PROBIOTIC SUPPLEMENTATION IMPROVES LAMB PERFORMANCE WHEN TRANSITIONING TO HIGH CONCENTRATE DIETS

A Thesis

by

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ABSTRACT

Lamb ruminal function is integral to their post-weaning health and performance. Lambs are often fed a high concentrate diet to enhance growth, and while this strategy is common, issues can arise with ruminal acidosis from excess grain consumption, including reduced weight gain and increased mortality. We hypothesized that administering probiotics orally prior to the transition to concentrate feeds would improve lamb performance in the post-weaning period. To assess this, finewool lambs (n = 103; males: n = 47, females: n = 56) were weaned from their dams at approximately 100 days of age at an average body weight (BW) of 25.8 ±0.2 kg and placed in a feedlot. Lambs were assigned randomly to treatment of 0g (CON), 5g (TRT-5), or 10g (TRT-10) of an orally administered probiotic paste (ProBios, Menomonie, WI) containing live lactate producing bacteria. Lambs were limit fed for 9 days and gradually transitioned to ad libitum access to a high concentrate ration. For six weeks, body weight (BW), average daily gain (ADG) and fecal egg counts (FEC) were collected on a weekly basis. Lambs receiving TRT-10 were significantly heavier ($P = 0.01$) than TRT-5 and CON lambs (30.3±0.2 kg versus 29.9±0.2 kg and 29.6±0.2 kg, respectively). There was no significant effect of probiotic treatment on ADG ($P = 0.17$). Fecal egg counts were lower ($P = 0.05$) in lambs receiving TRT-10 compared to those receiving CON and TRT-5. Four incidences of mortality were recorded, one death was recorded during week two, two during week four, and one during week five. There was no significant treatment ($P = 0.5$) or sex ($P = 0.8$) effects on lamb mortality. These data suggest that probiotic supplementation may be an effective strategy to decrease detrimental effects of diet transition on feeder lambs and may increase lamb performance while transitioning to a high concentrate diet.

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NOMENCLATURE

ADG	Average Daily Gain
BW	Body Weight
CF	Crude Fiber
CFU	Colony-forming Unit
CP	Crude Protein
D	Day
DFM	Direct-fed Microbial
DM	Dry Matter
DMI	Dry Matter Intake
EPG	Eggs Per Gram
FCR	Feed Conversion Ratio
FEC	Fecal Egg Count
GIT	Gastrointestinal Tract
H	Hour
HD	Head
LAB	Lactic Acid Producing Bacteria
LUB	Lactic Acid Utilizing Bacteria
TDN	Total Digestible Nutrients
TMR	Total Mixed Ration
VFA	Volatile Fatty Acid

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Probiotics, also known as direct-fed microbials (DFM), are used to enhance gut health and performance. While beneficial to ruminant livestock, they are also included in human and monogastric animal diets. Fuller (1989) defined probiotics as ‘a live microbial feed supplement that benefits the host animal by improving its intestinal microbial balance’. Probiotics can be supplemented in many forms, including pelleted feed, capsules, paste, powder, or granules. They can also be included in the diet as part of a ration or administered directly to each animal. Probiotics are included in various livestock diets; however, they are most common in the transitioning and weaning diets of ruminant animals because probiotics stimulate immunity and improve nutrient absorption (Teeler and Vanabelle, 1991), as well as enhance feed intake average daily gain (ADG), feed conversion ration (FCR), and increase protein availability (Antunovic et al, 2006). The digestive system harbors a self-establishing gut flora that typically keep the rumen very stable. However, these microorganisms can be influenced by dietary and environmental factors. The most common reason for gut flora to be disrupted in livestock is due to abrupt changes in the diet. Additional reasons include stress from weaning, transportation, or processing, and antibiotic therapy (Fuller, 1989).

In livestock diets, probiotics have been marketed and used as growth promoters. Marketing of probiotics have been used to reduce antibiotics and synthetic feed supplements added to transitioning diets. Although the DFM should be consistent with the label, the ‘growth stimulatory effect in itself is bound to be variable,’ as it will only work when the animal is stressed by the presence of growth depressing microflora (Fuller, 1989). Regardless of the stimulatory effect required for DFM to entirely benefit the host, probiotics are of potential value in livestock diets, especially when there is an increased risk for emotional or physical stressors to the animal (Simon et al., 2001).

1.2 Literature Review

Direct-fed Microbials

As concerns surrounding antibiotics and growth stimulating hormone usage grow in the livestock industry, interest in the effects of direct-fed microbials (DFM) on growth performance and animal health is also growing. Direct-fed microbials have been used in efforts to replace or reduce the use of antibiotics in ruminant animals, as well as improve feed efficiency and daily gain. Furthermore, probiotics may restore and maintain desirable microorganisms in times of stress or disease risk of immature animals' growth. Microorganisms do this by stimulating immunity, and improving food digestion and absorption (Antunovic et al., 2006). Moreover, microorganisms improve ruminal functions that directly affect the host animal with different modes of action when DFM is supplemented in the diet.

Effect of DFM on the Rumen Environment

In ruminant animals, the rumen is the first part of the digestive tract affected by DFM upon ingestion. The rumen benefits from DFM by its ability to grow microbes, modifying the microbial ecosystem, and fermentation characteristics (Seo et al., 2010). This alteration to the ecosystem primes the rumen for an increase in lactic acid production when ruminant animals are transitioned to a highly fermentable diet. The modes of action of DFM in the rumen also play a role in animal performance and health. Seo et al. (2010) describes three modes of action of DFM in the rumen, two types of bacterial DFM and one type of fungal DFM, as well as a mode of action in the post-rumen gastrointestinal tract (GIT). Lactic acid producing bacteria (LAB) provide a constant lactic acid supply to the rumen, adapt microflora to the lactic acid accumulation, stimulate lactate utilizing bacteria, and stabilize ruminal pH. Lactic acid producing bacteria such as *lactobacilli* and *enterococci* are used primarily to prevent or reduce the risk of ruminal acidosis, especially in feedlot rations (Nocek et al., 2002). Lactic acid utilizing bacteria (LUB) convert lactate to volatile fatty acids (VFA), produce propionic acid rather than lactic acid, increase feed efficiency, decrease methane production, and increase ruminal pH (Seo et al., 2010). Lactic acid utilizing bacteria such as *M. elsdenii* may utilize lactate and prevent drastic drops in pH (Kung and Hession, 1995), improving energetic efficiency and reducing ketosis (Weiss et al., 2008). Fungal DFM reduce oxygen and prevent excess of lactic acid in the rumen. Additionally, fungal DFM provide growth factors, increase rumen microbial activity and numbers, improve ruminal end products, and increase ruminal digestibility (Seo et al., 2010). Fungal DFM such as *S. cerevisiae* are most used in ruminants to improve performance and normalize rumen fermentation by increasing ruminal populations via providing growth factors such as organic acids and vitamins (Chaucheyras et al., 1995). Direct-fed microbials are also utilized in the post-rumen GIT to produce antibacterial compounds, compete with pathogens for colonization of mucosa and other nutrients, as well as produce and stimulate enzymes. Direct-fed microbials also stimulate immune responses by the host and metabolize and detoxify desirable compounds (Dicks and Botes, 2010).

