Sudan Journal of Science and Technology
Journal homepage:
http://jst.sustech.edu/

Effect of PROTEXIN® Probiotics on Najdi Newborn Lambs Rumen Fermentation and Histomorphology.

Mohamed A. S. Abdalla[1]; Khalid Rodwan[1]; Mutassim M. Abdelrahman[2]

Collage of Vet. Medicine, Department of Microbiology, Sudan University of Science & Technology, Sudan.
College of Food and Agriculture Sciences, Department of Animal production, King Saud University, Saudi Arabia

ARTICLE INFO

ARTICLE HISTORY
Received: 31/12/2019
Accepted: 20/2/2020
Available online:

KEYWORDS:
PROTEXIN®, probiotics, lambs, rumen, fermentation, histomorphology, VFA.

ABSTRACT

Globally meat production and consumption has increased rapidly in recent decades, which in turn increases the need to develop techniques and products for improving animal production. Probiotic is one of these impoved products. This study was performed at AL-Khalidiah farm, Riyadh city, Saudi Arabia. It was designed to investigate the effect of two different probiotic (PROTEXIN®) formulation in relation to different dosing methods on newborn Najdi breed lamb's rumen fermentation and histomorphology. Fourty newborn lambs were divided into four equal groups; First group was a control group (group C) without any probiotic supplementation, second group (group T1) supplemented with a single dose of 5 ml PROTEXIN® LIFE START at the first day of age, third group (group T2) supplemented with daily dose of 0.25 gm PROTEXIN® COMPOUNDER and the forth group (group T3) supplemented with a single dose of 5 ml PROTEXIN® LIFE START at the first day of age then continued with a daily dose of 0.25 gm PROTEXIN® COMPOUNDER till the end of the study. The study continued up to 60 days of age. Rumen fluid (collected by a stomach pump) and rumen tissues (at postmortem) were collected for measuring fermentation and histomorphology effects of the treatment respectively.The results were analyzed using (ANOVA). Rumen fluid showed increased pH with no effect on lactic acid concentration. Rumen TVFA, propionic acid and valeric acid increased with the treatment, but no significant variations between the groups. Molar proportion between VFA increased in propionic acid and valeric acid in contrast to a decrease in acetic acid compared to control group. No morphological or histological effects

regarding rumen papillary length, width and surface area except increased of papillary density per cm² and thickness of papillary stratum corneum layer. PROTEXIN® probiotic improved lambs' rumen fermentation and histomorphology of rumen papillae with no significant differences between application methods.

Introduction:

Globally, meat production and consumption have increased rapidly in recent decades and the demand for livestock products will soon nearly double in sub-Saharan Africa and South Asia in 2050 (Nierenberg and Dannielle, 2011). Globally meat production is projected to be 16% higher in 2025 than the base period (2013-2015), compares to almost 20% increase in the previous decade. Livestock supply responses to market signals, continue to be influenced by environmental and food safety regulations, in addition to availability of natural resources, technical and technological opportunities for productivity gains (FAO, 2016). This increases the need for developing techniques and products to improve animals' production in order to meet rising demands for livestock products worldwide specially after Antibiotic Growth Promotors (AGP) pan in Europe and other countries in 2006, to avoid risk of antibiotic resistance in human.

One of the important substitutes for AGP are probiotics which has different types and ingredients. Probiotics can be defined as preparations containing live microorganisms as feed supplement for ruminant (Dawson, 2002). Real idea of administering microorganisms to animals is associated with feeding of large quantities of "beneficial" microbes to livestock when they were "stressed" or ill (Denev *et al.,* 2007). Microbial products used in this way were originally called "probiotics" or products "for life" (Beev *et al.,* 2007). The current definition formulated by FAO and WHO working group experts' in 2002 states that probiotics are "live strains of strictly selected microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO, 2002).

Probiotics help preventing and controlling gastro-intestinal track (GIT) pathogens and/or improve performance and productivity of animals' production through various mechanisms. Closely related strains may differ in their mode of action (Fioramonti *et al* 2003; Roselli *et al,* 2007; Lodemann, 2010). One of the healthy GIT major determinants is microbial population composition. Probiotics can change microbial population dynamics in GIT eventually creating a more favorable microbial population due to shift in beneficial and harmful microbes balance (Mountzouris *et al.,* 2007; An *et al.,* 2008; Mountzouris *et al.,* 2009). Healthy microbial populations in GIT are often associated with enhanced animal performance, reflecting more efficient digestion and improved immunity (Niba *et al,* 2009; Hung and Shu. 2012).

