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Comparing the effects of monensin and sodium butyrate on coccidia in post-weaned heifers

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COMPARING THE EFFECTS OF MONENSIN AND SODIUM BUTYRATE ON

COCCIDIA IN POST-WEANED HEIFERS

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ABSTRACT

The emergence of antibiotic resistances has raised concerns in society about the wide-spread use of antibiotics, such as monensin, as growth enhancers in agriculture. Pressure to find alternatives has increased since the European Union banned the use of ionophorous antibiotics. Butyrate supplementation has been found to enhance growth in pre-weaned calves and it has been recently suggested to enhance growth in post-weaned heifers. In a recent study by Rice in 2017, there was a quadratic (P=0.03) response for coccidia counts as sodium butyrate was increased, with the lowest counts being at the intermediate doses. This suggested that butyrate has the potential to decrease coccidian in post-weaned heifers. Monensin is also used as a coccidiostat. While the objective of the study as a whole was to determine if sodium butyrate can replace monensin in the prevention of coccidiosis and in enhancing growth and feed efficiency, this report focuses only on the prevention of coccidiosis. At this time, only six of the forty Holstein heifers entered the study. The design was a 2 X 2 factorial arrangement of treatments in a randomized complete block design. Heifers were randomly assigned to 1 of 4 treatments: (1) carrier (control; **C**); (2) 0.75 g SB/kg of body weight + carrier (**SB**); (3) 1.0 mg M/kg of body weight + carrier (**M**); (4) (0.75 g SB/kg of body weight and 1.0 mg M/kg of body weight + carrier (**SB/M**). Heifers entered the study and were trained to Calan doors from week 12 to 13 age of life. Treatment began on week 14 of life and continued for 12 weeks. Fecal samples were taken from each calf weekly beginning in the covariate period for determination of coccidia. (Measurements were also taken to evaluate the growth of post weaned calves and apparent total tract nutrient digestibility). Oocyte counts were determined through the modified Wisconsin sugar floatation method. Due to the small sample size a statistical analysis was unable to be completed. However, the preliminary data suggested a negative trend in coccidian oocyte counts

as treatment progressed. This indicates that both sodium butyrate and monensin have the ability to decrease the shedding of coccidian oocytes, though the statistical significance is not known at this time.

INTRODUCTION

IMPORTANCE OF DAIRY INDUSTRY

The dairy industry is extremely important to the economy of the globe. In 2011, there was estimated to be about 260 million dairy cows globally (IDF, 2013). In 2010, global milk production represented 8.9% of the value of all agricultural products (IDF, 2013). In some countries, milk production accounts for over 20% of their total agricultural value (IDF, 2013). The dairy industry employs up to 8% of the labor force in some countries (IDF, 2013). The dairy industry is expected to steadily grow due to the high demand for quality animal protein as the global population increases and economies emerge in developing countries (IDF, 2013). The dairy industry is especially important to developing countries, and it is responsible for the employment of approximately 750 million people on a small-scale dairy farms (IDF, 2013). In the United States in 2012, milk production totaled at $35.5 billion, or 9% of United States agricultural sales (USDA NASS, 2012). The USDA predicts that the export of dairy products will increase from $4.6 billion in 2016 to $9.5 billion in 2026 (USDA, 2017). Quality dairy products are in constant demand and this is unlikely to change. The dairy industry is a crucial part of the global economy and the economy of the United States. It is imperative for research to be conducted to make the industry more efficient and lucrative.

DEVELOPMENT OF CALVES

Calves are born with underdevelopment digestive system which develops as the calf ages. The food that the calf ingests in the beginning of its life has a huge impact on the development of the calf's digestive system. When a calf is born, it cannot digest feed in the same way that mature cows digest feed. The rumen, reticulum, and omasum are not developed or functional. There is an esophageal groove that allows the ingested liquid material to pass directly into the abomasum (Hegland et. al., 1957). The abomasum, the gastric stomach compartment, is where early digestion occurs in young calves, making young calves function like monogastric animals (Heinrichs, 2005). Because of this, the diet of a calf in the first 8 weeks of its life is mainly liquid, colostrum and milk or a milk replacer. Milk replacers are used instead of milk because they are less costly than milk and do not significantly affect the growth rate of the calves (Jaster et. al., 1989). However, a purely liquid diet does not promote optimal ruminal development.