Effect of DFM on Growth

The rumen undergoes further development after the animal is born. During the suckling period, milk bypasses the rumen via the esophageal groove and deposits it directly into the abomasum. The esophageal groove prevents milk from being fermented by the microorganisms in the rumen. Introducing lambs to feed with DFM, while still suckling their dam, accelerates rumen and reticulum development and performance prior to weaning (Amran et al., 2021). Lambs on creep feed have a value-added advantage when entering the feedlot and being fed a grain-based diet. Providing a creep feed to lambs from birth stimulates VFA concentrations early, preparing the rumen for increased VFA and ruminal ammonia production following weaning (Poe et al., 1971). Supplementing probiotics with creep feed has been shown to have significant effects on lamb performance and growth. Saleem et al. (2017) supplemented lambs with dietary probiotics at various concentrations during a pre- (15-90 d) and post-weaning (91-174 d) period. While lamb performance was not affected in the pre-weaning period, there was a positive correlation in dry matter intake (DMI) and probiotic supplementation during the post-weaning period. Intake was not affected by the different probiotic concentrations. Furthermore, final body weight, average daily gain (ADG), total weight gain, and feed conversion ratio (FCR) all tended to be higher when lambs were fed diets containing probiotics (Saleem et al., 2017). In a comparative study by Vosooghi-poostindoz et al. (2013), lambs were fed various concentrations of protein (pre-weaning, 16 vs. 18% CP of DM; post-weaning, 14.5 vs. 16.5% CP of DM) and probiotics (0 vs. 2 g Protexin®/d) during a pre-weaning phase (10-70 d), while the lambs were still on the ewe, and post-weaning phase (78-138 d). Pre-weaning performance results showed there to be increased weaning weight and ADG when fed an 18% protein versus a 16% protein. However, the inclusion of probiotics did not affect weaning weight or ADG. The crude protein by probiotic supplementation interaction resulted in a statistical difference in feed intake, showing that lambs fed 18% protein and probiotics had a higher intake than those in other treatment groups (Vosooghi-poostindoz et al., 2013). Improved performance reached with protein supplementation may be because of increased digestible organic matter intake and enhanced efficiency of metabolizable energy utilization (Owens et al., 1991). Furthermore, increased crude protein and amino acids available in the small intestine result in improved protein and glucose absorption as the small intestine is more efficient than ruminal fermentation (Meissner et al., 1996). Blood cortisol concentrations were found to be lower when probiotics were supplemented, 24 h and 48 h after weaning (Vosooghi-poostindoz et al., 2013). All other treatment groups were equal. Post-weaning performance results showed similar final body weight, feed intake, FCR, and ADG when supplementing probiotics with various levels of protein (Vosooghi-poostindoz et al., 2013). The lower blood cortisol concentrations reported in lambs that were fed probiotics prior to and after weaning suggest that DFM aid in decreasing the undesirable consequences of stress, ultimately stemming positive performance.

As mentioned, DFM help reduce the negative effects on digestion and growth caused by stress. Weaning is considered the most stressful period in a feeder lamb's life. The digestive system, specifically the rumen, is not fully developed during this period (Saleem and Zanouny, 2016). Such stress often alters the microorganisms in the rumen, increasing morbidity and mortality, in addition to decreasing growth and feed efficiency (Krehbiel et al., 2003). Pond and Goode (1985) reported 24.7% increased daily gain in lambs on a high concentrate diet during the first two weeks and 6.4% higher during the third and fourth week when feeding Probios. Similarly, feed efficiency was improved by 17% and 0.30%, respectively. Saleem and Zanouny (2016) found feed intake to be greater with increasing levels of probiotics in a weaned lamb's high concentrate diet. As a result, intake of digestible crude protein and total digestible nutrients (TDN) was also greater. In addition, the digestibility of dry matter (DM), organic matter, crude protein (CP), crude fiber (CF), and nitrogen-free extract were improved by lambs that received higher concentrations (1.0 g/hd/d) of probiotics when compared to other treatments (0.5 g/hd/d and 0 g/hd/d). Improved digestibility with probiotic supplementation may be due to increased ruminal microbial populations and improved ruminal pH (Saleem and Zanouny, 2016).

In cattle, newly received beef cattle entering a feedlot undergo a multitude of stress factors, such as recent weaning, transportation, vaccination, and a new environment. Krehbiel et al. (2003) studied research trials that administered DFM to reduce changes in the rumen microbial populations when beef calves were most stressed, usually when entering the feedlot. Feeding DFM at processing, through the receiving period (average = 30 d), or both, resulted in an increase in feed consumption (2.5%), ADG (13.2%), and feed to gain ratio (6.3%). However, the greatest response to DFM was in the first 14 d of the feeding period. Likewise, Ponce et al. (2015) found probiotic supplementation to increase finishing beef steer performance throughout the feeding period, with the greatest significance during the first 35 d of the feeding period. Even though other studies found that there was no significance in administering DFM when receiving feedlot calves (Kiesling and Lofgreen, 1981; Krehbiel et al., 2001), calves supplemented with DFM were less likely to be treated a second time with antimicrobials, suggesting that DFM might improve recovery of morbidity. Krehbiel et al. (2003) also found that daily supplementation of microbials in the diet throughout the feeding period improved daily gain and feed efficiency in feedlot cattle; but did not affect the DMI, yield grade, or quality grade of the carcass. In addition to reducing antimicrobial treatment, DFM reduced the occurrences of ruminal acidosis during the transitioning period in the feedlot.

Effect of DFM on Ruminal Acidosis

One of the greatest concerns when introducing ruminant animals to a high concentrate diet is ruminal acidosis. Ruminal acidosis is characterized by low ruminal pH and high ruminal concentrations of VFA (Krehbiel et al., 2003). A sudden increase in intake of a highly fermentable diet causes ruminal acidosis. This most commonly occurs when rapidly transitioning ruminants from a low- to high-concentrate or highly fermentable diet without an appropriate conditioning period (Meissner et al., 2010). Ruminal acidosis has both short- and long-term effects. Short term effects include discomfort, inflammation, and in some cases poor growth performance (Ametaj et al., 2009). Long term effects consist of liver abscesses, continued inflammation and lameness, and alterations to the immune system (Plaizier et al., 2008). Meissner et al. (2010) found that weight gains in feedlot steers fed DFM were more rapid (1.4-1.9%) and required less (1.9-3.9%) feed/unit of gain than cattle not fed DFM. Similarly, in sheep, DFM improved weight gains in wethers and post-weaned lambs. Furthermore, the health of the lambs that received DFM was improved (Meissner et al., 2010). In two separate trials, mortality was 2% and 8.9% in the control group compared to zero and 2.5% for the DFM treatment group. Morbidity was 0.7% for the control group in trial one and zero for all other parameters (Meissner et al., 2010). Supplementing DFM when introducing ruminants to grains might help prevent ruminal acidosis by allowing the ruminal microorganisms to maintain a normal ruminal pH and concentration of VFA in the rumen.

