($P < 0.03$) were significantly higher for cows fed ADY than fed EOC. Similarly, ND for crude protein (CP) ($P < 0.06$), acid detergent fiber (ADF) ($P < 0.09$), and magnesium ($P < 0.09$) were tended higher for ADY fed cows. This study demonstrated that while milk production and FE were similar and the cows fed EOC were cooler during the hot summer/fall season, whereas ND was significantly higher for the cows fed ADY.

Keywords: Essential oils, dried active yeast, lactation performance, robotic,

INTRODUCTION

Use of antibiotics in ruminant animal production system has been declining in recent years and due to its negative health impact to humans and bacterial resistance due to their consumption, it has been a great concern to the public. The dairy nutritionist and rumen microbiologists are more focused on exploring antibiotic alternatives for improving feed efficiency and animal productivity. The essential oils are phytochemicals present in metabolites of some plants and they can act as antibiotics. They can be potential alternatives to manipulate microbial activities in the rumen for better productivity (Benchaar, et. al., 2008). Several studies have been done to evaluate the effects of EO on animal production. In a review, conducted by Calsamiglia et. al. (2007), it was reported that EO has several effects on rumen function: (a) There is inhibition of deamination and methanogenesis resulting in lower ammonia nitrogen, methane and acetate but higher propionate and butyrate concentrations without reducing total VFA production; (b) There are variable responses due to specific EO alone or in combination; (c) The effects of some EO are ruminal pH and diet dependent. In dairy cows, there is effect of increasing ruminal true organic matter and N digestibilities, total tract ADF and starch digestibilities, and milk yield, DMI and milk fat have been reported (Tassoul and Shaver, 2009).

A commercial proprietary blend of essential oil and cobalt lactate (EOC) (produced by Ralco Nutrition, Marshall, MN as Stay Strong) has been promoted to increase immunity, protect feed quality and release more energy from the feed to improve animal productivity. In a study conducted by Pretz and Casper, (2015) at South Dakota State University, it was demonstrated that feeding additional Co as cobalt-lactate did not

influence milk production, milk components, DMI or BW for lactating dairy cows fed a high forage ration, but did appear to alter ruminal fermentation to increase fiber digestion and microbial protein synthesis. The samples from the same study were analyzed in Gansu, China, demonstrated that feeding additional Co as cobalt-lactate visually appeared to increase fiber digestibility of particles when evaluated by stereo and scanning electron microscopy (Pretz et. al. 2015).

OmniGen-AF is a commercial feed product (produced by Prince Agri Products, Inc., Quincy, IL) which contains a proprietary blend of B-vitamins, active dry yeast (*Saccharomyces cerevisae*), enzyme (*Trichoderma longibrachiatum*), and aluminosilicates and it is a product based on active dried yeast (ADY). Different studies on the performance of OmniGen-AF has shown that OmniGen-AF can support the dairy cow's natural immune system, aid in the maintenance of health and production of dairy cows during the times of heat stress (Chapman, 2001; Chapman et.al., 2005). The supplementation of OmniGen-AF to the diet restored the normal level of L-selectin, which indicated that it has potential for restoring the ability of neutrophils to monitor the endothelial cell lining for infection (Wang et. al., 2004) and reduce the risk of infection. L-selection is a molecule essential for neutrophil adhesion and increased expression may aid in the innate immune response to the infection (Barkley & Waldron, 2013). The field trials conducted have shown that Omnigen-AF fed to heat stressed cows had reduced rectal temperatures, higher feed intakes, and lower SCC.

So, the study objective was to compare feeding EOC and ADY on the lactational performance and nutrient digestibility of lactating Holstein dairy cows on a commercial dairy farm using 2 Lely robotic milking systems. The study hypothesis was that lactating

dairy cows fed two different nutritional technologies (EOC and ADY) to improve gut health and immune response would result in similar lactational performance and nutrient digestibility.

MATERIALS AND METHODS

Animal and Diets

This study was carried out at a commercial dairy in southwest Balaton, Minnesota for 12 weeks from July to September 2015 on lactating Holstein cows and all the procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee before the start of the study. The dairy farm was equipped with two Lely Astronaut A4 robotic milking units (Gorter's Clay and Dairy Equipment of MN, Inc., Pipestone, MN). The cows (n = 78) were kept separated in two free stall pens each having a robot milker (n = 40 in robot 101 and n = 38 in robot 102). The cows were blocked by parity, DIM and milk production. The cows were randomly assigned to one of the treatments: 1) a product based on a proprietary blend of essential oils and cobalt lactate (EOC) and 2) Active dried yeast product (ADY) (OmniGen-AF, Prince Agri). EOC was added to a soy hull carrier (0.199 kg soy hulls to 0.027 kg EO) which made the feeding rate approximately 7.216 gm of EOC per cow per day. Similarly, the ADY at 0.038 kg was also added to a soy hull carrier (0.188 kg soy hulls and 0.038 kg of ADY).

Cows were fed a TMR (Table 2.1) consisting of 55 % forage and 35% of grain on a DM basis and both experimental groups received the same basal diet. The composition of the protein mix used in the TMR is given in Table 2.2. The nutrient composition of the

TMR (Table 2.3) was based on three representative samples taken throughout the experimental period. A salt lick drum was placed between the two pens to prevent animals consuming feed in the adjacent pen throughout the experimental period. The cows also received a pelleted concentrate (Table 2.4) in the milking unit/parlor, which was dispensed according to the current milk production of the individual cow. The nutrient composition of corn silage, alfalfa hay, and alfalfa baelage are given in Table 2.4. The EOC-soyhull carrier was added to the wagon TMR and well mixed for 5 min before distributed to the cows in the pen. Similarly, ADY was also added into the TMR mixer and offered to the cows. The TMR diets were formulated by Hubbard Feeds Inc. (Worthington, MN). The feed was supplied once daily to the experimental cows between 0900 h and 1200 h and orts were collected once weekly for each pen and recorded. Cows were provided water ad libitum.

Sample Collections

The production data: average milk production, BW, rumination, milk conductivity, milk protein, and milk fat were recorded electronically by Lely Time for Cows (T4C) milking software (Gorter's Clay & Dairy Equipment of MN, Inc. Pipestone, MN) during each individual cow's milking time. Each day, at around 1200 h, the data report was remotely transferred to a computer at Dairy Science Department, South Dakota State University from the dairy farm. The data collected also included DIM, parity, and total amount of concentrates intake during the 24 h period by individual cows.

Throughout the experimental period, forages and TMR samples were collected monthly by Hubbard Feeds, Worthington, MN and submitted to Dairyland Laboratories, Inc., Arcadia, WI for nutrient analysis. Milk samples were collected at every two weeks

using a Lely shuttle milk sampling unit and were sent to DHIA, Zumbrota, MN for analysis of fat, protein, lactose, MUN (Fourier Transformed Infrared Technology) and SCC (Flow cytometry by using ethidium bromide for white blood cell staining, Fossomatic FC 5000, Foss Electric, Denmark).

Body surface temperatures of each cow was taken weekly by using a thermal imaging camera (Fluke Infrared Camera, Fluke Inc., Everett, WA). The camera was targeted at the thoracic region and the temperature was taken within 10 feet or less from the animal. Similarly, BCS was taken weekly by two individuals on scale 1-5 (1 is emaciated and 5 is obese) as described by Wildman et al., (1982). Each week, from each pen, representative fecal samples were collected based on a composite of 15 cows by using a bucket and scoop. One representative sample from each of the groups were sent out to Rural Technologies Inc., Brookings, SD for E. coli, salmonella, clostridia and campylobacter tests and another was frozen at -20^0C for further analysis.

Samples of TMR and concentrate were collected weekly from each pen and composited once every month. One composite was submitted to Research Technology Inc, Brookings, SD for *Aspergillus fumigatus* screening, while another TMR composite and concentrates composite sample were frozen at -20^0C for further analysis.

From each experimental group after feed distribution, two buckets were filled up with the respective TMRs and a smart button temperature data logger (ACR System Inc., Surrey, B. C., Canada) were placed inside the TMR. To monitor the ambient temperature, another smart button was placed outside of buckets. To control the variation in outside temperature, each time the buckets were kept in the room adjacent to the milking parlor, where the temperature was maintained at approximately 21 ^{0}C. The smart button was

programmed to log temperatures at half hour intervals beginning at 1600 h. The buttons were collected the following week and the temperatures were recorded for each treatment. The bucket was emptied, washed thoroughly with hot water and soap, and dried before resetting for another week of data collection. The data from the loggers was transferred each week from the smart button to TrendReader for smart button (Version TR3 3.02.000.12407-10, ACR Systems Inc., Surrey, B. C. Canada) by using an ACR systems connection driver (ACR smart button adapter connection driver, ACR systems, Inc., Surrey, B. C. Canada). The data was transferred to excel spreadsheet for further analysis.