Studies have shown that main ruminal bacteria which affecting ruminal fermentative capacity are classified as lactic acid-producers and consumers (Belanche *et al.,* 2012), and both bacterial groups are use as probiotics. Probiotics which composed of various microbial components are known to improve ruminal fermentation by activating rumen microbiota (Chiquette *et al.,* 2008; Nocek *et al.*, 2002; Timmerman *et al.,*2005) and directly increasing ruminal performance in dairy cattle (Belanche *et al*., 2012; Weinberg, *et al.*, 2004). Researches on microbial composition and ruminant digestive ecosystems functional diversity suggests, consecutive probiotic supplementation to improve animal performance by altering ruminal microbiota and increasing digestion capability (Nocek *et al.,* 2002; Timmerman *et al.,*2005).

Probiotic reduces organic acid accumulation. Study done by Hiroko *et al. (*2016), showed a 24-hr mean ruminal pH to be higher in probiotic treated groups compared to control group during SARA (subacute ruminal acidosis) challenge. This indicated increased ruminal fermentation in cows, same as reported by Ghorbani *et al.* (2002) and Weinberg *et al*. (2003). However, Chiquette *et al.* (2012) reported no effects on ruminal pH when a single strain of probiotic was administered in SARA-challenged cows. However, he found ruminal pH to be increased compared to control group when using a combination of *E. faecium* and *yeast.* Probiotic appears to increase ruminal bacteria' ability to metabolize lactic acid and regulate ruminal pH (Qadis *et al.,* 2014). It has been hypothesized that functionality and efficacy of probiotics can be determined based on their effects on predominant rumen microbiota (Ghorbani *et al.,* 2002; Chiquette *et al.,* 2012).

Lactate is a common product of carbohydrate fermentation produced by lactate producing bacteria species such as *Streptococcus bovis* (Chiquette, 2009; Chaucheyras-Durand *et al.,* 2012). Lactic acid bacteria (LAB) probiotics administration is thought to help rumen microbiota to adapt to presence of lactic acid (Ghorbani *et al.,* 2002), and prevent lactate accumulation in rumen (Russell and Wilson, 1996). In a study done by Qadis *et al*. (2014) significantly lower lactic acid concentrations in weaned calves receiving probiotic were observed compared to control.

Ruminal VFAs pattern differs in different diets fed animals and can reflect ruminal microbes' population make up (Chen *et al.* 2011). Some studies found increased VFA production in small ruminants which fed probiotics (Sadiek and Bohm, 2001; Abd El-Ghani, 2004). Moreover, Abd El-Tawab *et al.,* (2016) reported probiotics to increase VFA production. However, other studies recorded a significant reduction in ruminal VFA formation in growing lambs and adult goats which were given probiotic supplemented diets (Kowalik *et al.* 2011; Tripathi and Karim 2011).

Concerning rumen histology, some studies showed increased stratum corneum thickness with probiotic supplementation (Steele *et al.* 2012; Garcia *et al.* 2018). Probiotics in poultry diets can affect intestinal mucosa histology. Villus height and villus: crypt ratio in intestinal mucosa were increased by *B. subtilis* (Jayaraman *et al.,* 2013; Afsharmanesh and Sadaghi, 2014), *B. coagulans* (Hung and Shu. 2012), lactic acid producing bacteria *L. salivarius, P. parvulus* (Biloni *et al.,* 2013) and *E. faecium* (Cao *et al.,* 2013). *B subtilis PB6* reconstituted chicken intestinal villi that distorted and damaged as a result of necrotic enteritis caused by *Cl. perfringens* to their normal structure (Jayaraman *et al.,* 2013).

Materials and Methods:

The study was performed at AL-Khalidiah sheep farm, Riyadh city, Saudi Arabia. Forty newly born lambs (one day old) with average body weight 6.00 ± 0.1 kg (Najdi breed) was divided randomly into 4 equal groups of 10 animals each. All groups were kept in separate pens with their mothers at the same environmental conditions (feeding and vaccination programs.)

First group (C) was a control group without any probiotic supplementation.