Gastrointestinal Nematodes in Feeder Lambs

The most common type of gastrointestinal nematode recognized in the sheep industry is *Haemonchus contortus,* also known as Barber's pole worm. *H. contortus* is a highly prolific internal parasite. *H. contortus* is capable of producing over 5,000 (Selemon, 2018) to 10,000 (Roberts and Swan, 1981) eggs per day. These blood sucking parasites are found in the abomasum and are detrimental to overall health, productivity, and can be fatal (Roberts and Swan, 1981). *H. contortus* is prevalent in most regions of the United States, especially in the southern regions (Kaplan, 2005) with suitable climates of elevated temperatures, rainfall, and humidity (Maphosa et al., 2010). Diagnosis of *H. contortus* infection include clinical signs of anemia, submandibular edema, weight loss, illness, and finding large numbers of eggs in the feces (Selemon, 2018). Resistance to internal parasites varies by genetic selection and breed of sheep (Gonzalez-Garduno et al., 2013); however, young animals are typically more susceptible to *H. contortus* compared to adults (Selemon, 2018). Hair breeds of sheep tend to be more resistant to parasitic nematodes compared to wool breeds (Gonzalez-Garduno et al., 2013). Notter et al. (2003) reported that 4–8-week-old wool lambs had an average fecal egg count (FEC) of 4011 eggs per gram (EPG) while hair sheep retained under the same conditions had an average FEC of 1135 EPG. Furthermore, susceptibility increases during times of stress, poor nutrition, and decreased immunity (Selemon, 2018).

The life cycle of *H. contortus* begins with the adult female laying eggs that are passed out in the feces of the host animal. Excreted eggs hatch when temperature and humidity are suitable. Immature larva feed on the bacteria in the feces during the developing stages. When developed, larva remain in their cuticle and crawl up grass blades to await ingestion by a host animal. After ingestion, the larva settles in the abomasum and sheds the cuticle to burrow and develop to an adult and feed on the blood of the host (Hale, 2006). During unsuitable conditions, cold or dry, hypobiosis, or development cessation occurs (Gatongi et al., 1998).

Effect of DFM on Gastrointestinal Nematode Infection

Yang et al. (2020) found the bacterial class *Bacillales* were of particular interest as the main contributor as a probiotic to protect sheep from *H. contortus* within the abomasal microbiota. Data demonstrated a significant reduction ($P < 0.005$) in *Bacillales* amid *H. contortus* infection suggesting a potential protective role of probiotic bacteria. Furthermore, recombinant and wild type *Bacillus subtilis,* a member of the *Bacillales* family, spores were used to study the protective effect on sheep against *H. contortus*. Body weights of infected sheep receiving recombinant *B. subtilis* spores (10^{10} CFU/animal) were close to those of the uninfected controls. Infected sheep receiving wild type *B. subtilis* spores had 27.7% more body weight than the infected sheep without treatment. Additionally, parasite load was determined by EPG. Recombinant *B. subtilis* reduced EPG levels by 71.5% compared to sheep infected with *H. contortus* alone (Yang et al., 2020). In a similar study by Pinto et al. (2020), sheep infected with *H. contortus* were supplemented *Saccharomyces cerevisiae* (400 million CFU/d of suspension for 49 d) to evaluate effectiveness of DFM. Data demonstrated that sheep supplemented with *S. cerevisiae* exhibited a decreased ($P < 0.05$) number of larvae and increased ($P < 0.05$) serum immunoglobulin levels when compared to the control group that was infected but not supplemented (Pinot et al., 2020).

Conclusions

An increase in the consumer's desire to know where their food comes from and a shifting preference to all natural and antibiotic free products requires the food animal industry to make appropriate transitions based on consumer demand. The use of DFM may help the ruminant livestock industry minimize the use of antibiotics and anthelmintics while maintaining proper animal health and performance, particularly during stressful event such as transitioning range lambs to a feedlot diet.

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CHAPTER II

PROBIOTIC SUPPLEMENTATION IMPROVES LAMB PERFORMANCE WHEN TRANSITIONING TO HIGH CONCENTRATE DIETS

2.1 Materials and Methods

2.1.1 Animals and Management

Newly weaned finewool lambs (n=103) (initial BW = 25.8 ± 0.2 kg; age = 100 ± 10 d) from a single flock were brought into the Texas A&M AgriLife Research feedlot in San Angelo, TX. Lambs were individually weighed and equipped with an electronic identification tag.

Lambs were classified by sex (females, n=56; males, n=47), and each lamb was assigned randomly to one of three treatment groups. Treated lambs were assigned to receive Probios Max, an oral probiotic paste containing four live, lactate producing bacteria, *Enterococcus faecium*, *Lactobacillus acidophilus, Lactobacillus casei*, and *Lactobacillus plantarum*. The control group (CON) received 0 g of Probios Max (n=35; females, n=19; males, n=16), the second treatment group received 5 g of Probios Max (TRT-5) (n=35; females, n=19; males, n=16), and the third treatment group received 10 g of Probios Max (TRT-10) (n=33; females, n=18; males, n=15). Lambs were housed in a feed yard (30.48 x 30.48 m) with two bulk feeders and free choice access to an automatic watering system. The pen was partially covered for access to shade.

Lambs were group-fed a 50% concentrate diet containing cracked milo, cottonseed hulls, alfalfa pellets, cottonseed meal, and mineral premix (Denis Ranch #5; Denis Ranch, Vancourt, TX) as a total mixed ration (TMR). Nutrient composition of the ration is described in Table 1. Lambs were fed daily at increasing amounts (250 to 750 g/hd/d) for the first nine days and then provided an *ad libitum* access to TMR feed. During the first 9 d of the feeding period, the lambs were split randomly into two equal groups and housed in separate pens. After the period of limit feeding, the two groups of lambs were mixed and provided access to self-feeders. The lambs remained in this pen for the remainder of the study. Lambs were fed and monitored throughout the entire 42 d post-treatment period. Morbidity and mortality were monitored at feeding time, daily. Morbidity was visually determined by reduced alertness of the lamb. Morbid animals were monitored for elevated temperatures, and treatment was administered on a case-by-case basis.

2.1.2 Sample Collection and Measurements

Body weights (kg) of each individual lamb were obtained at weekly intervals for six subsequent weeks. Average daily gain (kg/d) was determined by subtracting the initial weight on day 0 from the final weight on day 42 and then dividing by the duration of the study (42). Average daily gain from weeks one to six were also determined. Data from four of the lambs (two CON, one TRT-5, and one TRT-10) were removed from the statistical analysis; all four died from an unknown cause.