Laboratory Analysis

DM and nutrient analysis of feeds and forages were determined by Dairyland Laboratories, Inc., Arcadia, WI during the experimental period. The DM was determined by the Method 2.1.4 of national Forage testing Association Reference (NFTA, Omaha, NB) by drying the samples for 3 h in oven at 105 ^{0}C. Nutrient analysis for the samples were done by using the methods: CP (AOAC 990.03; 1998), NDF (AOAC 2002.04; 2005), ADF (AOAC 973.18, 1996), ADICP and NDIP (AOAC 990.03; 1998), lignin (AOAC 973.18; 1996), Ash (AOAC 942.05; 1996), pH (Orion Research, Inc., 1997), SP (AOCS Method, Ba 10-65), CF (AOAC 920.39), NDFD (Goering and Van Soest, 1970), NH3-N through distillation from recommended Methods of Manure Analysis, lactic acid and acetic acid (Muck and Dickerson, 1988), NFC (NRC, 2001), NE_L (NRC, 2001), and RFQ (Rohweder et. al., 1978), and Fe, Cu, Zn, and Mn (AOAC 953.01). Ca, P, Mg, K and Na were analyzed by adding 5 mL of HCl to 0.5 g of sample and by adding 10 mL of Aquaregia for 10 min on a hot plate, dilutes to 100 mL water, and it was analyzed by

using inductively-coupled plasma (ICP). Cl was measured by using a Corning 926 Chloride analyzer and S was analyzed by using ICP-OES and microwave digestion.

The frozen samples of TMR, concentrates, and fecal composite samples were submitted to Analab, Fulton, IL for digestibility analysis by using acid insoluble ash (AIA) used as an internal marker for determining nutrient digestibility (ND) coefficients (Van Keulen and Young, 1977) for DM, CP, ADF, NDF, hemicellulose, starch, and minerals (Ca, P, Mg, and S). The samples were analyzed by the AOAC (1998) methods: dry matter (DM) (935.29), CP (990.03), nutrient detergent fiber (NDF) (2002.04), acid detergent fiber (ADF) (973.18), acid detergent insoluble crude protein (ADICP) (973.18 and 976.06), neutral detergent insoluble crude protein (NDICP) (2002.04 without sulfite and 976.06), ash (942.05), lignin (973.18), Calcium(Ca), Phosphorus (P), Magnesium (Mg), Sodium (Na), Iron (Fe), Copper (Cu), Potassium (K), Zinc (Zn), and Manganese (Mn) (985.01), Cl (915.01), and Sulfur (S) (923.18). The other nutrient parameters were measured by using the following methods: soluble protein (Krishnamoorthy et. al., 1982), starch (Glucose Reagent Set, AMRESCO, Solon, OH and ALPKEM Corporation, 1990), oil (Damon, 1996), *in vitro* dry matter digestibility (IVDMD) (24 h ruminal and 24 h enzymatic digestion using Kansas State Buffer (Marten and Barnes, 1980), neutral detergent fiber digestibility (NDFD) (Van Soest et. al., 1991) incubated for 30 h using Kansas State Buffer (Marten and Barnes, 1980), ammonia nitrogen (NH_3-N) (United States Environmental Protection Agency, 1993, method 351.2 and International Organization for Standardization, 2013, method 11732), non-fiber carbohydrate (NFC) (NRC, 2001), and net energy for lactation (NE_L) (NRC, 2001).

The fecal samples were analyzed for fecal pathogens by using non-selective media (blood agar) to estimate normal flora and others at Rural Technologies Inc., Brookings, SD. A sterile swab was used to inoculate the sample and the plates were incubated at 37 ^{0}C for 48 h for E. coli and Salmonella; and for clostridia and campylobacter, it was incubated in anaerobic chamber for 41^{0}C. After growth of pathogens, their amount was described as: few (1-10 colonies), small (growth from initial streak only; 10-50 colonies), moderate (growth in second quadrant; 50-100 colonies), and large (growth in third or greater quadrants; more than 100 colonies). Two observations were done to record the results: at 24 h and 48 h. The TMR submitted to Rural Technologies Inc., Brookings, SD for the presence of *Aspergillus fumigatus* were mixed and 450 mL of peptone diluent was added on 50 g of sample. This was then mixed in a stomacher for two min before diluted serially down to 10^{-4}. After dilution, 100 µl of each of the dilutions were plated on to potato dextrose agar and the plates were incubated at 30 ^{0}C before result interpretation.

Fat-corrected milk (FCM) (3.5%) was calculated by using the equation: (0.432 x kg milk) + (16.216 x kg fat) and energy-corrected milk (ECM) was calculated by using the equation: (0.327 x kg milk) + (12.95 x kg fat) + (7.65 x kg protein) as described by Orth (1992). Whereas, management level milk (MLM) 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. Similarly, nutrient digestibility (ND) was calculated by using the equation: Nutrient Digestibility (ND, %) =100-(100 × ((Acid Insoluble Ash (AIA) in feed, %) / (AIA in feces, %) × (Nutrient in feces, %) / (Nutrient in feed, %))).

Statistical Analysis

All data were subjected to least squares ANOVA for a randomized complete block design (Steel and Torrie, 1980) having 2 treatments using the PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Cows were blocked by parity, DIM, and milk yield. The statistical model used was: $Y_{ijk} = \mu + B_i + T_j + W_k + (T_j \times W_k) + e_{ijk}$, where Y_{ijk} = dependent variable, μ = overall mean, B_i = Block effect, T_j = treatment effect, W_k = week, and $(T_i \times W_k)$ = treatment by week interactions, and e_{ijk} = random error. Experimental wk was considered a repeated measurement in time having an autoregressive covariance structure. Treatment, wk, and treatment × wk were considered to be fixed effects with cow within treatment as a random effect. Whenever significant differences attributed to treatment were detected, the Fischer's least significant difference test (Steele and Torrie, 1980) was used to separate least squares (PDIFF statement) treatment means. Significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$.

Fecal pathogens were subjected to least squares ANOVA for a randomized complete block design (Steel and Torrie, 1980) having 2 treatments 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

The ingredients and chemical composition of diets offered for both the experimental groups of EO and ADY are given in Table 2.1, 2.2, and Table 2.3. Both treatment groups were on the same basal diet with the same forages. The post analysis of chemical composition of TMR shows that the diets formulated were at or above the NRC, (2001) recommendations.

Milk production and management level milk (MLM) were similar ($P > 0.10$) between the two treatments (*$P < 0.25$ & 0.38*), but numerically higher for the cows fed with ADY than cows fed with EOC (Table 2.5). These results are similar to the finding of various studies reported by Kung et al, (2008); Spanghero, et al., (2009); Yang, et al., (2007) and Benchaar et al., (2006) for EO and their different blends, where they found no differences in the milk production. However, Chapman et al. (2010) found an increase of 200 kg of milk compared to cows fed than control in 305 days of milking period and also Chapman et al., (2005) found increased in absolute milk production by addition of ADY in the diet.

Feed efficiency (FE) was similar for cows fed EOC and ADY. Similarly, FCM and ECM were similar ($P > 0.10$) with both the treatments. There were no differences between treatments for BW, milk conductivity, and BCS (Table 2.6), which is in agreement with the finding of Kung et al. (2008); Tassoul and Shaver, (2009) and Yang, et al., (2007) where they fed EO and its different blends.

Whereas, the cows fed EOC were found to be cooler than cows fed ADY which was highly significant ($P < 0.0004$) (Table 2.6) indicating that cows fed EOC were cooler and able to better withstand heat stress compared to cows fed ADY because this study was conducted during the summer/fall. Cows fed EOC had lower DMI than cows fed ADY ($P < 0.01$). Lower DMI would reduce the amount of energy (heat) required for nutrient digestion. Improved digestion reduced DMI to achieve the same amounts of absorbed nutrients (Casper, 2008). So these cows would be cooler. Fifty percentage of the oxygen consumption is used by the gut and liver tissues. So improving the efficiency of digestion and reduction in DMI would reduce oxygen consumption by the gut and liver, thereby reducing heat of digestion for the cows fed EOC. Reza-Yazdi et al. (2014) conducted a similar study to determine the effect of dietary EO compounds containing cinnamaldehyde, eugenol, peppermint, coriander, cumin, lemongrass, and an organic carrier on feed intake, milk composition, and rumen fermentation of dairy cows during heat exposure and they found decreased ($P < 0.01$) DMI and milk yield in control fed cows compared to cows fed EO. Hall et al., (2014) did an evaluation of OmniGen-AF in 30 lactating heat stressed Holstein cows in a commercial dairy in a controlled environment chamber. They found reduced thermal stress in OmniGen-AF fed cows as there were reduced rectal temperatures and respiration rates. Feed intake was higher on OmniGen-AF fed cow during heat stress.

There was no difference ($P > 0.10$) between treatments on milk compositions parameters such percentages of milk fat, milk protein, and lactose, MUN and SCC (Table 2.7) which is similar to the findings of Kung et al. (2008), Wall et al. (2014), and Tassoul

and Shaver, (2009) for EO fed cows and Barkley and Waldron (2013) for OmniGen-AF fed cows.

