The Effects of Probiotics on Growth and Development of Ram Lambs

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Introduction

- Probiotics are microbes that are naturally occurring in the gastrointestinal tract of an organism, and when administered to an animal, they can reduce the population of pathogenic microbes (Wang et al., 2017).
- Probiotics have been suggested as an alternative to sub-therapeutic antibiotic use to improve growth efficiency, gut health, and metabolism (Abas et al., 2007, Alhidary et al., 2016, Meng et al., 2010).
- The mechanisms by which probiotics improve nutrient bioavailability and growth performance are not well understood (Abd El-Tawab, 2016).
- The objective of this study was to determine if growth performance, metabolism, and gut microbiomes could be modified in ram lambs with daily administration of probiotics from two to six months of age.
- We hypothesized that probiotic treatment would increase growth measurements, improve feed efficiency, and alter circulating factors and gut microbial composition.

Materials and Methods

- Twenty ram lambs (mean 59.85 ± 2.74d of age, mean 25.4 ± 3.91kg body weight) were individually housed from 2 to 6 months of age. Each lamb was fed lamb grower grain (17.2% CP, 3.56 Mcal/kg) at 3.6 % of body weight and hay ad lib to meet NRC requirements.
- One group of lambs (n = 10) were fed 10g of Probios (Chr. Hansen Inc., Menomonie, WI) probiotic powder daily with their grain and the remaining lambs served as controls.
- Each week, body weights (BW) and body condition scores (BCS) were determined. Feed intake (FI) was recorded daily and averaged on a weekly basis. Feed to gain ratio (F:G) was calculated on a weekly basis.
- At week 0 and week 16, loin eye area (LEA), back fat depth (BF), heart girth (HG), crown-rump length (CR) were measured. Heart girth (HG), crown-rump length (CR) were measured by tape measure. Loin eye area (LEA) and back fat depth (BF) were measured by ultrasound. Ultrasound images were quantified with Auskey software.
- Malondialdehyde (MDA) plasma concentration was measured by assay kit (NWLSS, Vancouver, WA) at weeks 0, 6, and 16.
- Fecal samples were collected at week 16. Sequencing (16S Bacteria and Archaea) was completed by the MARS center at the University of Connecticut (Storrs). Analysis was completed using mothur v 1.43.0 (Schloss et al., 2009).
- Statistical analysis of HG, CR, LEA and BF was completed using the SAS PROC GLM function. Statistical analysis of MDA, ADG, FI, F:G, BW, and BCS was completed using the PROC MIXED function with repeated measures in SAS. A $P \leq 0.05$ was considered statistically significant.

	Week 0		Week 4		Week 8		Week 12		Week 16		S.E.M.		*P*-Value		
	Con	Prob	Con	Prob	Con	Prob	Con	Prob	Con	Prob	Trt	Time	Trt	Time	Trt x Time
BW (kg)	25.92	24.80	39.04	37.50	50.02	48.40	58.20	58.04	66.32	65.94	1.33	1.03	0.566	<.0001	<.0001
BCS	3.29	2.90	3.03	2.98	3.05	2.90	3.08	3.05	3.05	3.00	0.05	0.05	0.461	<.0001	0.0007
ADG (kg)	0.53	0.48	0.39	0.40	0.40	0.43	0.39	0.40	0.37	0.37	0.01	0.04	0.528	<.0001	0.0240
FI (kg)[1]	*8.70	*9.52	12.19	12.69	15.02	14.62	16.88	15.55	17.50	17.90	0.43	0.41	0.895	<.0001	0.1089
F:G[1]	*2.39	*3.39	4.15	5.29	10.48	5.65	17.72	8.09	6.83	11.19	0.95	1.85	0.466	<.0001	0.0642

Table 1: Growth measurements from week 0 to week 16 Body weight (BW) and body condition score (BCS) were measured weekly. Average daily gain (ADG), feed intake (FI), and feed to gain (F:G) were calculated weekly. No effect of probiotics was observed for any growth measurement (BW P =0.566, BCS P = 0.461, ADG P = 0.529, FI P = 0.896, F:G P = 0.467), which may indicate that daily treatment with Probios may not have an effect on growth or feed efficiency in healthy ram lambs. *Feed intake and feed:gain data presented are for Week 1. [1] Calculated on a dry matter basis.