The 2nd group (T1) received only one dose of treatment within the first 12 hours of life using the product PROTEXIN LIFE START® manufactured by PROBIOTIC INTERNATIONAL COMPANY – UK, at manufacturer dose rate of 4 ml paste orally within the first 12 hour of life. The product is a **combination of probiotic bacteria** (*Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Streptococcus faecium, Streptococcus thermophilus, Bifidobacterium bifidum*) total viable count $8X10^8$ CFU/ gm, **natural colostrum (**IgG1, IgG2, IgGA, igGm) 10%, **vitamin** B^{12} - 0.000001 mcg/ml, **minerals:** Cobalt - 0.003% and Cod liver oil base - 84.997%).

The 3rd group (T2) received a daily dose of probiotic combination using the product PROTEXIN COMPOUNDER® manufactured by PROBIOTIC INTERNATIONAL – UK, the product is a **combination of probiotic bacteria** (*Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Streptococcus faecium, Streptococcus thermophilus, Bifidobacterium bifidum*), total viable count $2X10^8$ CFU/ gm and **Dextrose Monohydrate** 96.67%. The dose rate was 0.25 gm/animal/day continuously till the 60 days of age. **4th group (T3)** was treated with 4 ml single dose using the product PROTEXIN LIFE START® at the first 12 hours of age then continued treatment by receiving daily dose of PROTEXIN COMPOUNDER® the dose rate was 0.25 gm/animal/day till the 60 days of age.

Rumen fluid was collected from the 4 groups at the age of 30 days and 60 days using stomach pump though the mouth, the collected ingesta was squeezed by hand through cheesecloth to get the rumen fluid, the collected fluid was centrifuged at 3000 rpm for 30 min to remove the large particles then the supernatant fluid was collected into clean tubes and stored at -20 C for further analysis. The pH of the collected rumen fluid was measured immediately after collection using calibrated pH meter (Model pH 211, Hanna Instruments) and the results were recorded for statistical analysis.

For assay of lactic acid, the ruminal fluid was centrifuged immediately at 2,000 × g for 30 min, and concentrations in the supernatant were determined using a commercial kit (F-kit; D-lactate/L-lactate, J. K. International, Tokyo, Japan). For the VFA analysis, 10 ml of ruminal fluid was added to 2 ml of 25% metaphosphoric acid in 3 N H_2SO_4 for assay of VFA. Total VFAs and three individual VFAs (acetic acid, propionic acid and butyric acid) were separated and quantified with gas chromatography (Model 135, Hitachi, Tokyo, Japan) using a packed glass column (3% Thermon-3000) on a Shimalite TPA 60–80 support (Shinwa Chemical Industries Ltd., Kyoto, Japan).

At the end of the experiment (60 days of age), randomly three lambs per each treatment were slaughtered. A $3cm^2$ samples were taken from rumen without straining the wall for histological analysis. Samples were fixed in 10% (vol/vol) phosphate-buffered formalin for at least 72h, after which they were dehydrated in graded alcohol and embedded in paraffin. Sections of 5 µm were cut from each sample by the Microtome System and stained with hematoxylin and eosin according to the method described by Alhidary *et al*. (2016). Measurements of height (L), width (W) of the papilla, the stratum corneum (SC), the width of the epithelium (WE), lamina propria (LP), the number of papillae/cm^2 mucosa (papillae density) and sub mucosa were based on randomly 5 well-oriented papilla per section per animal using an IX71 Inverted Olympus Microscope (Eyepiece: WH10X, Objective Lens: 4X) and a PC-based image analysis system (Olympus DP72 Microscope Digital Camera; Olympus NV, Aartselaar, Belgium) with software Analysis (Cellsens Digital Imaging Software for Research Application). Papilla height and width data were used to calculate papilla surface area [$2\pi \times (W/2) \times L$], where W = papilla width and L = papilla length. In addition, the total surface of papillae per cm^2 mucosa was determined as length × width × 2, multiplied by the number of papillae/cm^2 (Alvarez-Rodríguez *et al*., 2012; Alhidary *et al*., 2016).

Data were subjected to analysis of variance (ANOVA) using General Linear Model (GLM) procedure of Statistical Analysis System institute, Inc. (SAS 2009) JMP software version 11 for a Completely Randomized Design

Results:

Lactic Acid and PH

The effects of the three types of probiotics treatments on rumen Lactic acid are shown in Table 1. Lactic acid was not affected significantly by treatments (P > 0.3717) compared with the control group. Lactic acid increased significantly (P < 0.0001) with the increase of age when comparing 30 with 60 days of age.

As shown in Table 1. the pH of the rumen of treatment groups have significantly higher pH (P < 0.0123) compared to the control group, T2 group and T3 group were the highest within the treated groups.