In order to transition from a monogastric animal to a ruminant, the rumen must be stimulated to develop. Certain feed requirements must be met to optimize ruminal development (Heinrichs, 2005). The epithelial layer of the rumen is responsible for the absorption if the nutrients digested in the rumen. Papillae exist in the rumen to increase the surface area of the epithelial layer to increase absorption. It has been found that early introduction of solid feed can stimulate the rumen to develop more quickly (Heinrichs, 2005). The ingestion of dry feed and the introduction of the microbial community result in the production of volatile fatty acids, which have been found to stimulate the development of the rumen. Concentrates and diets with casein, starch, cellulose, and minerals induce the development of the rumen better than a purely forage diet (Heinrichs, 2005). Butyrate and propionate are two volatile fatty acids that have been shown to enhance the development of the rumen (Heinrichs, 2005). These molecules are produced by

microbes in the rumen as a by-product of carbohydrate digestion. The types of microbes in the rumen determine the amount of volatile fatty acids produced. Gram-negative bacteria increase the amount of propionate while gram-positive bacteria increase the amounts of acetate and butyrate.

Calves that have a more developed rumen at a younger age are more beneficial to farmers. They grow more quickly and more efficiently and can mature at a younger age. This is important to farmers because heifers consume resources without producing milk, meaning that farmers must spend money on heifers without making money from them in milk sales. Consequently, the sooner a heifer matures, the sooner she can be bred and become a source of income to the farmer, and this decreases the cost of feeding heifers (Heinrichs et. al., 2013). Optimizing the development, growth, and feed efficiency of calves and heifers can help farmers become more lucrative.

COCCIODIOSIS

Coccidiosis is an economically significant disease caused by parasitic protozoans from the genus *Eimeria* that infect the intestinal tract of many species, including sheep, chickens, turkeys, and cattle. There are many species of *Eimeria*, and each species of *Eimeria* infects a specific host species. The species that cause coccidiosis in cattle are primarily *Eimeria bovis* and *E. zurnii* (Quigley et al., 1997). The parasites reproduce in the intestines and cause damage to the intestinal tissue. The eggs produced are called oocytes. They are released in the feces and can infect other cattle that ingest them.

Oocytes are essentially ubiquitous in the environment, so it is hard to prevent cattle exposure to the parasite without a prevention plan. Young animals are most susceptible to

coccidiosis because of their weak immune system, but adults in poor condition with weakened immune systems can also develop clinical symptoms. Clinical symptoms include diarrhea, bloody diarrhea, dehydration, weakness, and lack of appetite. Most cattle are infected subclinically when they are calves and develop a strong immunity towards the parasite by adulthood. Subclinical coccidiosis is also detrimental to the calf and the farmer. The parasites destroy the lining of the intestine, causing inefficiency of nutrient absorption and decreased appetite. This is detrimental to the growth and development of the calves and can lead to future inefficiency in the mature animal. The economic loss due to coccidiosis in the United States has been estimated to be $100 million (Taylor, 2016). In order to optimize the growth and feed efficiency of calves and heifers, coccidiosis must be prevented.

MONENSIN: COCCIDIOSTAT AND GROWTH ENHANCER

Monensin is an ionophorous antibiotic which damages bacteria, and to a lesser extent coccidian, by damaging the cell membrane so that the cell membrane can no longer effectively maintain its proper ion concentrations (Hilton et. al., 1990). Ionophores have been found to have coccidiostatic effects on dairy cattle, specifically in younger animals. In one experiment, calves fed the ionophore lasalocid shed less oocyts than the control group (Quiglet, et. al., 1997). Monensin has been shown to have coccidiostatic affects in cattle as well (Stickdale, 1981). Monensin prevented clinical signs of coccidious in 10-week-old Holstein-Friesian calves inoculated with oocytes (Fitzgerald and Mansfield, 1973). However, this antibiotic must be fed daily due to the resilience of the protozoans. The only time monensin was found to affect the parasites was after they reached the stage of sexual reproduction, because it is after this point that they burst through the host cell and are able to come into contact with the drug (Melhorn et. al.,

1983). Supplementing ionophores such as monensin at an early age has been shown to reduce coccidian oocyst shedding.

Ionophores have the ability to change the microbial population in the rumen. As previously stated, ionophores disrupt the ion gradient in the cell membrane of bacteria and prevent the bacteria from maintaining adequate rates of cellular growth to sustain survival (Russell and Strobel, 1989). However, ionophores do not affect all bacteria equally. Gram-positive bacteria have a cell membrane that makes them more susceptible to damage from ionophores (Callaway et al., 2003). With ionophorous treatment, the population of Gram-positive bacteria is decreased, which provides more nutrients for Gram-negative bacteria to proliferate. As previously mentioned, decreasing the population of Gram-positive bacteria will lower concentrations of acetate and butyrate, and increasing the population of Gram-negative bacteria will increase the concentration of propionate. These conditions increase feed efficiency in heifers. Because of this, ionophores such as monensin have also been found to be growth enhancers. Much research has been done to support monensin's ability to increase growth and feed efficiency. In one study, cattle fed monensin as a supplement to their diet gained weight 1.6% faster, ate 6.47% less, and required 7.5% less feed than the cows fed a normal diet (Goodrich et. al., 1984). In another study, Heifers fed monensin at 200 and 600 mg/d levels gained 0.09 kg/d more than animals on the control diet and had lower dry matter intakes, leading to 12.6 and 13.4% greater efficiency in converting feed to body weight gain than control heifers (Baile et al., 1982). The supplementation of ionophores in dairy cattle diets increases feed efficiency. Monensin decreases feed intake while allowing cattle to maintain similar body weight gains, making them more efficient.