Fecal samples from each individual lamb were collected directly from the rectum on days 0, 7, and 14. Fecal samples were placed in an insulated cooler with ice packs immediately after collection and analyzed the same day. A modified McMaster technique protocol was used to analyze each sample (Whitlock, 1948). Two grams of feces were pulverized and mixed with 28 mL of sodium nitrate solution and filtered through cheesecloth to remove solids. The solution was placed on a double-sided McMaster slide (Chalex LLC, Wallowa, OR, USA) and *H. contortus* eggs were counted under a microscope using the 10X objective (Thorne et al., 2022). Fecal egg count (FEC), reported in eggs per gram (FPG), were calculated as the total *H. contortus* eggs counted, multiplied by 50.

2.1.3 Statistical Analysis

Data for lamb body weight, ADG, and FEC were analyzed using the PROC GLIMMIX procedure (SAS 9.4, SAS Institute, Cary, NC, USA). Lamb body weight was analyzed using a normally distributed ANCOVA model with sex, week, and treatment as fixed effects, and day 0 weight as the covariate. Average daily gain was analyzed using a normally distributed ANOVA model with sex, week, treatment, and interactions therein as fixed effects. Fecal egg counts were not normally distributed and were log transformed to achieve normality with sex, week, and treatment as fixed effects and individual lamb as a random variable. Means of transformed FEC were back transformed for reporting. Error bars for FEC data are reported as the 90% confidence interval. Lamb mortality was analyzed using a binary logistic regression model accounting for the fixed effects of sex, week, treatment, and interactions therein. Data was reported as least squares means with greatest standard errors.

2.2 Results

Treatment effect on average body weight of the lambs are shown in Fig. 1. Lambs in TRT-10 were significantly heavier ($P = 0.01$) after six weeks when compared to lambs in CON or TRT-5 (30.3 ± 0.2 kg vs. 29.9 ± 0.2 kg vs. 29.6 ± 0.2 kg). Effects on overall lamb BW of treatment by week are shown in Fig. 2. There was a significant interaction of treatment and sex on lamb body weight ($P < 0.01$) (Fig 3). CON ewe lambs were heavier than CON wether lambs, TRT-5 wether lambs were heavier then TRT-5 ewe lambs, and TRT-10 lambs were not different. Wether and ewe lamb weights by week are shown in Fig. 4 and Fig. 5, respectively. Statistical differences in treatments in wether lambs are seen in weeks three through six (P < 0.01). Ewe lamb weights by treatment did not differ. In addition, there was a tendency ($P = 0.07$) of interaction of treatment, sex, and week on lamb body weight and gain.

Average daily gain differed by week ($P < 0.01$) (Table 2). It should be noted that ADG for week three of the study is significantly reduced due to a significant amount of rainfall (4.38 inches) between weeks three and four. Additionally, ADG had a tendency ($P = 0.17$) to be higher among lambs receiving TRT-5 and TRT-10 than those receiving CON. Similarly, ADG had a tendency ($P = 0.14$) to be higher in wether lambs receiving TRT-5 and TRT-10 than those receiving CON (Table 3). Ewe lamb ADG tended to be higher ($P = 0.14$) in lambs receiving CON and TRT-10 than those receiving TRT-5 (Table 3).

The effects of Probios Max supplementation on FEC of the lambs by treatment are shown in Fig. 6. Effects on treatment by week are shown in Fig. 7. Fecal egg counts were lower ($P = 0.05$) in lambs receiving TRT-10 compared to those receiving CON and TRT-5. Furthermore, there was a significant interaction of sex and week on FEC ($P < 0.05$) (Fig. 8).

2.3 Discussion

Supplementing lambs with probiotics when transitioning to high concentrate diets poses an opportunity for improved performance. Lambs treated with either 5 g (TRT-5) or 10 g (TRT-10) of Probios Max were treated to study the effects of probiotics on lamb performance. Overall, lambs in TRT-10 were heavier than lambs in the TRT-5 and CON groups. Pond and Goode (1985) reported that when feeding lambs a high concentrate diet, daily gain in DFM supplemented lambs tended to be 24.7% higher in the first and second week and 6.4% higher in the third and fourth week when compared to control. Saleem et al. (2017) also found a positive correlation between supplementing lambs with dietary probiotics and DMI, resulting in a higher ADG, FCR, and total weight gain over the feeding period. Furthermore, Krehbiel et al. (2003) found that feeding DFM to cattle at receiving and through the feeding period increased feed consumption by 2.5%, ADG by 13.2%, and feed to gain ratio by 6.3%. Although individual lamb or treatment feed consumption was not measured, we can assume that increased weight gain and ADG in lambs receiving probiotic supplementation is because more feed was consumed. Saleem and Zanouny (2016) reported increased feed intake when lambs were supplemented with high concentrations of probiotics. In this study, statistical differences were found during weeks three through six in wether lambs supplemented with Probios Max. However, other data suggested the greatest response to DFM has been seen during the first 14 (Krehbiel et al., 2003) to 35 d (Ponce et al., 2015) of the feeding period. It is difficult to compare the difference in result based on sex, as the comparative studies did not discuss the sex of the lambs in studies.

Due to the microbial ecosystem of the rumen and modes of action of the bacteria, probiotics should be supplemented more than once during the feeding period. Krehbiel et al. (2003) found that daily supplementation of DFM improved ADG and feed efficiency and reduced the occurrences of ruminal acidosis. Furthermore, probiotic supplementation improves digestibility and increases ruminal microbial populations resulting in improved ruminal pH (Saleem and Zanouny, 2016). Although no clinical cases of ruminal acidosis occurred in this study. More work is necessary to understand the effect of Probios Max supplementation on the rumen environment, as subclinical cases may have occured.

Vosooghi-poostindoz et al. (2013) found that DFM supplementation correlated with lower blood cortisol concentrations. Lower concentrations suggest that stress was reduced in lambs supplemented with DFM compared to lambs that were not. Blood cortisol concentrations were not analyzed in this study however, a positive correlation of weight gain and TRT-10 could suggest that blood cortisol concentrations were lower in lambs supplemented with Probios Max compared to CON. Reducing stress allows for a greater opportunity to optimize feed intake. Stress during the transition period often increases the opportunity for morbidity and mortality.

Krehbiel et al. (2003) found calves treated with DFM at processing were less likely to be treated with antimicrobials a second time.