Rumen pH increased significantly (P < 0.0001) with the increase of age when compared 30 with 60 days of age.

Table (1) Effect of probiotics and age on Lactic Acid and pH in rumen fluid of the growing lambs

	Parameters	
Treatment[2]	L. Acid	pH
C	4.69	7.10[ab]
T1	3.96	6.84[b]
T2	4.36	7.31[a]
T3	5.77	7.34[a]
SEM[1]	0.522	0.111
P-value	0.3717	0.0123
Age		
30	2.34[b]	6.90[a]
60	6.85[a]	7.40[b]
SEM[2]	0.419	0.081
P-value	<0.0001	0.0001
Treatment*Age		
P-value	0.7849	0.980

[a - c] Mean value within a column with different superscripts are significantly different, P≤ 0.05. SEM[1], standard error of the mean, Treatment[2] = **T1, T2 and T3,** Control = group without probiotic supplementation, L. Acid = Lactic acid

Volatile Fatty Acids (VFA)

The effect of treatment on rumen concentrations of total volatile fatty acids, acetic acid, propionic acid, butyric acid and valeric acid were compared to the control group are shown in Table 2. The results showed significant increase in TVFA (P-value 0.018) within the treatment groups than the control group, regarding the result of individual VFA were showed in the same table. The concentration of propionic acid in rumen increased (P-value 0.001) within the treated lambs. Proportion of acetic acids in TVFA lowered (P- value 0.002) and that of propionic acid increased (P-value <0.001) in treated lambs compared to control group. Proportion of valeric acid was also higher (P-value 0.016) in treated groups as compared to control group. T3 group showed the highest levels of TVFA, propionic acid and valeric acid within the treatment groups but the differences were not significant.

Table (2) Effect of probiotics on lamb's rumen total volatile fatty acids (TVFA), acetic acid (A), propionic acid (P), butyric acid (B) and valeric acid (V)

Parameters	Treatments[2] Control	T1	T2	T3	SEM[1]	P- value
			Total and Individual Fatty Acids (meq/L)			
TVFA	87.621[b]	97.834[a]	99.647[a]	100.608[a]	4.641	0.018
A	52.392	5.3811	54.019	54.219	3.182	0.751
P	23.520[b]	32.117[a]	32.292[a]	33.519[a]	2.483	0.001
B	10.524	9.206	9.817	10.143	0.896	0.855
V	1.132[b]	2.809[a]	3.024[a]	3.129[a]	0.293	0.003
			Molar Proportion (%)			
A	59.80[a]	50.10[b]	51.30[b]	51.40[b]	1.502	0.002
P	26.90[b]	36.70[a]	36.80[a]	37.00[a]	1.453	<0.001
B	12.00	10.00	9.20	9.00	0.712	0.112
V	1.30[b]	3.20[a]	2.70[a]	2.60[a]	0.284[a]	0.016

[a - c] Mean value within a column with different superscripts are significantly different, P≤ 0.05. SEM[1], standard error of the mean, Treatment[2] = **T1, T2 and T3,** Control = group without probiotic supplementation.

Rumen Histomorphology

The effects of treatments on rumen papilla measures are shown in table 3 and figure 1 The results showed that there was slight increase in Papilla length (L), Papilla width (W), Papillae surface area (SA) and total surface area of papilla within the treatment groups (T1, T2 and T3) compared to the control group, although this increase was not significant (P > 0.5667, P > 0.0645, P > 0.7391 and P > 0.3886 respectively). On the other hand, there was significant increase (P < 0.0005) in Papilla density per cm² in all treated groups (T1; T2; T3) compared to control group. T2 showed that highest density within the treated groups.

Table (3) Effect of probiotics on lamb`s Rumen Papilla Length (L), Width (W), Surface Area (SA), Density of papilla and Total Surface of Papillae (TSP).

Treatments[2]	Parameters L (mm)	W (mm)	SA (mm)	Density (per cm²)	TSP (per cm²)
Control	3.9667	0.43778	5.3011	62.547[c]	246.98
T1	3.8911	0.50556	6.0067	68.489[bc]	244.81
T2	4.6411	0.40889	5.6156	86.363[a]	298.68
T3	4.0022	0.50111	5.6567	78.062[ab]	269.99
SEM[1]	0.4186	0.0292	0.4448	3.7710	24.632
P-value	0.5667	0.0645	0.7391	0.0005	0.3886

[a - c] Mean value within a column with different superscripts are significantly different, P≤ 0.05. SEM[1], standard error of the mean, Treatment[2] = T1, T2 and T3, Control = group without probiotic supplementation.