Both the TMR with EOC and ADY prevented the growth of *Aspergillus fumigatus*. Both diets were < 10 cfu/g, which was below the detection limit (Table 2.9). *Escherichia coli* and yeast were numerically lower ($P < 0.02$ & $P < 0.10$) on the feces of cows fed with EOC than cows fed ADY (Pathogen Scoring: none=0, rare=1, slight=2, moderate=3, large=4, and excessive=5). This absorption would result in a cleaner gut with less *E. coli*; therefore, less nutrients will be used by pathogens; thereby increasing nutrient availability to the cow.

Nutrient digestibility (ND) for dry matter (DM) ($P < 0.05$), nutrient detergent fiber (NDF) ($P < 0.04$), hemicellulose ($P < 0.02$), calcium ($P < 0.02$), and phosphorus (P < 0.03) were significantly higher for cows fed ADY than cows fed with EOC. Similarly, ND for crude protein (CP) ($P < 0.06$), acid detergent fiber (ADF) ($P < 0.09$), and magnesium ($P < 0.09$) were tended higher for cows fed ADY compared to EOC fed. Cows fed ADY has better nutrient digestibility because the product contains a fibrolytic enzyme *Trichoderma longibrachiatum* in it, but no enzymes are in EOC. However, there was no increase in milk and greater feed efficiency with feeding ADY despite having better nutrient digestibility. So, it can be concluded the cows wasted the nutrients.

CONCLUSION

There were no significant differences in milk yields and feed efficiency when cows were fed EOC or ADY. The DMI and body surface temperatures were significantly lower for cows fed EOC indicating that cows were cooler and able to better withstand heat stress, because this study was conducted during the summer/fall. Both EOC and ADY prevented the growth of *Aspergillus fumigatus* in the TMR. Thus, a cleaner gut with less *E. coli* will waste less nutrients increase the nutrient is availability to the cow. Nutrient digestibility was higher for ADY fed cows than cows fed EOC. Further studies are recommended to incorporate the status of ruminal VFAs profile, ammonia and pH if an impact occurs by collecting rumen fluid as well as analyze immune parameters to determine if an impact occurs in the immune status of the lactating cow.

Table 2.1 Composition of Total Mixed Ration (TMR) for cows fed EOC and ADY

Ingredients	% of DM
Corn Silage	37.8
Grass Hay	0.85
Alfalfa Hay	8.47
Alfalfa Baelage	17.1
High moisture ear corn	10.1
Molasses	1.89
Protein Mix*	14.4
Corn/cotton mix	8.69
Energy Booster	0.79

[1]EOC = Blend of Essential Oil and Cobalt Lactate.
[2]ADY = Active Dried Yeast.

Table 2.2 Composition of Protein Mix*used in Total Mixed ration (TMR)

Ingredients	% of DM
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	0.6
Corn gluten meal	0.39
Corn starch	0.29
Urea	0.21
Yeast culture concentrate	0.11
DCAD (Dietary Cation Anion Difference) plus	0.1
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 2.3 Nutrient composition of TMR fed during trial for EOC and ADY

Nutrient	EOC[1]	ADY[2]
DM, %	47.34	46.97
CP, %	15.22	15.31
SIP[3], %	41.38	40.67
ADF, %	23.01	21.20
NDF, %	36.23	33.86
ADIP[4]	0.78	0.74
NDIP[5]	1.78	1.71
Starch	23.67	26.08
Oil	3.32	3.47
Nitrate, ppm	78.00	76.67
IVDMD[6]	74.79	76.27
IVTD[7]	81.16	82.74
NDFD[8]-(30 h)	48.35	49.03
Lignin	4.31	3.79
Ash	7.18	7.13
Na	0.55	0.55
Mg	0.27	0.27
P	0.35	0.35
S	0.22	0.22
K	1.48	1.44
Ca	0.92	0.91
Cl	0.63	0.62
Mn, ppm	89.67	89.00
Fe, ppm	330.33	304.67
Cu, ppm	24.33	23.67
Zn, ppm	105.33	103.33
AIA, %	1.16	0.97
NFC	39.82	41.93
NEL, MCal/kg	0.75	0.78
NEM, MCal/kg	0.80	0.82
NEG, MCal/kg	0.48	0.50

[1]EOC = Blend of Essential Oil and Cobalt Lactate. [2]ADY = Active Dried Yeast. [3]SIP = Soluble intake protein. [4]ADIP = Acid detergent insoluble protein. [5]NDIP = Neutral detergent insoluble protein. [6]IVDMD = *In vitro* dry matter digestibility. [7]IVTD = *In vitro* total digestibility. [8]NDFD = Neutral detergent fiber digestibility.

Table 2.4 Nutrient composition of corn silage, alfalfa Baelage, alfalfa hay used in TMR and pelleted concentrate fed during trial

Nutrient	Corn Silage	Alfalfa Baelage	Alfalfa Hay	Pellet
DM, %	33.77	50.03	77.40	88.10
pH	3.87	4.76	--	--
CP,%	7.43	22.78	20.52	21.90
NDF	37.59	40.52	44.14	26.60
ADF	24.54	34.79	37.71	16.20
Lignin	3.64	8.97	21.75	--
ADICP[1]	0.50	2.01	1.66	--
NDICP[2]	1.33	5.11	4.50	--
SIP[3], %	53.53	59.27	44.48	--
Ammonia CP	0.62	1.41	--	--
Starch	33.46	--	--	20.10
Fat	24.65	3.32	1.78	--
Ash	4.00	11.17	13.11	--
AIA[4]	--	--	--	0.22
Ca	0.25	1.37	1.44	0.42
P	0.26	0.41	0.34	0.81
Mg	0.15	0.28	0.28	0.41
K	1.16	2.82	2.87	1.88
S	0.10	0.26	0.21	0.07
Fe, ppm	--	--	--	237.00
Cu, ppm	--	--	--	15.80
Zn, ppm	--	--	--	118.00
Mn, ppm	--	--	--	80.30
Sugar	1.61	2.16	3.60	--
Lactic Acid	3.72	4.14	--	--
Acetic Acid	1.15	0.17	--	--
Propionic Acid	0.01	0.01	--	--
Butyric Acid	--	0.01	--	--
NDFD[5], % NDF (30h)	53.05	42.84	--	--
TDN[6]	71.04	59.97	52.23	--
NFC	48.76	28.02	23.94	
NE_L (Mcal/kg)	0.72	0.62	0.52	--
RFV[7]	--	146.39	125.71	--
RFQ[8]	--	133.64	--	--

[1]ADICP = Acid detergent insoluble protein. [2]NDICP = Neutral detergent insoluble protein. [3]SIP = Soluble intake protein. [4]AIA = Acid insoluble ash. [5]NDFD = Neutral detergent fiber digestibility. [6]TDN = Total digestible nutrients. [7]REV = Relative feed value. [8]RFQ = Relative forage quality.

Table 2.5 Effects of feeding EOC and ADY on milk and milk components

Measurement	EOC[1]	ADY[2]	SEM	*P-value*
Milk, kg/d	36.11	37.74	2.19	0.25
MLM[3], kg/d	37.28	39.28	2.80	0.26
Fat, %	3.46	3.30	0.06	0.41
Protein, %	2.98	2.98	0.02	0.99
FCM[4], kg/d	32.88	33.52	1.93	0.60
ECM[5], kg/d	32.56	33.29	1.86	0.53
Rumination, min/d	476.0	482.5	12.5	0.72
Pellet, kg/d	4.90	5.03	0.11	0.06
DMI, kg/d	21.82	22.68	0.1	0.01
Feed Efficiency	1.66	1.69	0.04	0.53

[1]EOC = Blend of Essential Oil and Cobalt Lactate.
[2]ADY = Active Dried Yeast.
[3]Management level milk (MLM) = ((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.
[4]Fat corrected milk (FCM) = (0.4 x kg of milk) + (15 x kg of milk fat).
[5]Energy corrected milk (ECM) = (0.327 x kg of milk) + (12.95 x kg of milk fat) + (7.2 x kg of milk protein).

Table 2.6 Dry matter intake (DMI), feed efficiency (FE), body weight (BW), body surface temperature and BCS for cows fed EOC and ADY

Measurement	EOC[1]	ADY[2]	SEM	*P-value*
DMI[3], kg/d	21.82	22.68	0.1	0.01
Feed Efficiency[4]	1.66	1.69	0.04	0.53
Body weight, kg	634.94	665.74	16.7	0.01
Body surface temperature, ^{0}C	32.95	33.57	0.3	0.0004
BCS[5]	3.098	3.128	0.037	0.433

[1]EOC = Blend of Essential Oil and Cobalt Lactate.
[2]ADY = Active Dried Yeast.
[3]DMI includes both DMI of total mixed ration (TMR) and concentrate.
[4]FE = Feed efficiency = Kg of milk produced per kg of DM consumed.
[5]BCS=Body condition score, 1-5.