RISE OF ANTIBIOTIC RESISTANCES

Because of their effect as a growth enhancer, antibiotics including monensin are fed to livestock on a large scale. It has been estimated that about 3.7million pounds of antibiotics are used in cattle farms as grow enhancers annually in the United States (Mellon et. al., 2001). The extensive use of antibiotics can lead to populations of resistance pathogens, and coccidian is no exception. One study on broiler chickens showed that of the 10 *Eimeria* field isolates, 9 were resistant to antibiotcs, and 6 of those were resistant to monensin specifically (Stephan et. al., 1997). Another study showed that *Eimeria meleagrimitis,* parasite of turkeys, has the ability to develop resistance to monensin as well (Jeffers and Bentley, 1980). However, bacterial resistance to ionophores is not well understood. Even so, humans are not treated with ionophores, so it is unlikely that ionophore-resisitant bacteria would affect people (Russell and Houlihan, 2003). Nevertheless, the European Union has banned antibiotics including ionophores, so there is a need to find growth enhancers that are not antibiotics.

BUTYRATE

Butyrate is a volatile fatty acid that is produced by microbial fermentation. Butyrate has been found to have many functions in gut cells. Butyrate functions as a source of energy and as a cellular mediator (Beford and Gong, 2017). Butyrate has been found to be able to alter gene expression, induce cell differentiation, stimulate gut tissue development, induce immune modulation, and control diarrhea in multiple species (Bedford and Gong, 2017). Butyrate can act as a histone deactylase inhibitor. Butyrate blocks the actions of histone deactylase molecules, resulting in the activation of silent genes (Marks et. al., 2000). Butyrate has been found to have anti-inflammatory properties. In humans, one of the most studied mechanisms is the inhibition of nuclear factor kappa B. This controls the expression of genes encoding pro-inflammatory

cytokines, inflammation-inducing enzymes, growth factors, heat shock proteins, and immune receptors (Vinolo et al., 2011). In vitro, sodium butyrate induced expression of host defense peptides in chicken cell types including macrophages, monocytes, bone marrow cells, jejunal cells, and cecal cells (Sunkara et.al., 2011). Butyrate has been found to have antimicrobial properties in chickens, in pigs, and in humans. Butyrate has been shown to reduce and prevent the colonization of *Salmonella* in broiler chickens (Fernandez-Rubio et. al., 2009). The reported beneficial effects of butyrate supplementation (antimicrobial and anti-inflammatory activities, enhancement of growth performance and gut tissue development, modulation of immune response and intestinal microbiota) in humans, poultry, and pigs suggest that butyrate could be a potential substitute for in-feed antibiotics (Bedford and Gong, 2017).

Butyrate is also an importance source of energy in cows as well as cellular modulator. Supplementing dietary butyrate to newborn calves has been shown to cause the acceleration of gastrointestinal tract (GIT) development, including the rumen, abomasum, small intestine, and pancreas (Gorka et. al., 2011a). Butyrate can also modulate GIT secretion of peptides and hormones (Guilloteau et. al., 2009b). In calves, administration of butyrate directly into the rumen stimulated proliferation of epithelial cells and reduced epithelial cell apoptosis, resulting in longer papillae (Mentschel et. al., 2001). When butyrate is supplemented in dry feed, it affects the rumen. When butyrate is supplemented in liquid feed, it affects the abomasum and small intestine. Consequently, butyrate should be added to both dry and liquid feed in calves to stimulate development of the entire GIT (Gorka et. al., 2011a). Butyrate has been shown to improve growth, but the exact mechanism has not been determined in detail in ruminants (Gorka et. al., 2018). When butyrate was supplemented to calves in milk replacer, the calves has reduced incidences of scours (Gorka et. al., 2011a).

Most of the research on butyrate has focused on its effect on pre-weaned calves. In work completed at the University of New Hampshire by Rice in 2017, different concentrations of sodium butyrate were supplemented in the heifer's diet (0g/kg of body weight, 0.25g/kg body weight, 0.5g/kg of body weight, and 0.75g/kg of body weight). This was believed to be the first experiment to evaluate the supplementation of sodium butyrate to post-weaned heifers. Results of this research indicated that as sodium butyrate increased in the diet, dry matter intake was unchanged. However, overall weight gain and average daily gain increased with an increase in sodium butyrate. While not statistically significant, the observations suggested that as sodium butyrate increased in the diet, there was improvement in feed efficiency with no negative effects seen in the heifers. There was also a significant quadratic response in coccidian counts as the amount of sodium butyrate increased. The results indicated that at a dosage of 0.25g SB/kg of body weight was most effective in reducing the shedding of coccidian oocytes.