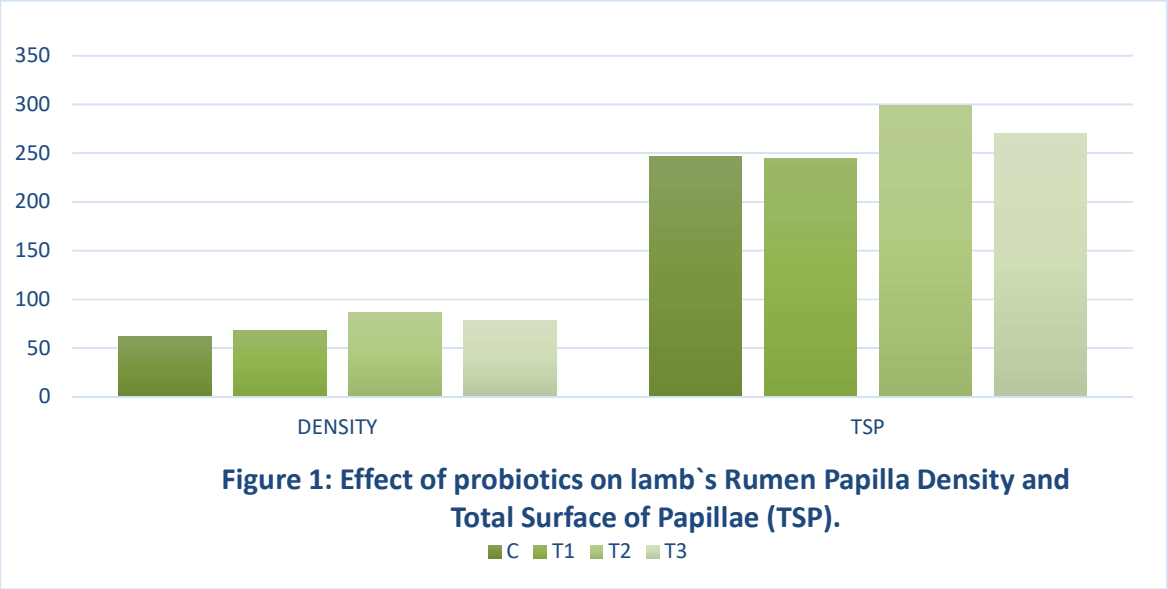

Figure 1: Effect of probiotics on lamb`s Rumen Papilla Density and Total Surface of Papillae (TSP).

Treatment[2] = T1, T2 and T3, Control = group without probiotic supplementation.

The effects of treatments on rumen papillae histology measures are shown in Table 4. And figure (2) The results showed that there was slight increase in Papillae width of the epithelium (WE), Lamina Propria (LP) and sub-mucosa layers in the treatment groups (T1; T2; T3) compared to the control group, although this increase was not significant ($P > 0.0800$, $P > 0.1754$ and $P > 0.7275$ respectively). On the other hand, there was significant increase ($P < 0.0234$) in Papilla Stratum Corneum (SC) in all treated groups

Table (4) Effect of probiotics on measures of lamb`s rumen Papillae Stratum Corneum (SC), Width of the Epithelium (WE), Lamina Propria (LP) and Sub-mucosa.

Treatments[2]	Parameters SC	WE	LP	Sub-mucosa
Control	0.0144[b]	0.4133	0.3156	0.7656
T1	0.0233[a]	0.5433	0.4244	0.8933
T2	0.0144[b]	0.4456	0.4178	0.8256
T3	0.0178[ab]	0.4489	0.3567	0.7856
SEM[1]	0.0022	0.0356	0.0391	0.0852
P-value	0.0234	0.0800	0.1754	0.7275

[a - c] Mean value within a column with different superscripts are significantly different, P≤ 0.05. SEM[1], standard error of the mean, Treatment[2] = T1, T2 and T3, Control = group without probiotic supplementation.