Table 2.7 Milk Components data from Dairy Herd Improvement Association (DHIA) for cows fed EOC and ADY

Measurement	EOC[1]	ADY[2]	SEM	*P-value*
Fat, %	3.37	3.34	0.11	0.74
Protein, %	3.03	3.02	0.69	0.04
Lactose, %	4.90	4.95	0.04	0.15
MUN, mg/dL	13.00	12.40	0.10	0.85
SCC, log10, cells/mL	4.98	4.99	0.28	0.04

[1]EOC= Blend of essential oil and cobalt lactate.
[2]ADY = Active Dried Yeast.
SCC=Somatic cell count.
MUN=Milk urea nitrogen.

Table 2.8 Nutrient Digestibility (ND[5], %) of cows fed EOC and ADY by using Acid Insoluble Ash (AIA) as digestibility marker.

Measurement	EOC[1]	ADY[2]	SEM	P-value
Dry Matter, %	76.5	80.1	1.62	0.05
Crude Protein, %	76.4	79.8	1.46	0.06
ADF[3], %	55.1	60.6	4.49	0.09
NDF[4], %	60.4	66.6	3.47	0.04
Hemicellulose, %	69.3	76.6	2.21	0.02
Starch, %	97.4	97.2	0.86	0.71
Ca, %	18.7	29.8	6.11	0.02
P, %	62.1	66.8	2.06	0.03
Mg, %	28.8	39.0	5.62	0.09
S, %	60.2	66.0	2.82	0.27

[1]EOC= Blend of essential oil and cobalt lactate.
[2]ADY = Active Dried Yeast.
[3]ADF= Acid Detergent Fiber
[4]NDF= Neutral Detergent Fiber
[5]Nutrient Digestibility (ND, %) =100-(100 × ((Acid Insoluble Ash (AIA) in feed, %) / (AIA in feces, %) × (Nutrient in feces, %) / (Nutrient in feed, %))).

Table 2.9 Measurement of Fecal pathogens and TMR *Aspergillus fumigatus* for cows fed EOC and ADY

Measurement	EOC[1]	ADY[2]	SEM	*P-value*
		Fecal		
Normal Flora	3.69	3.77	0.09	0.66
Salmonella sp.	ND	ND	---	----
Campylobacter sp.	ND	ND	---	----
Clostridia sp.	ND	ND	---	----
Coliforms sp.	3	3.31	0.06	0.02
Mold	2.15	2.15	0.08	1
Yeast	1.22	1.85	0.18	0.1
Bacillus sp.	1.46	1.15	0.16	0.33
		TMR		
Aspergillus fumigatus	ND	ND	----	-----

[1]EOC= Blend of essential oil and cobalt lactate.
[2]ADY = Active Dried Yeast.
Pathogen Scoring: none = 0, rare = 1, slight = 2, moderate = 3, large = 4, and excessive = 5
ND=not detected or too low to count, i.e. essentially 0.

CHAPTER 3:

EVALUATION OF COBALT CARBONATE AND COBALT LACTATE ON RUMINAL FERMENTTATION AND NUTRIENT DIGESTIBILITY OF GRASS HAY USING IN VITRO GAS PRODUCTION SYSTEM

ABSTRACT

This study was carried out to evaluate cobalt carbonate and cobalt lactate (CoL, CoMax: Ralco, Marshall, MN) on ruminal fermentation and nutrient digestibility *in vitro*. Dried and ground grass hay samples were prepared in individual containers by adding required amounts of the treatments. Treatments included 1) Blank: No feed and no treatment, 2) $CoCO_3$ - 0.1 ppm, 3) $CoCO_3$ -3.5 ppm, 4) CoL - 0.11 ppm, 5) CoL - 0.22 ppm, 6) CoL - 0.875 ppm, 7) CoL - 1.75 ppm, and 8) CoL - 3.5 ppm. One g of feed with treatment were placed in fiber filter bags having a 57 µm pore size, heat sealed, and placed into gas fermentation bottle (GFB) for fermentation. The treatment block was replicated 3 times and each treatment was replicated 4 times, which gave 12 observations per treatment with a total of 96 fermentation bottles. Rumen fluid was collected from a ruminally cannulated lactating dairy cow and strained through 4 layers of cheese cloth and flushed with CO_2. Gas fermentation bottle was pre-warmed in water bath at 39 ^{0}C with 200 ml of McDougall's Buffer solution. Fifty ml of prepared rumen fluid was poured into each GFB. The gas pressure in each bottle was recorded at 5 min interval for a 30 h incubation period. All the GFB were opened and the pH was measured for each GFB and samples were taken for VFA and NH_3-N analysis. The filter bags were taken out, washed, dried, and weighed to determine dry matter digestibility (DMD) and neutral detergent fiber digestibility (NDFD). Rate of gas production was lower ($P < 0.05$) for 0.1 ppm of $CoCO_3$ (0.08) and 3.5 ppm of $CoCO_3$ (0.10), and 0.11, 0.22, 0.875, and 0.11 ppm of CoL (0.10,

0.10 0.11, and 0.11 respectively) was intermediate. Concentration of total VFA (mmol/L) was lower ($P < 0.05$) for 0.22 and 3.5 ppm of CoL than other treatments (44.23 and 47.68 for 0.1, 3.5 of $CoCO_3$ and 48.62, 45.40 and 43.22 for 0.11, 0.875, 1.75 ppm of CoL respectively). Acetate (molar percentage) was lower ($P < 0.05$) for 1.75 and 3.5 ppm than other treatments (63.97, 64.09 for 0.1, 3.5 of CoCO3 and 63.93, 63.22, and 63.70 for 0.11, 0.22, and 0.875 ppm respectively). Rumen ammonia concentration was similar for all treatments. Dry matter digestibility was highest ($P < 0.05$) for 0.11 ppm of CoL (22.28) and was intermediate for 0.1, 3.5 ppm of $CoCO_3$, and 0.22 ppm of CoL. NDFD is highest for was higher ($P < 0.05$) for 0.11 ppm of CoL (51.31), intermediate for 0.1, 3.5 ppm of $CoCO_3$ and 0.22 ppm of CoL and lower ($P < 0.05$) for 0.875, 1.75, and 3.5 ppm of CoL (45.79, 44.52, 44.05 respectively) than other treatments. Results show that lower doses of CoL are more effective for fiber digestion of grass hay than $CoCO_3$.

Key Words: Cobalt, gas production, nutrient digestibility, grass hay

INTRODUCTION

Cobalt is an essential trace mineral in the diet of ruminants for the production of vitamin B_{12} by the ruminal microflora. The microbes in the rumen can produce the required amount of vitamin B_{12} when adequate cobalt is provided in the diet (NRC, 2001). The production of vitamin B_{12} and its analogues in the rumen during fermentation is primarily dependent on the concentration of cobalt, roughage content in the diet and cobalt source (Sutton and Elliot, 1972; Dryden and Hartman, 1971; Kawashima et. al., 1997a).

The dietary requirement for cobalt is 0.11 mg/kg of dietary DM for dairy cattle and it is based on the amount of cobalt that must be supplied to keep the tissue concentrations of vitamin B_{12} above 0.3 μg/L (NRC, 2001). However, various projects have been done by increasing the dose of cobalt *in vitro* and *in vivo* with a higher level of dietary level of cobalt has been suggested both in beef (Stangla et. al., 1995) and dairy cows (Tomlinson and Socha, 2003). Mills, (1981) found increase in ruminal B_{12} synthesis by 20 fold in sheep when dietary Co was increased from 0.1 to 0.5 mg/d. Similarly, Kawashima et al. (1997b) found the concentrations of vitamin B_{12} were increased until 40 ppm in serum and liver of sheep whereas there was increased synthesis of vitamin B_{12} as the dietary concentration of cobalt was in increased from 0.1 to 1.0 mg/kg (Tiffany et. al., 2006) using mixed ruminal microorganisms grown in continuous culture fermenters. Multiparous cows fed diet with 1.26 mg of Co/kg of DM had greater milk and 3.5% FCM yields than multiparous cows fed a diet with 0.37 or 0.68 mg of Co/kg of DM and there was no effect of cobalt on milk production in primiparous cows. In another study, it was

reported that 0.3-0.5 mg Co/kg DM increased the microbial activity, fermentation and synthesis of vitamin B_{12} (Singh and Chhabra, 1995).

There are different sources of cobalt, but the rate of bioavailability is different. Cobalt chloride and nitrate, and cobaltous carbonate and sulfate are suitable sources of cobalt for ruminants (NRC, 2001) and cobalt lactate has been found to be a highly soluble source of cobalt in the rumen (Pretz et al., 2015). Ruminal cultures containing inadequate cobalt had unstable fermentation patterns, which could be due to insufficient vitamin B12 synthesized and the shifting of the microbial population (McDonald and Suttle, 1986). After addition of 2 ppm cobalt from cobalt sulfate increased the number of protozoa of genera *Isotricha* and *Dasyfticha*, but the total number was not changed in sheep (Zelenak et. al. 1992). The feeding of additional cobalt as cobalt-lactate did not influence milk production, milk composition, DMI or BW for lactating dairy cows fed a high forage ration, but did appear to alter ruminal fermentation to increase fiber digestion and microbial protein synthesis (Pretz and Casper, 2015). They reported increased fiber digestibility when particles were evaluated by stereo and scanning electron microscopy (Pretz et. al. 2015). Similarly, the addition of Cu and Co above NRC recommended level, improved the DM digestibility of low quality forages like corn residues in an *in situ* study done in heifers by Lopez-Guisa and Satter, (1992).