Because of the effects of sodium butyrate (SB) on weight gain, average daily gain, feed efficiency, final weight and coccidian counts, it appears that sodium butyrate is acting in the gut producing responses similar to those when monensin (M) is fed to cattle. With the effort to decrease the use of antibiotics across the globe, sodium butyrate is a potential replacement for ionophores. The aim of this study is to see if SB is an effective replacement for monensin for aiding in prevention of coccidiosis and nutrient absorption through altering the rumen environment. While the focus of the study as a whole (completed by Tess Stahl) is to evaluate the growth of post-weaned calves and apparent total tract nutrient digestibility of diets containing no treatment, 0.75 g SB/kg of body weight, 1 mg of M/kg of body weight, or the combination (0.75g SB/kg of bodyweight and 1mg M/kg of body weight), this report is focused specifically on evaluating the coccidian counts in the post weaned calves feed diets containing 0, 0.75 g/kg

SB, 1 mg/kg monensin or the combination. It is hypothesized that sodium butyrate will be able to replace monensin and the two treatments will yield similar coccidian oocyte counts.

METHODS

Forty Holstein heifers will be included in this study. At this point in the study, only six of the forty calves entered the study. The design was a 2 X 2 factorial arrangement of treatments in a randomized complete block design. Heifers were randomly assigned to 1 of 4 treatments: (1) carrier (control; **C**); (2) 0.75 g SB/kg of body weight + carrier (**SB**); (3) 1.0 mg M/kg of body weight + carrier (**M**); (4) (0.75 g SB/kg of body weight and 1.0 mg M/kg of body weight + carrier (**SB/M**). The carrier used was soybean meal. Treatments were adjusted weekly according to body weight. Heifers entered the study and were trained to Calan doors from week 12 to 13 age of life. Each heifer was assigned a specific door and assisted with opening the door during this week until they were able to enter and exit on their own. Treatment began during week 14 of life and the heifers remained on the study for 12 weeks.

Pre-trial measurements (body weight, heart girth, paunch girth, body length, DMI, blood concentrations of β-hydroxybutyrate (BHB), glucose, fecal oocyte count) served as covariates. Daily dry matter intake was measured and calves were fed a haylage based diet at the same time daily. Total mixed ration samples were taken weekly and composited monthly for nutrient analyses. Orts samples were taken daily and composited by month for each calf. Feed ingredient samples were taken monthly for nutrient analyses. Blood samples for BHB and glucose analyses, body weight and skeletal measurements (heart girth, paunch girth, body length), and fecal samples for oocyte determination were taken once every Tuesday at 8:00am. Body length, heart girth, and paunch girth were measured with weight tape. Body weight was determined with a platform scale. Blood samples were taken from the jugular vein using a 20-

gauge needle before the administration of treatments. Samples were collected in 2 10-mL vacutainer tubes, 1 containing anticoagulant EDTA and 1 without anticoagulant. Concentrations of BHB were obtained utilizing a hand-held electronic blood glucose and ketone monitoring system. Apparent total tract digestibility was determined twice during the experiment, once during the third week and once during the ninth week. Chromium oxide (4 g/d) was dosed twice daily (12 h intervals) for 7 d, and fecal samples taken over the last three days of the period to mimic a 24 h period.

The coccidian oocyte counts were determined using the Modified Wisconsin sugar float test. Sheather's solution was made by dissolving 90.8 grams of sugar into 71.0 milliliters of hot water. The solution was covered and cooled to 38°F before use. To complete the sugar float test, 3 grams of fecal matter was mixed thoroughly in 10mL of Sheather's solution. The mixture was strained into a 15mL test tube and centrifuged at 1200rpm for 5minutes at 4°C. After centrifugation, the test tubes were slightly over-filled with Sheather's solution, until a meniscus formed at the top of the tube. A coverslip was then placed on the meniscus. After 10 minutes, the coverslip was placed a slide and viewed under light microscopy. Oocytes were counted at 10x over the entire area of the coverslip.

At this stage of the study, significant statistical analysis was not possible. The sample size was too small due to time restrictions to accurately run a statistical analysis. However, the preliminary data was analyzed. The oocytes counts were converted to oocytes per kilogram of feces. The oocytes per individual heifer were plotted in scatterplots to observe trends. The data from each heifer was plotted in two charts. The first was a simple scatterplot with connected points to visualize the progression of the oocyte counts per week. The second was a scatterplot

with a linear regression to determine if there was a relationship between the number of oocytes and the progression of treatment throughout the weeks of the experiment.