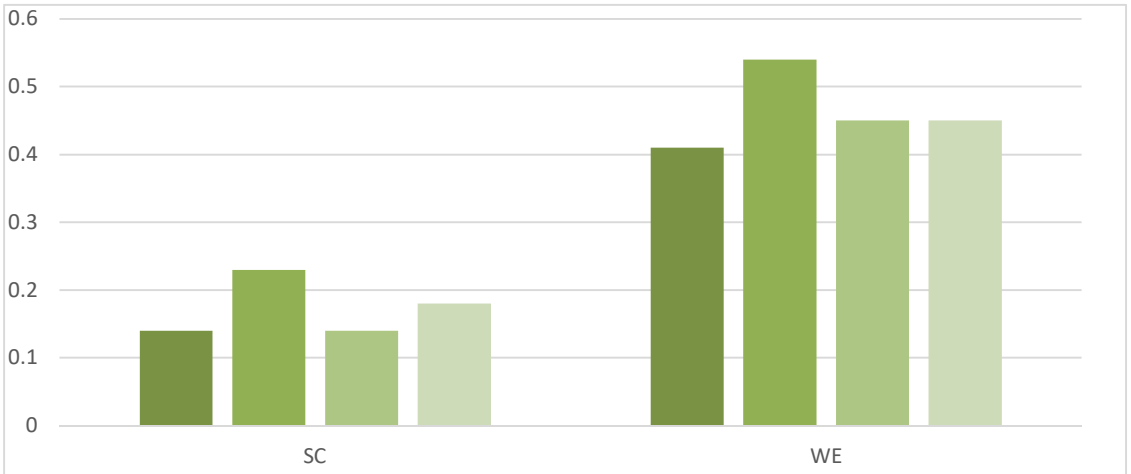

Figure 2: Effect of probiotics on measures of lamb`s rumen Papillae Stratum Corneum (SC) and Width of the Epithelium (WE).

Treatment[2] = T1, T2 and T3, Control = group without probiotic supplementation.

Discussion:

Ruminal fermentation in ruminants can be determined by changes in ruminal pH and lactic acid concentrations. The current study (Table 1) showed significant increase in pH levels (P-value 0.0123) within the treatment groups (T1, T2 and T3) than the control group, this agrees with Ghorbani *et a*l. (2002); Chiquette *et al.* (2012); Hiroko *et al*.(2016), this can be justified that probiotic bacteria increased the ability of ruminal bacteria to metabolize lactic acid and regulate ruminal pH (Qadis *et al*., 2014). Other authors correlated the increase of ruminal pH to the increased activities of lactate-consuming bacteria and greater lactate absorption affect ruminal pH (Khafipour *et al*., 2009). Some studies correlated the amount of increase in pH with the probiotic dose supplemented, the high dose might increase lactic acid bacteria (LAB) numbers in the rumen and hence more increase in pH (Khafipour *et al*., 2009; Jianbiao *et al*., 2017) which consist with the present study results where T2 and T3 groups (received continuous daily supplementation of Protexin® Probiotic) showed the highest levels of pH than T1 group (received one dose of Protexin® probiotic). Regarding lactic acid the results of this study (Table 1.) showed no significant differences between control group and treatment groups regarding lactic acid concentration in the rumen liquor, on the other hand the results showed significant increase in lactic acid obtained when comparing one day old with 60 days old lambs, this may be due to the presence of *lactobacillus spp*. as main component of the probiotic used in the study (Protexin®) and the fact that lactobacillus bacteria are lactic acid producing bacteria as a result of sugar fermentation (Zaunmüller *et al*. 2006).

Volatile fatty acids are the primary products of rumen fermentation which contributed to rumen epithelium development of fattening sheep. The VFA profile is associated with effects on end-product composition and energy balance in ruminants. The results of current study (table 2.) showed significant increase in TVFA (P-value 0.018) within the treatment groups than the

control group which agreed with the results reported by Sadiek and Bohm (2001); Abd El-Ghani (2004) and Abd El-Tawab *et al*. (2016). The increase in VFA is mainly due to the improvement of rumen fermentation by probiotics. The results of individual VFA concentrations (table 2) showed increased propionic acid concentration (P-value 0.001) within the treatment groups. Concerning molar proportion of VFA, propionic acid (P-value <0.001) and valeric acid (P-value 0.016) increased however, acetic acids (P- value 0.002) decreased within treatments group compared to control group, this agrees with the results obtained by Bhatt and Sahoo (2018). Zitnan *et a*l. (2005) who stated that higher sum of VFAs, especially propionic, butyric and valeric acid concentration in the rumen fluid of calves indicate enhanced rumen fermentation. Propionate is the main source of glucose and a substrate for gluconeogenesis for the ruminant, while acetate and butyrate are precursors for long-chain fatty acid synthesis, high glucogenic to non-glucogenic VFA ratio is beneficial for growth of finishing cattle (Abd El-Tawab *et al*., 2016)