Limited study has been done on the effect of cobalt carbonate and cobalt lactate in dairy cattle diets on their lactational performance. The cobalt is a very interesting ingredient for enhancing the ruminal digestion of DM and fiber, however, not much is known about the dietary rate of supplementation. Thus, the objective of this *in vitro* titration study was to determine the optimal rate of CoCO3 and Co-lactate

supplementation to maximize ruminal fermentation and digestion of DM and fiber by using gas fermentation system when fed grass hay as substrate.

MATERIALS AND METHODS

Treatments

Eight different treatments were selected which were based on the NRC recommendation and rates (mg/hd/d) being fed in the field. The treatments were as follows:

Blank: No feed and no treatment. Rumen fluid only.

Cobalt carbonate ($CoCO_3$) 46% -0.1 ppm (NRC; 2.27 mg/hd/d)

$CoCO_3$ 46% -3.5 ppm (79.4 mg/hd/d)

Cobalt Lactate (CoL) 10% - 0.11 ppm (2.5 mg/hd/d)

CoL 10% - 0.22 ppm (5.0 mg/hd/d)

CoL 10% - 0.875 ppm (19.8 mg/hd/d)

CoL 10% - 1.75 ppm (39.7 mg/hd/d)

CoL 10% - 3.5 ppm (79.4 mg/hd/d)

The treatment block was replicated 3 times and each treatment was replicated 4 times, which gave 12 observations per treatment with a total of 96 fermentation bottles.

Preparation of samples

Grass hay samples were obtained from the SDSU cow calf research facility which was being fed to the beef cattle. The grass hay sample was dried at 55^0C for 48 hours in a force air oven and ground through a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) having a 1 mm screen. Individual samples were prepared in individual containers by adding required amounts of the treatments (Table 3.1). 1 g of ground hay with treatments were weighed and placed in fiber filter bags having a 57 µm pore size (ANKOM Technology, Macedon, NY) and sealed by using heat impulse sealer.

Preparation of McDougall's Buffer solution

500 ml (half of the desired buffer volume) of distilled water was taken in a flask and the chemicals (Table 3.2) were weighed and dissolved one by one as listed that required for in vitro gas fermentation. The final volume was made to 1 L and the buffer solution was dispersed with carbon dioxide (CO_2) before use to make the pH of the solution below 7. It was refrigerated overnight before use.

Rumen fluid preparation

Rumen fluid was collected from a ruminally cannulated lactating dairy cow. It was collected in a pre-warmed (39^0C) thermos flushed with CO_2 and transported to SDSU gas fermentation laboratory. The rumen fluid was strained through 4 layers of cheese clothes and continued to flush with CO_2 for 30 sec.

ANKOM gas production system operation

The ANKOM gas production system (ANKOM Technology, Macedon, NY) has gas fermentation bottles that have gas pressure sensor modules and are connected to a computer via a radio frequency without wire. The gas pressure is recorded by the computer from the system.

Each gas fermentation bottles (GFB) was pre-warmed in a circulating water bath at 39^0C with 200 ml of McDougall's Buffer solution and filter bag with treatment and grass hay samples in it. Fifty ml of prepared rumen fluid was poured into each GFB under continuous flushing of CO2 for 1.5 min. The modules were tightly closed for each GFB and the computer program was set to record the data for 30 hours. The gas pressure accumulated in each bottle was recorded at every 5 min for 30 h of incubation period. The release pressure was set at 1 psi. Each bottles were shaken every 2 hours during the fermentation period to ensure that the feed bags were completely immersed into the solution of buffer and rumen fluid. After 30 hours of incubation period, the system was turned off and the data recorded was saved. All the modules were opened and the pH was measured for each rumen fluid and samples were taken for VFA and NH_3-N analysis. The filter bags with the feed were taken out and washed with running tap water until clean. Bags were dried in an oven at 55^0C for 48 h and weighed for calculation of dry matter digestibility. The cumulative gas produced for 30 h was fitted to the equation: gas =b x (1-e^ (-c x h) by using non-linear regression to determine the kinetic rate of gas production.

Analysis of Samples

For determination of NH_3-N, 10 ml of rumen fluid samples obtained from after 30 h gas fermentation were collected in a vial containing 200µl of 50% (V/V) H_2SO_4 for each bottles and for determination of VFA, 10 ml of rumen fluid samples were collected in the vials containing 2 ml of 25% (W/V) meta-phosphoric acid. They were immediately stored at -20^0C for later determination. During the time of analysis, rumen fluid samples were thawed and centrifuged at 30,000 × g for 20 min at 20^0C (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). NH3-N was analyzed by using the procedures from Chaney and Marbach (1962). The samples stored with meta-phosphoric acid were analyzed for VFA concentrations by using automated gas-liquid chromatograph (model 6850, Agilent, Santa Clara, CA). The samples for VFA analysis were prepared according to Erwin et al., 1961. NDF analysis was done for the dried bag from fermentation and the original grass hay samples by using the filter bag technique method as described by ANKOM Technology Macedon, NY by using NDF solution, heat stable alpha amylase and sodium sulfite in fiber analyzer vessel developed by ANKOM Technology Macedon, NY. The NDFD was measured by taking difference between the amount of NDF in the grass hay samples before and after in vitro fermentation. NDF assay for original grass hay samples and the samples after in vitro digestion were used to calculate the NDFD.

Statistical Analysis

All data were subjected to least squares ANOVA using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, version 9.4) for a randomized complete block design

(Steele and Torrie, 1980) having 7treatments. The statistical model used was: $Y_{ijkl} = \mu + B_i$ + Rep (Block)j, $T_k + e_{ijk}$, where Y_{ij} = dependent variable, μ = overall mean, B_i = Block effect, Rep (Block) j = Rep nested within Block, T_k = treatment effect, and e_{ijk}= random error. Block and Rep (Block) were considered to be random, while treatment was considered fixed. Whenever significant differences due to treatment were detected, the Fischer's least significant difference test (Steele and Torrie, 1980) was used to separate least squares treatment means (PDIFF statement). Significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

The nutrient composition (% of DM) of grass hay used for feeding in the gas fermentation is presented in Table 3.2. The grass hay had 7 % CP with 67% NDF and 40% ADF.

The concentration of rumen VFA produced when grass hay was fed with CoCO3 and CoL in different doses is presented in Table 3.3. Concentration of total VFA (mmol/L) was lower ($P < 0.05$) for 0.22 and 3.5 ppm of CoL than other treatments (0.1, 3.5 of $CoCO_3$ and 0.11, 0.875, 1.75 ppm of CoL respectively). Molar proportion of acetate was lower ($P < 0.05$) for 1.75 and 3.5 ppm than other treatments (0.1, 3.5 of $CoCO_3$ and 0.11, 0.22, and 0.875 ppm respectively). Similarly, molar proportion of propionate was similar ($P > 0.10$) for all treatments. Rumen ammonia concentration (Table 3.4) was similar for treatment, which is similar with the finding of Tiffany et al. (2014) where there was no effect on rumen ammonia concentration by Co

supplementation in diet. There was no effect on production parameters by feeding Co-lactate but it decreased rumen ammonia concentrations and increased ruminal molar concentrations of acetate and increased fiber digestion numerically (Pretz et al., 2015).

The DMD was highest ($P < 0.05$) for 0.11 ppm of CoL and was intermediate for 0.1, 3.5 ppm of $CoCO_3$, and 0.22 ppm of CoL (Table 3.5). NDFD for was higher ($P < 0.05$) for 0.11 ppm of CoL (51.31), intermediate for 0.1, 3.5 ppm of $CoCO_3$ and 0.22 ppm of CoL and lower ($P<0.05$) for 0.875, 1.75, and 3.5 ppm of CoL than other treatments. Similar finding was reported by Hussein et al., (1994) who demonstrated higher *in vitro* digestibility of DM, OM, and NDF for inoculum from steers fed alfalfa than concentrate.

Rate of gas production was lower ($P < 0.05$) for 0.1 ppm of $CoCO_3$, intermediate for 3.5 ppm of $CoCO_3$, and 0.11, 0.22, 0.875, and 0.11 ppm of CoL, and highest for 3.5 ppm of CoL (Table 3.6). This indicates that addition of CoL aided ruminal nutrient digestion. The higher DMD and NDFD with 0.11 ppm of CoL support the above finding of increased nutrient digestion. However, there are intermediate molar proportions of acetate for 0.11 ppm of CoL and numerically higher total VFA, which can be related to increased fiber digestion. This is similar to the findings of Pretz and Casper, (2015) where they found alteration in ruminal fermentation due to increase fiber digestion and microbial protein synthesis by feeding additional Co as CoL for lactating dairy cows fed a high forage ration. They found increased digestibility of fiber particles when evaluated by stereo and scanning electron microscopy on the samples. Results show that lower doses of CoL are more effective for fiber digestion of grass hay than $CoCO_3$.