RESULTS

The individual observations of oocyte counts in eggs per kilogram of feces is summarized in Table 1. The observations vary among individual heifers, as seen by the standard deviations in Table 2. The total and average oocytes counts for each treatment is summarized in Table 2. In the preliminary data, the combination of treatments SB/M yielded the highest average oocyte count at 5309.091 eggs/kg. Monensin yielded the second highest oocyte count at 2925.556 eggs/kg. Sodium butyrate yielded 1512.308 eggs/kg. The lowest oocyte count occurred in the control treatment, with 1332.857 eggs/kg.

Table 1: Individual oocyte counts for each heifer

Week	962 SB	963 M	964 C	965 SB/M	966 SB/M	967 SB
0	0	0	2000	3000	14330	15330
1	0	0	2000	5530	0	2330
2	0	7000	1670	31330	2330	1000
3	0	19330	2000	500	0	
4	1000	0	1000	1250	330	
5	0	0	330	0		
6	0	0	330			
7	0	0				
8	0	0				
9	0					

Table 2: Total observations, oocytes counts, average oocyte counts and standard deviations per treatment

	SB	M	SB/M	Control
Total observations	13	9	11	7
Total oocyte count (eggs/kg)	19660	26330	58400	9330
Average oocyte count (eggs/kg)	1512.308	2925.556	5309.091	1332.857
Standard deviation (eggs/kg)	4210.165	6572.852	9600.136	771.4859

The trend in oocyte count over weeks of treatment of sodium butyrate for 962 is summarized in Figures 1 and 2. The oocyte count in this heifer remained low and stable. A negative relationship was observed. The equation for the linear regression is $y = -6.0606x + 127.27$ with an R-squared of 0.0536 (Figure 2). The trend for oocyte count during the course of treatment of monensin for 963 in summarized in Figures 3 and 4. The oocyte count in this heifer had a peak during week 3 of treatment, but dropped by week 4 and remained stable for the rest for the treatment (Figure 3). The negative relationship was observed. The equation for the linear regression is $y = -555.5x + 5147.6$ with an R- squared of 0.0536 (Figure 4). The trend for oocyte count during the control treatment for 964 is summarized in Figures 5 and 6. The oocyte count in this heifer remained stable, remaining between 1000 and 2000 eggs/kg, until dropping slightly in the last two weeks (Figure 5). The negative relationship was observed. The equation for the linear regression is $y = -322.14x + 2299.3$ with an R-squared of 0.8137 (Figure 6). The first heifer on combination treatment (965) saw a peak in oocyte count at week 2 (Figure 7). After week 2, the oocyte count dropped down. There was a negative trend with a linear regression equation of $y = -1676.3x + 11126$ with an R- squared of 0.067 (Figure 8). 966, the second heifer treated with combination began the study with a high oocyte count and it dropped over the course of the treatment (Figure 9). The linear regression was negative with an equation of $y = -$

2800x + 8998 and an R-squared of 0.5119 (Figure 10). 967, the second heifer treated with

sodium butyrate, had the highest counts in the covariate week and the counts dropped in the

following weeks (Figure 11). The linear regression was negative with an equation of y = -7165x
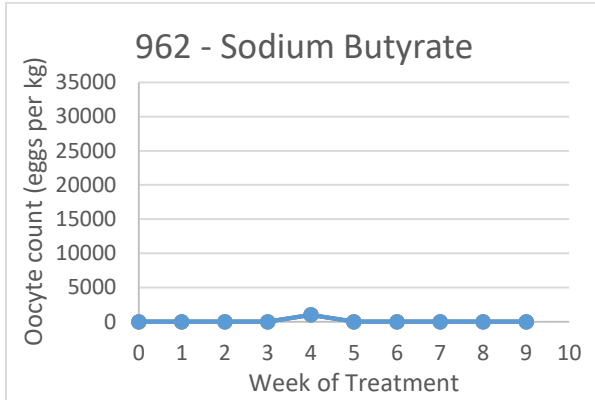
+ 13385 and an R-squared of 0.819 (Figure 12).