Fecal egg counts were performed during weeks one through three to determine the effect of DFM on intestinal parasitic infection. Fecal egg counts were lower in lambs receiving TRT-10 compared to CON and TRT-5; however, initial FEC was relatively low to begin the test and remained below a level that would warrant anthelmintic treatment or result in animal health issues. Yang et al. (2020) found *B. subtilis* to have a potential protective role to *H. contortus* infections in the abomasum. Pinto et al. (2020) reported a decrease in number of *H. contortus* larvae and increased serum immunoglobulin levels when probiotics were supplemented, compared to those lambs not supplemented. Increased immunoglobulin levels suggest that intestinal parasitic infection is reduced as *H. contortus* feed on the blood of the host (Hale, 2006). Yang et al. (2020) reported EPG levels to be reduced by 71.5% when probiotic bacteria were supplemented compared to sheep that were infected with *H. contortus* and not supplemented. There was a significant interaction of sex and week on FEC. Similar to performance data, it is difficult to compare the effects of sex with probiotic supplementation and FEC as sex of lambs were not discussed in these studies.

2.4 Conclusion

Probiotic supplementation may be an effective strategy to decrease detrimental effects of diet transition and increase growth of feeder lambs transitioning to a concentrate diet. Dosage and frequency for direct-fed microbials, specifically *Probios Max*, to newly received feedlot lambs needs further investigation. Furthermore, more research needs to be done to determine the effects of probiotic supplementation on feed intake, rumen characteristics, and blood metabolites.

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APPENDIX A

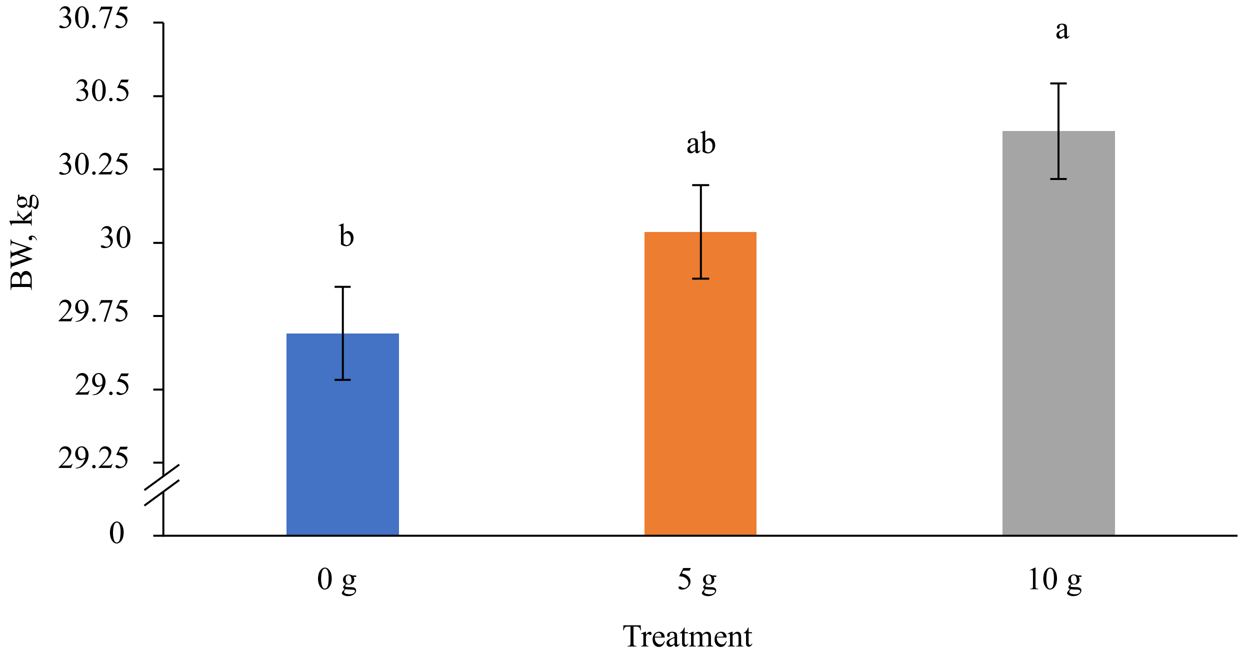

Fig. 1. Effects of supplementing Probios Max on lamb final BW.
LS-means of final body weight (kg). Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed ad libitum concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent SEM. Bars with different letters are significantly different ($P < 0.05$).

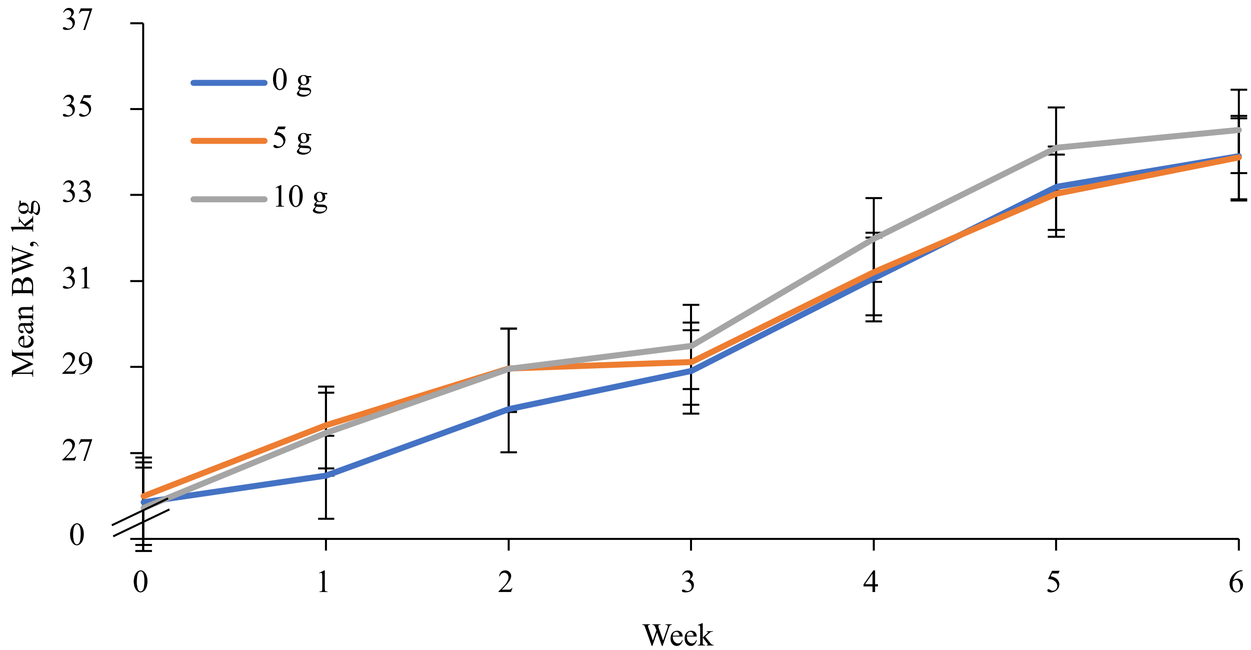

Fig. 2. Effects of supplementing Probios Max on overall lamb BW.
LS-means of overall lamb body weight (kg). Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent SEM.