CONCLUSION

Use of cobalt in higher doses in the diet of ruminants is beneficial for vitamin-B_{12} synthesis which is used by ruminal microflora as a growth factor and is needed for propionate metabolism. In this experiment using grass hay as substrate, total VFA and molar proportions of acetate, and acetate: propionate ratio were found to be higher for $CoCO_3$, but other VFAs like isobutyrate, butyrate, isovalerate, and valerate was higher for CoL. Increased in acetate should indicate increased fiber digestion, but DMD and NDFD were higher for lower doses of CoL and also the rate of gas production was higher for CoL, but they are lower with $CoCO_3$. The lower doses of CoL have better performance than the higher doses of $CoCO_3$, which might to due to greater bioavailability of CoL. Additional studies in this area might be needed to measure the microbial protein synthesis and vitamin B_{12} synthesis to determine the efficacy of the cobalt source and function on fiber digestion. An i*n vivo* trial is recommended to determine the animal performance to different doses of cobalt.

Table 3.1 Chemical Composition of McDougall's Buffer

Chemicals	g/L
Sodium Bicarbonate ($NaHCO_3$)	9.8
Sodium Phosphate (Na_2HPO4 Dibasic)	2.77
Potassium Chloride (KCl)	0.57
Sodium Chloride (NaCl)	0.47
Magnesium Sulfate ($MgSO_4.7H_2O$)	0.12
Calcium Chloride ($CaCl_2.2H_2O$)	0.16
^{15}N Ammonium Sulfate ($(^{15}NH_4)_2SO_4$ (10% ^{15}N))	2.12

Table 3.2 Nutrient composition (% of dry matter (DM) unless otherwise stated) of grass hay (GH) used for gas fermentation

Nutrients	GH
Dry Matter	94.45
Crude Protein	7.06
SP[1], % CP	35.92
Neutral Detergent Fiber	67.1
Acid Detergent Fiber	40.73
ADIP[2]	1.03
Non Fiber Carbohydrate	18.3
Net Energy of Lactation (NE_L, Mcal/kg	1.27
Oil	1.55
IVDMD[3]	49.12
NDFD[4], % NDF	42.09
Lignin	5.04
Ash	8.87
Calcium	0.34
Phosphorus	0.16
Magnesium	0.21
Potassium	1.63
Sodium	0.02
Chlorine	0.75
Sulfur	0.14
Iron, ppm	84
Copper, ppm	8
Zinc, ppm	18
Manganese, ppm	41

[1]SP = Soluble protein
[2]ADIP = Acid detergent insoluble protein
[3]IVDMD = In vitro dry matter digestibility
[4]NDFD = Neutral detergent fiber digestibility, 30 h

Table 3.3 Concentration of rumen VFAs when grass hay was used as substrate with different sources and doses (ppm) of Cobalt

Measurements	CoCO3		CoL					SEM
Ppm	0.1	3.5	0.11	0.22	0.875	1.75	3.5	
Total VFA	44.23^{ac}	47.68^{a}	48.62^{ad}	43.16^{bcd}	45.40^{ad}	43.22^{ad}	42.01^{bcd}	3.20
----------(mmol/100 mmol of total VFA----------								
Acetate	63.97^{a}	64.09^{a}	63.93^{ac}	63.22^{ad}	63.70^{ac}	62.67^{bd}	63.10^{bcd}	1.43
Propionate	21.59	21.47	21.76	21.98	21.75	21.83	21.82	1.48
IsoButyrate	1.55^{bc}	1.54^{bc}	1.50^{bc}	1.57^{ac}	1.56^{bc}	1.66^{a}	1.58^{ac}	0.07
Butyrate	9.33^{b}	9.35^{b}	9.28^{b}	9.55^{bc}	9.41^{bc}	9.96^{a}	9.76^{ac}	0.27
IsoValerate	1.82^{bc}	1.86^{bc}	1.82^{bc}	1.91^{cd}	1.84^{bc}	2.03^{a}	1.94^{ad}	0.16
Valerate	1.68^{bc}	1.67^{bd}	1.70^{bc}	1.75^{acd}	1.71^{bc}	1.82^{a}	1.77^{ac}	0.05
Acetate: Propionate	2.98^{a}	3.02^{a}	2.97^{ac}	2.91^{bc}	2.96^{ac}	2.90^{bc}	2.93^{ac}	0.24
(Ace+But): Propionate	3.42^{acd}	3.46^{a}	3.40^{acd}	3.35^{bc}	3.40^{acd}	3.36^{bd}	3.38^{ac}	0.27

^{abcd}Means in same row with different superscripts differ significantly for treatment effect.

Table 3.4 Rumen ammonia and pH when grass hay was used as substrate with different sources and doses (ppm) of Cobalt.

Measurements	CoCO3		CoL					SEM
	0.1	3.5	0.11	0.22	0.875	1.75	3.5	
Ammonia*	31.56	27.98	30.27	30.60	27.3	27.65	29.49	2.19
pH	6.87^{ac}	6.86^{bc}	6.86^{bc}	6.90^{a}	6.91^{a}	6.88^{ac}	6.87^{bc}	0.03

^{abc}Means in same row with different superscripts differ significantly for treatment effect.

Table 3.5 Dry matter digestibility (DMD) and neutral detergent fiber digestibility (NDFD) when grass hay was used as substrate with different sources and doses (ppm) of Cobalt.

Measurements	CoCO3		CoL					SEM
	0.1	3.5	0.11	0.22	0.875	1.75	3.5	
DMD[1]	19.40[b]	19.40[b]	22.28[a]	19.67[b]	16.90[c]	15.86[c]	16.00[c]	0.59
NDFD[2]	48.17[bd]	48.54[ad]	51.31[a]	48.26[bd]	45.79[c]	44.52[c]	44.05[c]	1.14

[abc]Means in same row with different superscripts differ significantly for treatment effect.

[1]DMD = Dry matter digestibility

[2]NDFD = Neutral detergent fiber digestibility

*not significant among the treatments

Table 3.6 Regression coefficients of gas production when grass hay was used as substrate with different sources and doses of Cobalt.

Measurements	CoCO3		CoL					SEM
Ppm	0.1	3.5	0.11	0.22	0.875	1.75	3.5	
b*	4.55	4.17	4.57	4.38	3.94	4.49	3.87	0.35
C	0.08^{bc}	0.10^{ac}	0.10^{ac}	0.10^{ac}	0.11^{ac}	0.11^{ac}	0.12^{a}	0.01

^{a,b,c} Means in same row with different superscripts differ significantly for treatment effect.

*not significant among the treatments

CHAPTER 4:

EVALUATION OF COBALT CARBONATE AND COBALT LACTATE ON FERMENTTAION AND DIGESTIBILITY OF A MIXTURE OF HIGH CORN SILAGE AND LOW ALFALA BAELAGE USING IN VITRO GAS PRODUCTION SYSTEM

ABSTRACT

This study was carried out to evaluate cobalt carbonate and cobalt lactate product (CoL, Ralco, Marshall, MN) on ruminal fermentation and nutrient digestibility *in vitro*. Dried and ground mixed samples (Corn silage-60% and alfalfa Baelage-40%, DM basis) were prepared in individual containers by adding required amounts of Cobalt. Treatments included: 1) Blank - No feed and no cobalt, 2) Cobalt carbonate ($CoCO_3$) -3.5 ppm, 3) CoL - 0.11 ppm, 4) CoL - 0.22 ppm, 5) CoL - 0.875 ppm, 6) CoL - 1.75 ppm, and 7) CoL - 3.5 ppm. One g of sample with were placed in fiber filter bags having a 57 µm pore size, heat sealed, and placed into a gas fermentation bottle (GFB). The treatment block was replicated in 3 times and each treatment was replicated 4 times within each block which gave 12 observations per treatment and for total 84 gas fermentation. Rumen fluid was collected from a ruminally cannulated lactating dairy cow and was strained through 4 layers of cheese clothes and flushed with CO_2. GFB was pre-warmed in water bath at 39 ^{0}C with 200 ml of McDougall's Buffer solution. A 50 ml of prepared rumen fluid was poured into each GFB. The gas pressure in each bottle was recorded at 5 min intervals for 30 h of incubation period. All the GFB were opened and the pH was measured and samples were taken for volatile fatty acid (VFA) and rumen ammonia (NH_3-N) analysis. The filter bags were taken out, washed, dried, and weighed to determine dry matter digestibility (DMD) and neutral detergent fiber digestibility (NDFD). Rate of gas production was similar ($P > 0.10$) among the treatments. Concentration of total VFA

(mmol/L) was higher ($P < 0.05$) for 0.11 ppm of CoL (66.45 mmol/L), intermediate for 0.22, 0.875, 1.75, and 3.5 ppm of CoL (62.88, 63.64, 63.67, and 62.84 mmol/L respectively) and lower ($P < 0.05$) for 3.5 ppm of $CoCO_3$ (57.40 mmol/L). Molar percentage of acetate was higher ($P < 0.05$) for 3.5 ppm of $CoCO_3$, intermediate for 0.11, 1.75, and 3.5 ppm of CoL (60.86, 61.12, and 61.71 respectively) and lower for 0.22 and 0.875 ppm of CoL (60.76, 60.31 respectively). Propionate was higher for all treatment (0.11, 0.22, 0.875, 1.75, and 3.5) of CoL than 3.5 ppm of $CoCO_3$. Rumen NH_3-N and pH was similar among the treatments. The DMD was lower ($P < 0.05$) for 3.5 ppm of $CoCO_3$, intermediate for 0.11, 3.5 ppm of CoL and highest ($P < 0.05$) for 0.1 ppm of $CoCO_3$ and 0.22, 0.875, and 1.75 ppm of CoL. Results show that CoL is more effective for fiber digestion of high forage mix of corn silage and alfalfa baelage than $CoCO_3$.