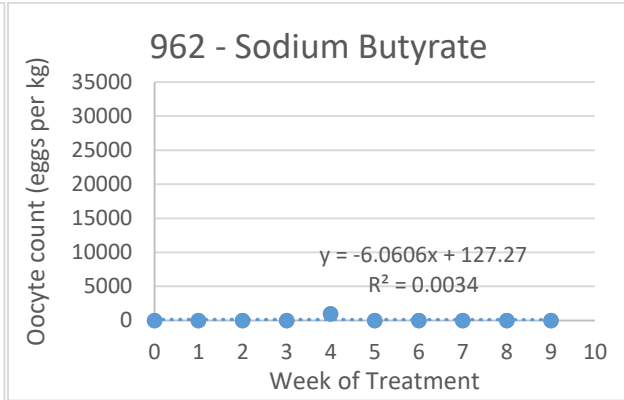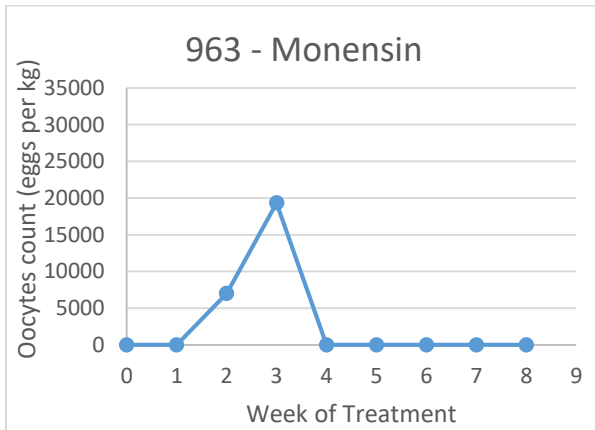| Figure 1: Line chart showing the progression of oocyte counts throughout the treatment of sodium butyrate in heifer 962	Figure 2: Linear regression of the number of oocytes in 962 as the treatment of sodium butyrate progressed

Figure 3: Line chart showing the progression of oocyte counts throughout the treatment of monensin in heifer 963	Figure 4: Linear regression of the number of oocytes in 963 as the treatment of monensin progressed
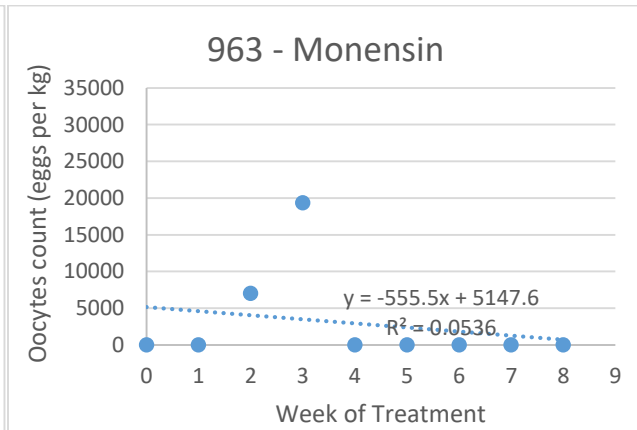
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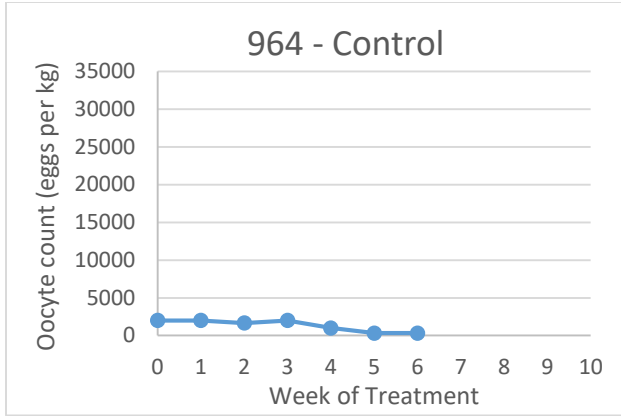
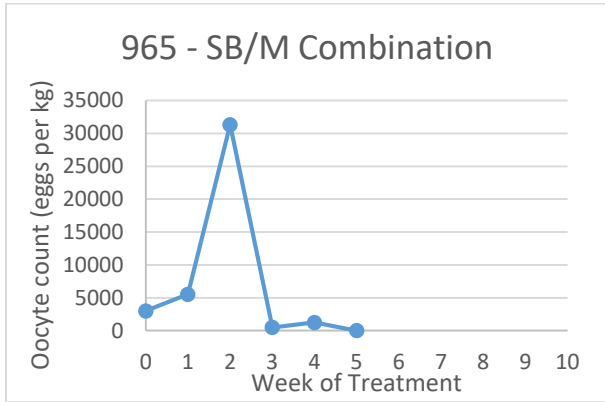| Figure 5: Line chart showing the progression of oocyte counts throughout the control in heifer 964	Figure 6: Linear regression of the number of oocytes in 964 as the control treatment progressed
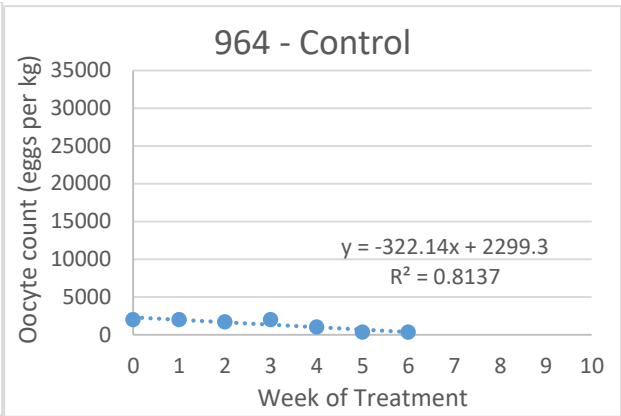
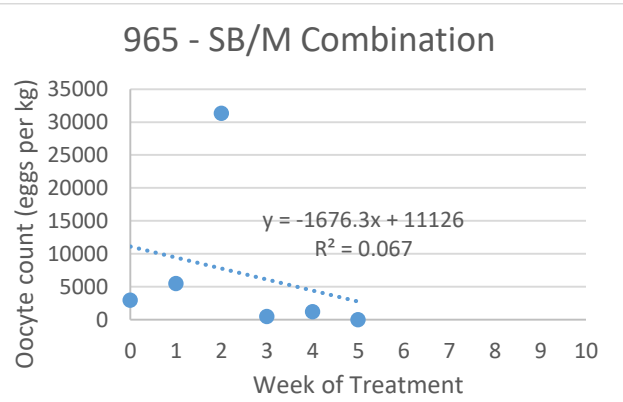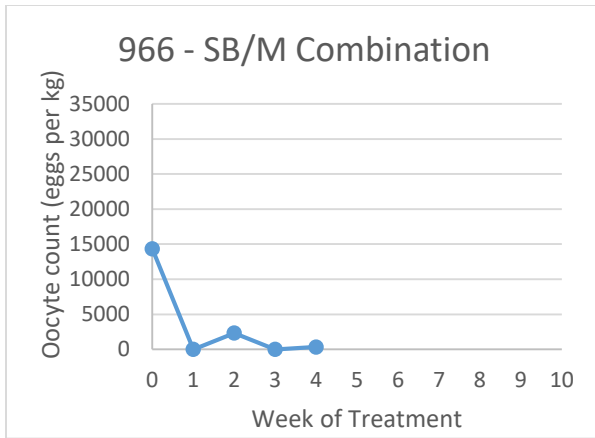| Figure 7: Line chart showing the progression of oocyte counts throughout the combination treatment in heifer 965	Figure 8: Linear regression of the number of oocytes in 965 as combination treatment progressed