The rumen and reticulum account for more than 70% of the total digestive tract volume in ruminants (Stobo *et al*. 1966). The results in (table 4) showed no significant influence in papillae width of the epithelium (WE), Lamina Propria (LP) and sub-mucosa layers (P-values: 0.08; 0.1754; 0.7275 respectively) which consist with the results of Garcia *et al*. (2018), this may be due to the fact that the rumen is incompletely developed both physically and metabolically at birth, representing only 30% of the total gastrointestinal capacity (Warner *et al*. 1956). On the other hand, the results showed significant increase (P-value 0.0234) in the stratum corneum (SC) that agrees with the results of Garcia *et al*. (2018), a possible justification for the better development of stratum corneum is that probiotics has protective effect on the ruminal epithelium against the damage caused by ruminal acidosis, the protection mechanism seems to be related to stabilization of the rumen pH, which may reduce the length of time during which the pH is below 5.8 (Bach *et al*. 2007., Chung *et al*. 2011., Vyas *et al*. 2014). Figure 2. Showed that treatment groups have increased papillae stratum corneum and width of the epithelium than control group which can result in better digestion and absorption of nutrients.

Regarding papilla size the results of current study (Table 3) showed no significant increase in Papilla length, width or surface area (P-values: 0.5667; 0.0645; 0.7391 respectively), this agrees with the results obtained by Kadir *et al*. (2016) in study done on goat kids. In this study there is significant increase in papilla density per cm² (P-value 0.0005) similar to the results obtained by Peng *et al*. (2011) in study done on diary calves.

Conclusion:

The supplementation of Protexin® Probiotic has positive effect in improving rumen fermentation indicated by increased pH levels, total volatile fatty acids specially, propionic acid which is the main substrate for gluconeogenesis, the effect is positively correlated to lamb`s age.

Treatment improved rumen histomorphology indicated by increased Papillae surface area and density per cm² accordingly, numbers of papillae protrude from the ruminal surface into the lumen increased the absorption surface area. Rumen epithelium is responsible for physiologically important functions, such as absorption, transport, VFA metabolism and protection.

However, differences between groups using different dosing methods or different products (Protexin® Life start; Protexin® Compounder) were not significant which may be due to the ability of live bacteria used to multiply within the rumen to compensate the dosing differences to reach the desirable balance.

References:

Abd El-Ghani, A. A. (2004). Influence of diet supplementation with yeast culture (Saccharomyces cerevisiae) on performance of Zaraibi goats. *Small ruminant research*, *52*(3), 223-229.

Abd El-Tawab, Mohamed ; Youssef, Ibrahim ; Bakr, H.A.; Fthenakis, G.C. and Giadinis, Nektarios. (2016). Role of probiotics in nutrition and health of small *ruminants. Polish Journal of Veterinary Sciences*. 19. 10.1515/pjvs-2016-0114.

Afsharmanesh, M. and Sadaghi, B. (2014). Effects of dietary alternatives (probiotic, green tea powder and Kombucha tea) as antimicrobial growth promoters on growth, ileal nutrient digestibility, blood parameters, and immune response of broiler chickens. *Comparative Clinical Pathology*, 23(3): 717–724.

Alhidary, I. A., Abdelrahman, M. M. and Khan, R. U. (2016). Comparative effects of direct-fed microbials alone or with a trace minerals supplements on the productive performance, blood metabolites, and antioxidant status in grazing Awassi lambs. *Environmental Science and Pollution Research*, *23*(24), 25218-25223.

Álvarez-Rodríguez, J.; Monleón, E.; Sanz, A., Badiola, J. J. and Joy, M. (2012). Rumen fermentation and histology in light lambs as affected by forage supply and lactation length. *Research in veterinary science*, 92(2), 247-253.

Bach, A.; Iglesias, C. and Devant, M. (2007). Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. *Animal Feed Science and Technology*, *136*(1-2), 146-153.

Beev G.; Todorov, P. and Tchobanova S. (2007). Yeast cultures in ruminant nutrition. *Bulg. J. Agric. Sci.* 13:357-374.

Belanche A; Doreau M; Edwards JE; Moorby JM; Pinloche E and Newbold CJ (2012). Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation. *J Nutr*. Sep; 142(9):1684-92.