	Week 0		Week 16		P-Values		
	Con	Prob	Con	Prob	Trt	Time	Trt x Time
HG (cm)	69.55 ± 4.83	69.85 ± 4.32	94.36 ± 2.90	93.09 ± 3.36	0.7134	<.0001	0.5528
CRL (cm)	79.95 ± 7.20	78.35 ± 6.57	101.35 ± 4.91	100.33 ± 3.45	0.4975	<.0001	0.8792
BF (cm)	0.81 ± 1.26	0.40 ± 0.10	0.38 ± 0.06	0.66 ± 0.74	0.8000	0.7146	0.1642
LEA (cm^3)	8.06 ± 0.66	8.03 ± 0.65	9.17 ± 0.95	9.13 ± 0.90	0.9061	0.0002	0.9863

Table 2: Growth measurements at week 0 and week 16 Heart girth (HG) and crown rump length (CRL) were measured by tape measure and back fat thickness (BF) and loin eye area (LEA) were measured by ultrasound at weeks 0 and 16 of study. Probiotic treatment did not effect HG (P = 0.713), CRL (P = 0.498), BF (P = 0.800), or LEA (P = 0.906). This indicates that Probios supplementation from 4 to 6 months of age may not affect HG, CRL, BF, and LEA in healthy growing ram lambs.

	Week 0		Week 6		Week 16		S.E.M.		P-Value		
	Con	Prob	Con	Prob	Con	Prob	Trt	Time	Trt	Time	Trt x Time
MDA (μM)	0.3852	0.5211	0.4052	0.3753	0.5113	0.4967	0.042	0.048	0.6160	0.2442	0.2617

Table 3: Malondialdehyde plasma concentration No change in MDA concentration was observed as a result of probiotic treatment (P = 0.6160). Probiotics have been suggested as a potential antioxidant and may decrease MDA concentrations in the blood (Wang et al., 2017). It is possible that healthy ram lambs are not producing enough free radicals for probiotics to have a significant effect.

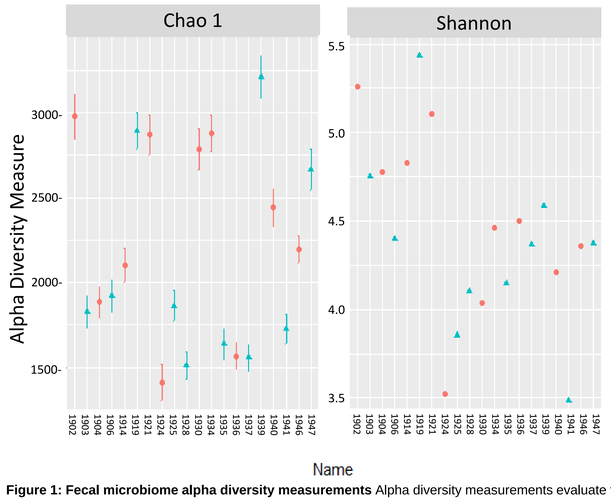

Figure 1: Fecal microbiome alpha diversity measurements Alpha diversity measurements evaluate the diversity of microbes for each individual animal. The majority of probiotic fed sheep had Chao1 alpha diversity measurements below 2000 and Shannon alpha diversity measurements below 4.5. This suggests that probiotic treatment may decrease alpha diversity in growing ram lambs however, more research is needed to evaluate if the absent species are pathogenic.

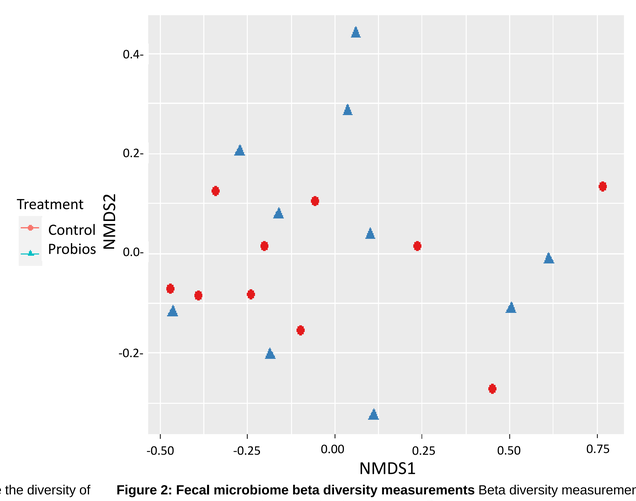

Figure 2: Fecal microbiome beta diversity measurements Beta diversity measurements were used to compare microbe diversity among treatment groups. Distance was measured using the Bray-Curtis Index and reported using Nonmetric Multidimensional Scaling (NMDS). The lack of clustering among probiotic and control groups suggest that probiotic treatment does not affect beta diversity in growing ram lambs.