15

Figure 9: Line chart showing the progression of oocyte counts throughout the combination treatment in heifer 966
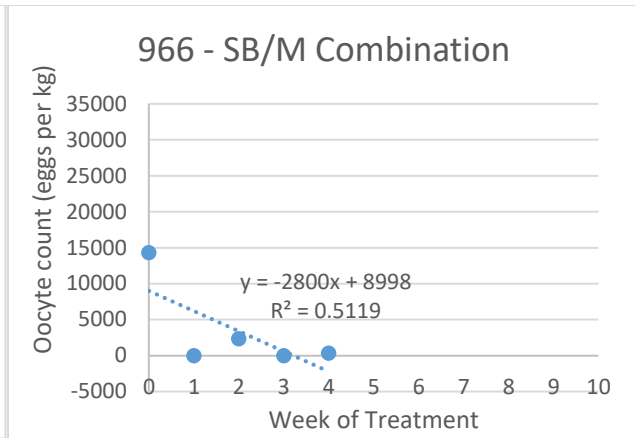

Figure 10: Linear regression of the number of oocytes in 966 as the combination treatment progressed
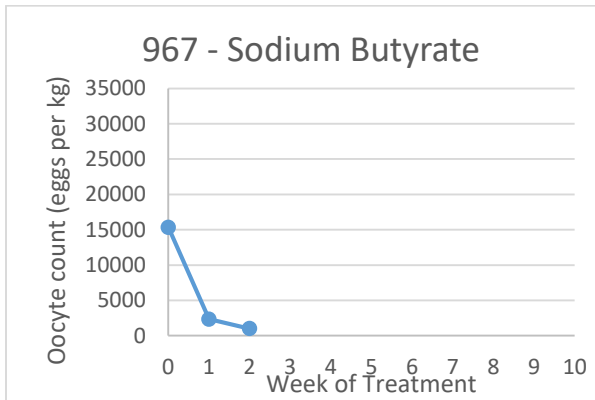

Figure 11: Line chart showing the progression of oocyte counts throughout the treatment of sodium butyrate in heifer 967
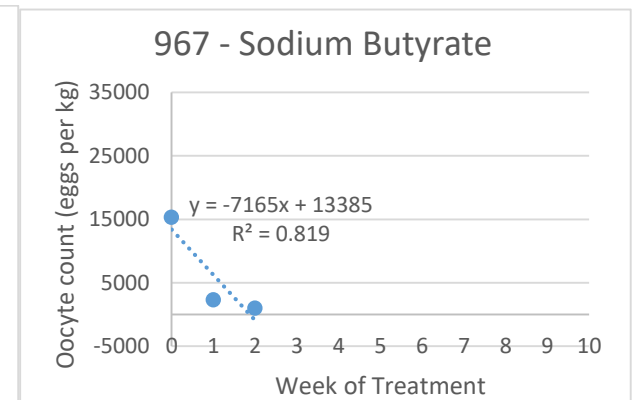