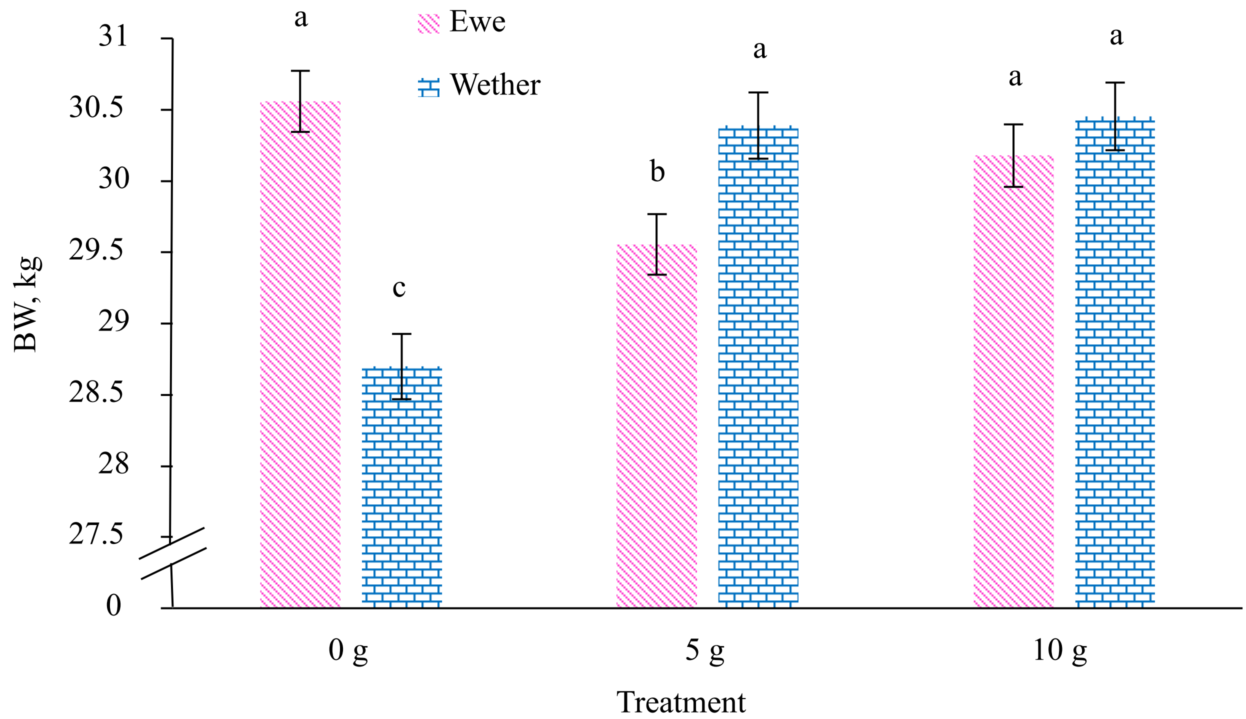

Fig. 3. Effects of supplementing Probios Max on lamb BW by sex.
LS-means of lamb body weight (kg) by sex. Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent SEM. Bars with different letters are significantly different ($P < 0.05$).

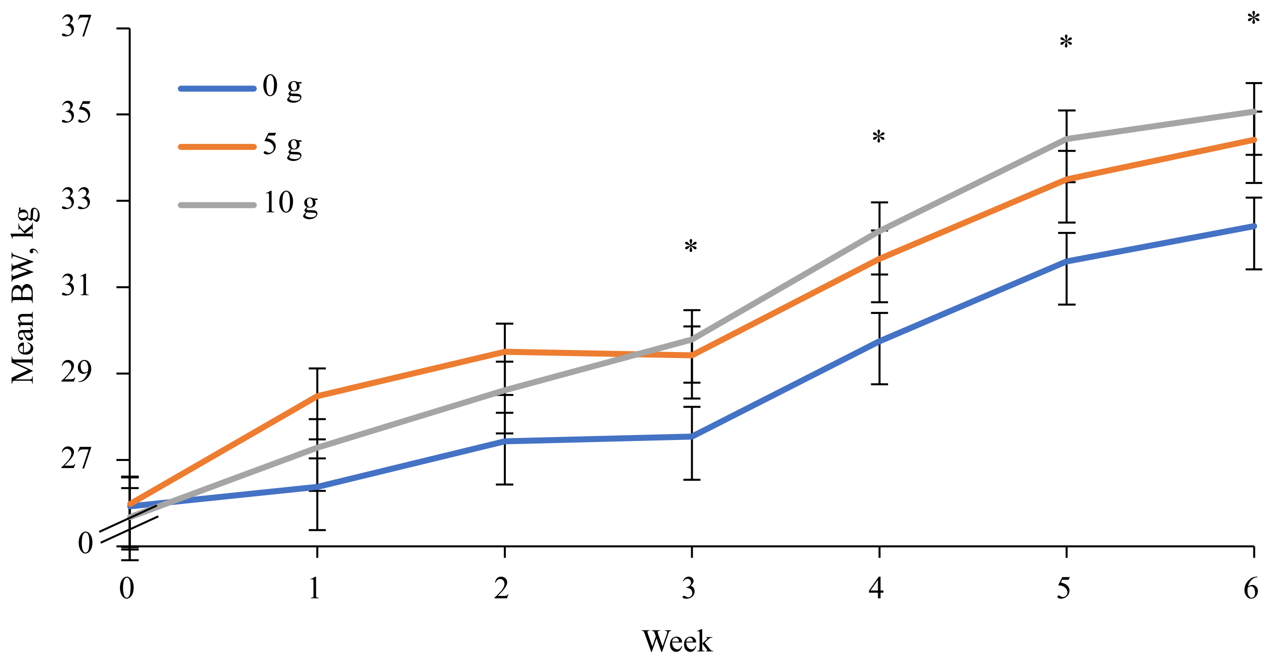

Fig. 4. Effects of supplementing Probios Max on wether lamb BW.
LS-means of wether lamb body weight (kg). Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent SEM. Bars with asterisks (*) are significantly different ($P < 0.05$).