Key Words: Cobalt, gas production, nutrient digestibility, high forage.

INTRODUCTION

Cobalt is an essential trace mineral in the diets of ruminants for the production of vitamin B_{12} by the ruminal microflora. The microbes in the rumen can produce the required amount of vitamin B_{12} when adequate cobalt is available in the diet (NRC, 2001). The production of vitamin B_{12} and its analogues in the rumen during fermentation is primarily dependent on the concentration of cobalt, roughage content in the diet and cobalt source used (Sutton and Elliot, 1972; Dryden and Hartman, 1971; Kawashima et. al.,1997a).

The dietary requirement for cobalt is 0.11 mg/kg of dietary DM for dairy cattle and it is based on the amount of cobalt that must be supplied to keep the tissue concentrations of vitamin B_{12} above 0.3 μg/L (NRC, 2001). However, various research studies have been reported by increasing the dose of cobalt *in vitro* and *in vivo* that a higher level of dietary level of cobalt has been suggested both in beef (Stangl et. al., 2000) and dairy cows (Tomlinson and Socha, 2003). Mills, (1981) found an increase in ruminal B_{12} synthesis by 20 fold in sheep when dietary Co was increased from 0.1 to 0.5 mg. Similarly, Kawashima et al. (1997b) found the concentrations of vitamin B12 were increased until 40 ppm in serum and liver of sheep whereas there was increased synthesis of vitamin B_{12} as the dietary concentration of cobalt was in increased from 0.1 to 1.0 mg/kg (Tiffany et. al., 2006) using mixed ruminal microorganisms grown in continuous culture flow through fermenters. Multiparous cows fed diet with 1.26 mg of Co/kg of DM had greater milk and 3.5% FCM yields than multiparous cows fed a diet with 0.37 or 0.68 mg of Co/kg of DM and there was no effect of cobalt on milk production in primiparous

cows. In another study, it was reported that 0.3-0.5 mg Co/kg DM increased the microbial activity, fermentation and synthesis of vitamin B_{12} (Singh and Chhabra, 1995).

There are different cobalt sources but the rate of bioavailability is different. Cobalt chloride, nitrate, carbonate and sulfate are suitable cobalt sources for ruminants (NRC, 2001) and cobalt lactate has been found to be a highly soluble source of cobalt in the rumen (Pretz et al., 2015). Ruminal cultures containing inadequate cobalt had unstable fermentation patterns, which could be due to insufficient vitamin B_{12} synthesized and shifting of microbial population (McDonald and Suttle, 1986). After addition of 2 ppm cobalt from cobalt sulfate increased the number of protozoa of genera *Isotricha* and *Dasyfticha,* but total number was not changed in sheep (Zelenak et. al. 1992). The feeding of additional cobalt as cobalt-lactate did not influence milk production, milk composition, DMI or BW for lactating dairy cows fed a high forage ration, but did appear to alter ruminal fermentation to increase fiber digestion and microbial protein synthesis (Pretz and Casper, 2015). They reported increased digestibility of fiber particles when evaluated by stereo and scanning electron microscopy on the samples from previous study (Pretz et. al. 2015). Similarly, the addition of Cu and Co than NRC recommended dose, improved the DM digestibility of low quality forages like corn residues in an *in situ* study done in heifers by Lopez-Guisa and Satter, (1992).

Limited studies have been conducted on the effect of cobalt carbonate and cobalt lactate in dairy cattle diets on their lactational performance. Cobalt is a very interesting ingredient for enhancing the ruminal digestion of DM and fiber, however, not much is known about the dietary rate of supplementation. Based on the finding of greater efficacy of feeding lower doses of CoL than higher doses of $CoCO_3$ for fiber digestion (Chapter

3), this study was conducted. Thus, the objective of this *in vitro* titration study was to determine the optimal rate of $CoCO_3$ and CoL supplementation in corn silage and alfala baleage as typical of a dairy ration as substrate to maximize ruminal fermentation and digestion of DM and fiber using a gas fermentation system.

MATERIALS AND METHODS

Treatments

Seven different treatments were selected which were based on the NRC recommendation and rates (mg/hd/d) being fed in the field. The treatments were as follows:

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The treatment block was replicated in 3 times and each treatment was replicated 4 times within each block which gave 12 observations per treatment and for total 84 gas fermentation.

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Ground samples (dried at 55^0C for 48 hours in a force air oven and ground through a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA by using a 1 mm screen) of corn silage and alfalfa baelage were obtained from the study conducted by Pretz et al., 2015. The TMR was prepared by mixing both corn silage and alfalfa hay at the DM ratio of 60:40. The nutrient composition of the samples is presented in the Table 4.2. Individual samples were prepared in individual containers by adding the required amounts of the Co treatments 1 g of forage blend being weighed and placed in fiber filter bags having a 57 µm pore size (ANKOM Technology, Macedon, NY) and sealed by using a heat impulse sealer.

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Each gas fermentation bottles (GFB) was pre-warmed in a circulating water bath at 39^0C with 200 ml of McDougall's Buffer solution and filter bag with treatment. A 50 ml of prepared rumen fluid was poured into each GFB under continuous flushing of CO_2 for 1.5 min. The modules were tightly closed for each GFB and the computer program was set to record the data for 30 hours. The bottles were continued to incubate in the circulating water bath at 39^0C. The gas pressure accumulated in each bottle was recorded at 5 min intervals for 30 h. The release pressure was set at 1 psi. Each bottle was shaken at every 2 hours during the fermentation period to ensure that the feed bags were completely immersed into the buffer solution and rumen fluid. After 30 hours of incubation, the system was turned off and the data recorded was saved. All the GFB were opened and the pH was measured for each GFB and the samples were taken for VFA and NH_3-N analysis. The filter bags with the feed were taken out and washed with running tap water until the rinse was clean. Bags were dried at 55^0C for 48 h and weigh for calculation of dry matter digestibility (DMD). The cumulative gas produced for 30 h was fitted to the equation: gas = b x (1-e^ (-c x h) by using non-linear regression to determine the kinetic rate of gas production.

Analysis of Samples

For determination of NH_3-N, 10 ml of rumen fluid samples obtained from after 30 h gas fermentation were collected in a vial containing 200µl of 50% (V/V) H_2SO_4. For the determination of VFA, 10 ml of rumen fluid was placed in a vial containing 2 ml of 25% (W/V) meta-phosphoric acid. Vials were immediately stored at -20^{0}C for later analysis. At the time of analysis, rumen fluid samples were thawed and centrifuged at 30,000 × g for 20 min at 20^{0}C (Eppendorf 5403, Eppendorf North America, Hauppauge, NY). NH3-N was analyzed by using the procedures from Chaney and Marbach (1962). The samples stored with meta-phosphoric acid were analyzed for VFA concentrations by using an automated gas-liquid chromatograph (model 6850, Agilent, Santa Clara, CA). The samples for VFA analysis were prepared according to Erwin et al., (1961). The NDF analysis was conducted for the dried bag from fermentation and the original forage blend by using the filter bag technique method as described by ANKOM Technology Macedon, NY by using NDF solution, heat stable alpha amylase and sodium sulfite in fiber analyzer vessel developed by ANKOM Technology Macedon, NY. The NDFD was measured by taking difference between the amount of NDF in the forage blend sample before and after *in vitro* fermentation. NDF assay for original samples and the samples after *in vitro* digestion were used to calculate the NDFD.

Statistical Analysis

All data were subjected to least squares ANOVA using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, version 9.4) for a randomized complete block design (Steele and Torrie, 1980) having 6 treatments. The statistical model used was: $Y_{ijkl} = \mu +$

$_{Bi}$ + Rep (Block) j, T_k + e_{ijk}, where Y_{ij} = dependent variable, μ = overall mean, B_i = Block effect, Rep (Block) j = Rep nested within Block, T_k = treatment effect, and e_{ijk} = random error. Block and Rep (Block) were considered to be random, while treatment was considered fixed. Whenever significant differences due to treatment were detected, the Fischer's least significant difference test (Steele and Torrie, 1980) was used to separate least squares treatment means (PDIFF statement). Significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

The nutrient compositions (% of DM) of corn silage and alfalfa baelage used for gas fermentation are presented on Table 4.1. The corn silage had 7.36% CP with 38.70 % NDF and 23.40 % ADF and alfalfa baelage had 26.90% CP with 33.40 % NDF and 25.90 % ADF.