Figure 12: Linear regression of the number of oocytes in 967 as the treatment of sodium butyrate progressed

DISCUSSION

The preliminary results suggest that both monensin and sodium butyrate are associated with a decrease in coccidian counts. The slopes of all the treatments were negative, indicating that as the heifers were being treated, the number of coccidian decreased. While the control group also had a negative slope, the control group had the slope of the least magnitude, with the exception of 962. 962 had extremely low coccidian counts, so it is very possible that in the final analysis this heifer will be considered an outlier. 963 experienced a spike in oocytes at 3 weeks of treatment with monensin and 965 experienced a peak at two weeks into treatment with the combination of SB/M. This could be due to the life cycle of the *Eimeria* species. The life cycle is 21 days, so when an animal is infected, the oocytes will not show in the feces until about 3 weeks later. This means that when 963 and 965 experienced peak coccidian counts at 2 and 3 weeks, they were showing the symptoms of an infection that likely began before the treatment began. As previously mentioned, monensin affects only the sexually mature protozoan. Depending on the stage of the life cycle of the parasites, monensin may not have been effective immediately.

Though preliminary, these data suggest that sodium butyrate could possibly replace monensin in the prevention of coccidiosis. The mechanism through which sodium butyrate could prevent coccidiosis in heifers is not definitively known. That being mentioned, it is possible that butyrate causes enough proliferation of the epithelial cells that the protozoan are not able to establish an infection in the epithelial cells before new epithelial cells take their place. It is also possible that butyrate induces the production of host defense peptides to aid the cells in the defense against an infection, as has been seen in other species like chickens.

17

While the preliminary data suggest that sodium butyrate has potential to be a replacement for monensin in the prevention of coccidiosis, there are details that could have affected the results. In this study, the concentration of sodium butyrate that was used was based off of the concentration in a previous study that yielded the best growth and feed efficiency, which was 0.75g SB/kg. It was not based off of the best concentration for the prevention of coccidiosis, which was 0.25g SB/kg. It is likely that the results would be different if a different concentration of sodium butyrate was used. This needs future research to be determined.

The source of butyrate, sodium butyrate, could have affected the results as well. Sodium butyrate has a high availability and a modest price, but there are other sources of dietary butyrate. Sodium butyrate dissolves easily in water (Mallo et al., 2012). As a result, dietary sodium butyrate rapidly dissociates in the stomach and it probably mostly absorbed there, instead of the small intestine or colon (Guilloteau et al., 2009b). When sodium butyrate is used, it is unsure how much, if any, of the butyrate is being delivered to the small intestine. Calcium butyrate is much less soluble in water solutions compared with sodium butyrate and could potentially deliver more butyrate to the small intestine (Mallo et al., 2012). Another way butyrate could be protected to change the site of absorption is in embedded in a lipid matrix of a vegetable saturated fat such as palm oil; however, the exact composition of the lipid matrix is rarely reported (Moquet et al., 2016). When protected in the lipid matrix, butyrate is only partially released in the stomach and most of active substance is released in the small intestine (Piva et al., 2007; Guilloteau et al., 2009a). This could very easily change the results because more butyrate would be acting directly on the epithelium of the small intestine, which is the site of coccidian infection.

There are many factors that can affect the incidence of coccidian infection in heifers, and ultimately skew the results. Individual differences in immunity strength could have affected the coccidian counts. Any type of stress can result in an infection because stress depresses immunity. There are many things that could have caused stress in the heifers. The process of moving from smaller hutches to the bigger pens could have caused stress. Stress from the enforcement of social hierarchy could have depressed the immunities of younger and smaller heifers. Fluctuating weather and over-crowding could have caused stress. Other illnesses such as ringworm and scours (not caused from coccidia) were observed in the heifers and they could have made heifers more susceptible to an infection. All of these factors could have had an effect on the results.

There is much potential for future research regarding butyrate. The mechanism of how butyrate effects the tissue on a molecular level in the heifers is not understood and needs to be studied. It is also unclear exactly where butyrate directly affects the tissue depending on the source and protection of butyrate. Different sources of butyrate could potentially have different affects in cattle of all ages. It also unknown exactly how butyrate affects the microbiota in the entirety of the GIT. The most effective dose of butyrate, source of butyrate, administration of butyrate, and time to be administered in the cow's life are all still to be determined. The long-term effects of butyrate supplementation from newborn to adulthood should also be studied. Despite this, current research has shown that butyrate has great potential to be a replacement for ionophores.

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