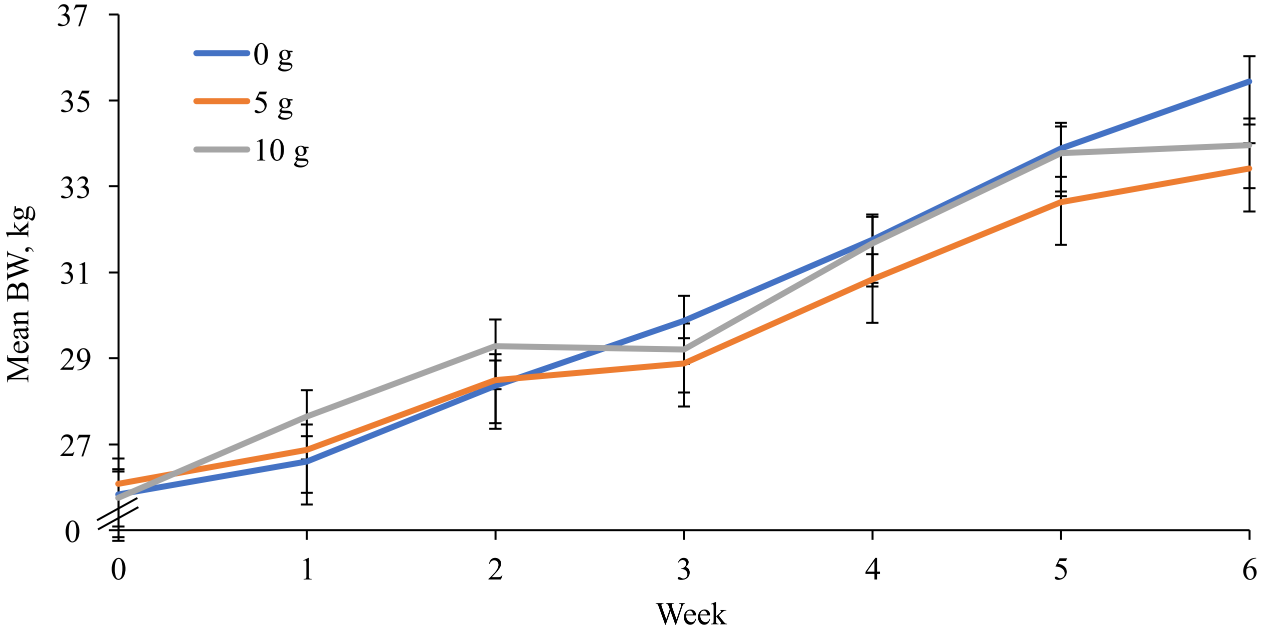

Fig. 5. Effects of supplementing Probios Max on ewe lamb BW.
LS-means of ewe lamb body weight (kg). Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent SEM.

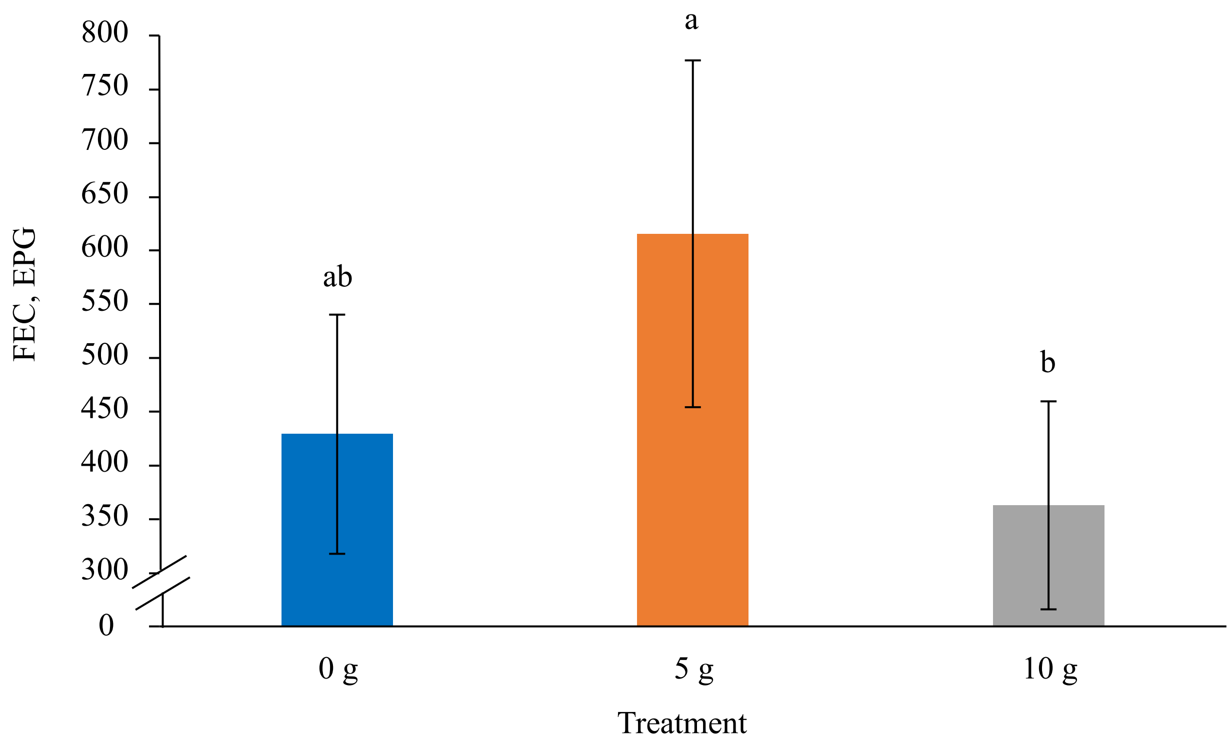

Fig. 6. Effects of supplementing Probios Max on lamb average FEC (EPG). LS-means of FEC (EPG). Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed ad libitum concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent the 90% confidence interval. Bars with different letters are significantly different ($P < 0.05$).