Concentration of total VFA (mmol/L) was higher ($P<0.05$) for 0.11 ppm of CoL, intermediate for 0.22, 0.875, 1.75, and 3.5 ppm of CoL and lower ($P<0.05$) for 3.5 ppm of $CoCO_3$ (Table 4.2). However, molar proportion of acetate was higher ($P<0.05$) for 3.5 ppm of $CoCO_3$, intermediate for 0.11, 1.75, and 3.5 ppm of CoL and lower for 0.22 and 0.875 ppm of CoL (Table 4.2). Molar proportion of propionate was higher for all treatment (0.11, 0.22, 0.875, 1.75, and 3.5) of CoL than 3.5 ppm of $CoCO_3$. The ratio of acetate to propionate was higher for 3.5 ppm of $CoCO_3$ (2.71) than other treatments of CoL. There was no difference by the addition of supplemental Co in steers in basal diets for total VFA and molar proportions of acetate, propionate, and isobutyrate and acetate:

propionate ratio. However, there was increase ($P < 0.05$) in molar proportions of butyrate, valerate, and isovalerate with supplementation of Co (Tiffany et al, 2003).

Rumen NH_3-N and pH was similar ($P > 0.10$) among treatments, which is similar with the finding of Tiffany et al. (2014) where there was no effect on rumen ammonia concentration by Co supplementation.

The DMD was lower ($P<0.05$) for 3.5 ppm of $CoCO_3$, intermediate for 0.11, 3.5 ppm of CoL and higher ($P<0.05$) for 0.22, 0.875, and 1.75 ppm of CoL (Table 4.4). Similarly, NDF was higher for 0.11, and 1.75 ppm of CoL and intermediate for 3.5 ppm of CoL and lower for 3.5 ppm of CoCO3 and 0.22, and 0.875 ppm of CoL (Table 4.4). Both the DMD and NDFD results show higher fiber digestion with 0.11 ppm of CoL than CoCO3. There was no effect of cobalt deficient hay on digestibility parameters but the acetate: propionate ratio was lowered in continuous cultures of rumen micro-organism given cobalt-deficient hay or barley as the substrate (McDonald and Suttle, 1986). Nagabhushana et al. (2008) also demonstrated no significant effect of supplementation of 1 and 6 ppm Co (Cobaltous chloride) on the digestibility of DM, OM, CP, EE and fibers like NDF, ADF, hemicellulose and cellulose in growing Holstein crossed calves.

The rate of gas production was similar among the treatments. However, Naseer and Ismail, (2011) found highest gas production *in vitro* study for the sample (70% wheat straw and 30% concentrate) with Co 0.70 mg/kg DM followed by 0.35 and 1.0 mg/kg for Egyptian ram. They also reported significant increases in VFA, DMD and OMD showing improved rumen fermentation with addition Co. Results demonstrate that CoL is more

effective for fiber digestion of higher forage mix of corn silage and alfalfa baelage than $CoCO_3$, which is due to high bioavailability of CoL than $CoCO_3$.

CONCLUSION

Feeding additional cobalt is helpful for ruminants for the synthesis of vitamin B_{12} and fiber digestion. The bioavailability of cobalt from the source is an important aspect for optimal use by the animal. In this experiment, by using corn silage and alfalfa baelage based forage as a substrate, the total VFA concentration was found to be higher for the lowest dose of CoL and intermediate for the higher doses. The lowest total VFA concentrations were for $CoCO_3$. But the molar proportion of acetate and acetate: propionate ratio was highest for CoCo3. Whereas, other VFAs like propionate, butyrate, and valerate were higher for CoL. But DMD and NDFD were higher for CoL at the lower doses. By performing future studies, the microbial protein synthesis could be correlated with the effects of cobalt on fiber digestion

Table 4.1 Nutrient composition (% of dry matter (DM) unless otherwise stated) of corn silage (CS), and alfalfa baelage (AB) used for gas fermentation

Nutrients	CS	AB
Dry Matter	41.30	47.20
Crude Protein	7.36	26.90
SP[1], % CP	54.50	67.90
Neutral Detergent Fiber	38.70	33.40
Acid Detergent Fiber	23.40	25.90
ADIP[2]	0.52	1.86
Non Fiber Carbohydrate	48.00	30.50
Net Energy of Lactation (NE_L, Mcal/kg	1.65	1.61
Oil	2.69	2.47
IVDMD[3]	70.50	78.20
NDFD4, % NDF	46.30	63
Lignin	2.29	5.68
Ash	3.82	8.62
Calcium	0.18	1.64
Phosphorus	0.20	0.38
Magnesium	0.17	0.36
Potassium	0.77	2.82
Sodium	0.03	0.08
Chlorine	0.16	0.68
Sulfur	0.05	0.27
Iron, ppm	67	258
Copper, ppm	2.75	8.25
Zinc, ppm	25	37.80
Manganese, ppm	32	48

[1]SP = Soluble protein
[2]ADIP = Acid detergent insoluble protein
[3]IVDMD = In vitro dry matter digestibility
[4]NDFD = Neutral detergent fiber digestibility, 30 h

Table 4.2 Concentration of Rumen VFAs when mixture of corn silage and alfalfa Baelage was used as substrate with different sources and doses (ppm) of Cobalt.

Measurements	CoCO3	CoL					SEM
	3.5	0.11	0.22	0.875	1.75	3.5	
Total VFA	57.39^{bc}	66.45^{a}	62.88^{ac}	63.64^{ac}	63.67^{ac}	62.84^{ac}	5.03
----------(mmol/100 mmol of total VFA----------							
Acetate (C_2)	62.3^{a}	60.86^{ac}	60.76^{c}	60.31^{b}	61.12^{ac}	61.71^{cd}	1.17
Propionate (C_3)	23.03^{b}	24.54^{a}	24.26^{a}	24.28^{a}	24.20^{a}	23.82^{a}	0.97
IsoButyrate (IC_4)	1.27	1.27	1.29	1.34	1.28	1.28	0.04
Butyrate (C_4)	9.71^{bc}	9.86^{ac}	9.97^{ac}	10.24^{a}	9.79^{ac}	9.61^{bc}	0.34
IsoValerate (IC_5)	1.80	1.69	1.78	1.83	1.75	1.73	0.13
Valerate (C_5)	1.83^{ac}	1.80^{a}	1.87^{ac}	1.95^{ac}	1.82^{ac}	1.79^{bc}	0.13
Acetate: Propionate	2.71^{a}	2.49^{bc}	2.51^{bc}	2.49^{b}	2.54^{bc}	2.61^{ac}	0.15
(Ace+But): Propionate	3.13^{a}	2.89^{b}	2.93^{bc}	2.92^{bc}	2.94^{bc}	3.01^{ac}	0.16

^{a,b,c,d} Means in same row with different superscripts differ significantly for treatment effect.

Table 4.3 Rumen ammonia and pH when grass hay was used as substrate with different sources and doses (ppm) of Cobalt.

Measurements	CoCO3	CoL				SEM
	3.5	0.11	0.22	0.875	1.75	3.5	
Ammonia*	30.62	32.32	31.18	30.73	31.52	28.94	1.85
pH*	6.7	6.71	6.74	6.74	6.75	6.75	0.025

*not significant

Table 4.4 Dry matter digestibility (DMD) and neutral detergent fiber digestibility (NDFD) when a mixture of corn silage (CS) and alfalfa Baelage (AB) was used as substrate with different sources and doses (ppm) of Cobalt.

Measurements	CoCO3	CoL					SEM
	3.5	0.11	0.22	0.875	1.75	3.5	
DMD_1	30.44^{c}	35.69^{ac}	36.65^{a}	34.85^{a}	35.27^{a}	34.34^{bc}	1.22
$NDFD_2$	44.85^{bc}	50.57^{a}	47.51^{bc}	47.66^{bc}	52.07^{a}	48.25^{ac}	2.98

^{a,b,c} Means in same row with different superscripts differ significantly for treatment effect.

[1]DMD = Dry matter digestibility.

[2]NDFD = Neutral detergent fiber digestibility.

Table 4.5 Regression coefficients of gas production when a mixture of corn silage (CS) and alfalfa Baelage (AB) was used as substrate with different sources and doses (ppm) of Cobalt.

Measurements	CoCO3	CoL					SEM
	3.5	0.11	0.22	0.875	1.75	3.5	
B	5.25[ac]	5.98[a]	4.52[bc]	6.54[a]	5.62[ac]	4.28[b]	0.47
C	0.05	0.11	0.07	0.07	0.08	0.07	0.02

[a,b,c] Means in same row with different superscripts differ significantly for treatment effect.

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