	Con	Prob	P-Value
Glucose (mg/dL)	80.80	82.80	0.48
Urea Nitrogen (mg/dL)	24.60	25.70	0.51
Creatinine (mg/dL)	0.62	0.64	0.59
Total Protein (g/dL)	6.45	6.72	0.16
Globulin (g/dL)	3.02	3.09	0.73
Calcium (mg/dL)	10.05	10.34	0.17
Phosphorus (mg/dL)	7.43	7.42	0.99
Total Bilirubin (mg/dL)	0.12	0.15	0.58
Direct Bilirubin (mg/dL)	0.00	0.00	-
*Albumin (g/dL)	3.43	3.63	0.03
Aspartate transaminase (U/L)	108.70	90.20	0.20
Gamma-glutamyl transferase (U/L)	56.20	45.40	0.13
Glutamate dehydrogenase (U/L)	87.20	35.20	0.24
Creatine Kinase (U/L)	122.60	133.20	0.87
Sodium (mEq/L)	140.60	144.70	0.26
Potassium (mEq/L)	4.87	4.94	0.66
Chloride (mEq/L)	106.40	105.40	0.28
Bicarbonate (mEq/L)	25.60	25.70	0.95
Anion Gap (mEq/L)	17.00	18.50	0.32
*Magnesium (mg/dL)	2.37	2.67	0.01

Table 4: Concentration of circulating factors Week 16 plasma samples were sent to University of Missouri VMDL to complete their Food Animal Maxi Panel. Plasma albumin (P = 0.03) and magnesium (P =0.01) concentrations increased as a result of probiotic treatment. Probiotic supplementation may increase albumin concentrations by increasing liver protein synthesis (Harding et al., 2008). Probiotics may also increase Mg absorption which may decrease blood cholesterol over time (Upadrasta and Madempudi, 2016). * indicates a statistically significant difference within the row.

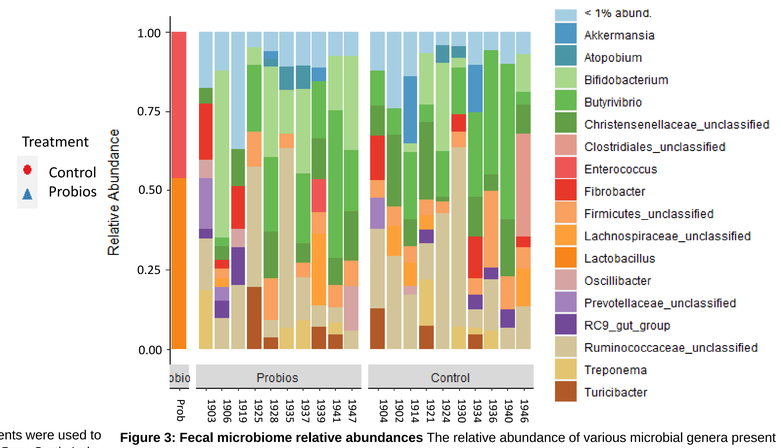

Figure 3: Fecal microbiome relative abundances The relative abundance of various microbial genera present in the sheep fecal samples are presented above. There are no distinct differences in the genera present in the probiotic fed sheep compared with control.

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Conclusions

- Probiotics supplementation did not have a direct effect on growth, feed intake, or body composition in the current study.
- Probiotic treatment did increase plasma albumin and magnesium concentrations at week 16 and may also reduce alpha diversity in healthy ram lambs.
- Additional studies should investigate the effects of probiotics on ram lambs at an earlier age, using different management techniques, or using different strains of probiotics to determine optimize supplementation to affect growth efficiency and the gut microbiome.