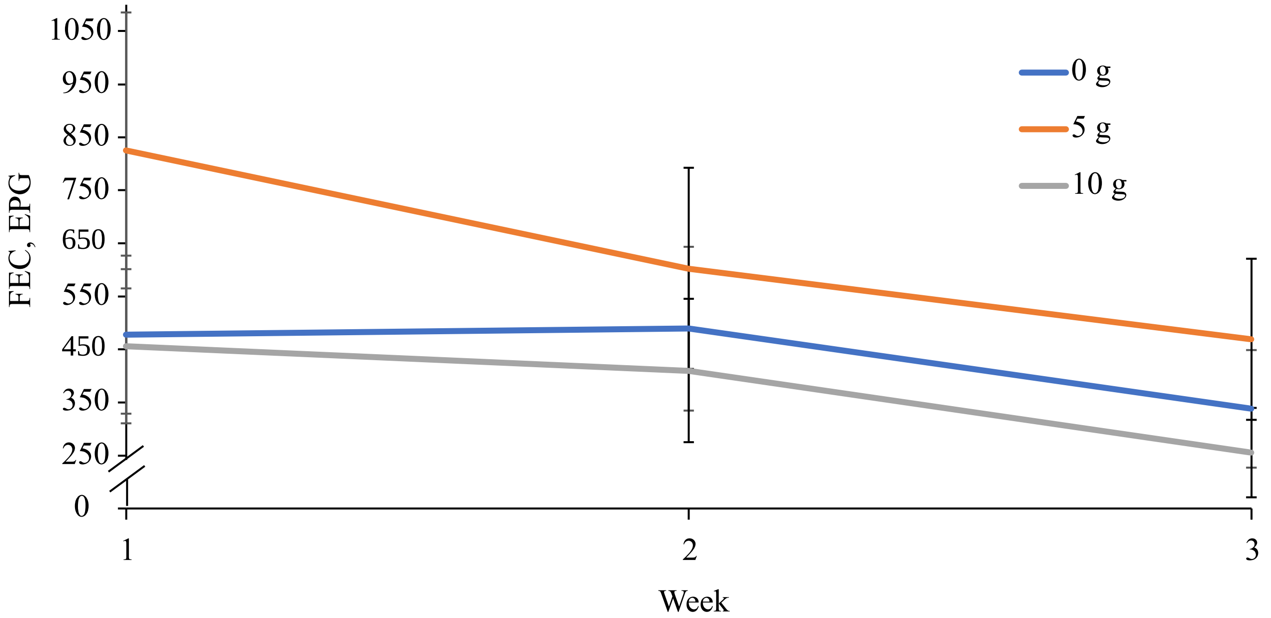

Fig. 7. Effects of supplementing Probios Max on overall lamb FEC (EPG). LS-means of lamb FEC (EPG). Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent the 90% confidence interval.

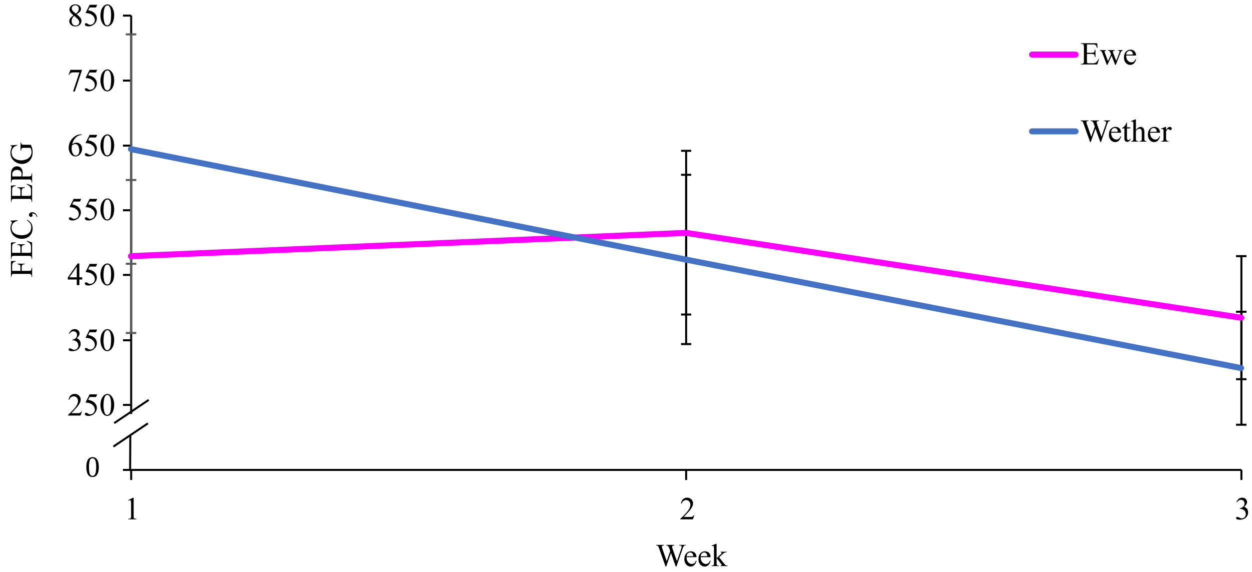

Fig. 8. Effects of supplementing Probios Max on sex of lamb FEC (EPG). LS-means of lamb FEC (EPG). Treatment diets only differed by grams of Probios Max administered on Day 0. Lambs were fed *ad libitum* concentrate 7diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max. Error bars represent the 90% confidence interval.

Table 1. Ingredients and nutrient composition (% DM basis) of diet.

Item	
Ingredients	
Milo	
Alfalfa	
Cotton seed hulls	
SUP-R-LIX	
BOVATEC	
Nutrient Composition	
DM %	89.3
CP %	13.8
CF%	14.9
TDN	69.8
ME, Mcal/kg DM	0.54

Treatment diets only differed by grams of Probios Max administered on Day 0. LS-means of average daily gain of lambs (units) by sex and treatment. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max.

Table 2. Body weight and average daily gain of lambs (unit) by sex, treatment, and week.

	Treatment									SE
	CON			TRT-5			TRT-10			
Week	Ewe	Wether	Overall	Ewe	Wether	Overall	Ewe	Wether	Overall	
BW, kg										
0	25.8	25.9	25.9	26.1	26.0	26.0	25.8	25.7	25.7	0.24
1	26.6	26.4	26.5	26.8	28.4	27.6	27.6	27.3	27.5	0.24
2	28.6	27.4	28.0	28.4	29.5	29.0	29.3	28.6	28.9	0.24
3	30.3	27.5	28.9	28.8	29.4	29.1	29.2	29.8	29.5	0.24
4	32.4	29.7	31.1	30.8	31.6	31.2	31.7	32.3	32.0	0.24
5	34.8	31.6	33.2	32.6	33.5	33.0	33.8	34.4	34.1	0.24
6	35.4	32.4	33.9	33.4	34.4	33.9	33.9	35.1	34.5	0.24
ADG, kg/d										
1	0.11	0.06	0.09	0.11	0.36	0.23	0.27	0.23	0.25	0.024
2	0.25	0.15	0.20	0.25	0.15	0.20	0.19	0.19	0.19	0.025
3	0.22	0.02	0.12	0.02	-0.01	0.005	0.02	0.18	0.10	0.025
4	0.27	0.30	0.28	0.28	0.32	0.30	0.33	0.35	0.34	0.025
5	0.30	0.26	0.28	0.26	0.26	0.26	0.30	0.31	0.30	0.025

Treatment diets only differed by grams of Probios Max administered on Day 0. LS-means of average daily gain of lambs (units) by sex and treatment. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max.

Table 3. Average daily gain of lambs (unit) by sex and treatment.

Sex	Treatment			SE	P-value
	CON	TRT-5	TRT-10		
Ewe	0.22[a]	0.19[b]	0.23[a]	0.03	0.04
Wether	0.16[b]	0.23[a]	0.26[a]	0.03	0.04
Overall	0.19[b]	0.21[ab]	0.24[a]	0.02	0.04

Treatment diets only differed by grams of Probios Max administered on Day 0. LS-means of average daily gain of lambs (units) by sex and treatment. Lambs were fed *ad libitum* concentrate diets. Each lamb in the control group (CON) was administered 0 g Probios Max, each lamb in treatment group 1 (TRT-5) was administered 5 g Probios Max, and each lamb in treatment group 2 (TRT-10) was administered 10 g Probios Max.