Bhatt R.S. and A. Sahoo (2018). Effect of Feeding Milk Replacer Alone or in Combination with Probiotic on Pre-weaning Performance, Nutrient Utilization and Rumen Fermentation in Malpura Lambs. *Animal Nutrition and Feed Technology* 18: 1-12 DOI: 10.5958/0974-181X.2018.00001. X.

Cao, G. T.; Zeng, X. F.; Chen, A. G.; Zhou, L.; Zhang, L.; Xiao, Y. P. and Yang, C. M. (2013). Effects of a probiotic, Enterococcus faecium, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged with Escherichia coli K88. *Poultry Science*, *92*(11), 2949-2955.

Chaucheyras-Durand F; Chevaux E; Martin C and Forano E. (2012). Use of Yeast Probiotics in Ruminants: Effects and Mechanisms of Action on Rumen pH, Fiber Degradation, and Microbiota According to the Diet In: Probiotic in animals.

Chen, Y.; Penner G. B.; Li, M. and Oba, M. (2011). Changes in bacterial diversity associated with epithelial tissue in the beef cow rumen during the transition to a high-grain diet. *Applied and environmental microbiology*, *77*(16), 5770-5781.

Chiquette J.; Allison MJ. and Rasmussen MA, (2008). Prevotella bryantii 25A used as a probiotic in early-lactation dairy cows: effect on ruminal fermentation characteristics, milk production, and milk composition. *Journal of Diary Science* Sep; 91(9):3536-43.

Chiquette J. (2009). The role of probiotics in promoting dairy production. WCDS *Advance. Dairy Technology* 21:143-157.

Chiquette J.; Allison MJ. and Rasmussen M. (2012). Use of Prevotella bryantii 25A and a commercial probiotic during subacute acidosis challenge in midlactation dairy cows. *Journal of Diary Science*. 20 Oct;95(10):5985-95.
Chung YH; Walker ND; McGinn SM and Beauchemin KA. (2011). Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in non-lactating dairy cows. *Journal of Diary Science* 2011; 94: 2431–2439. pmid:21524535
Dawson K. A. (2002). Manipulating Rumen Microbial Population to Improve Animal Productivity. Proceedings Intermountain Nutrition Conference „*Animal Nutrition, Health and Profit",* Utah State University, USA, pp 1-22.
Denev S. A., Peeva T.; Radulova P.; Stancheva N.; Staykova G.; Beev G.; Todorova P.; and Tchobanova S. (2007). Yeast Cultures in Ruminant Nutrition. *Bulg. J. Agric. Sci*. 13: 357-374.
FAO. (2002). Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Gidelines for the evaluation of probiotics in food. 2002;30.04–01.05.2002, London,
FAO AGRICULTURAL OUTLOOK. (2016). 2016-2025 © OECD/FAO 2016108
Fioramonti, J.; Theodorou, V. and Bueno, L. (2003). Probiotics: what are they? What are their effects on gut physiology? Best Practice & Research in Clinical Gastroenterology, 17(5): 711–724.
Garcia Diaz, T.; Ferriani Branco, A. and Jacovaci, F. A. (2018). Cabreira Jobim C, Bolson DC, Pratti Daniel JL (2018) Correction: Inclusion of live yeast and mannan-oligosaccharides in high grain-based diets for sheep: Ruminal parameters, inflammatory response and rumen *morphology. PloS one*, *13*(4), e0196184.Ghorbani GR,
Ghorbani GR.; Morgavi DP.; Beauchemin KA and Leedle JA, (2002). Effects of bacterial direct-fed microbials on ruminal fermentation, blood variables, and the microbial populations of feedlot cattle. *Journal of Animal Science*. Jul; 80(7):1977-85.
Hiroko GOTO; Abdul Qadir QADIS; Yo-Han KIM; Kentaro IKUTA; Toshihiro ICHIJO and Shigeru SATO, (2016). Effects of a bacterial probiotic on ruminal pH and volatile fatty acids during subacute ruminal acidosis (SARA) in cattle. J Vet Med Sci. 2016 Oct; 78(10): 1595–1600.
Hung, Alex Tsungyu and Shu-Yuan Lin. (2012). "Effects of Bacillus coagulans ATCC 7050 on growth performance, intestinal morphology, and microflora composition in broiler chickens." *Animal production science* 52, no. 9 (2012): 874-879.
Jayaraman, S.; Thangavel, G.; Kurian, H.; Mani, R., Mukkalil R. and Chirakkal, H. (2013). Bacillus subtilis PB6 improves intestinal health of broiler chickens challenged with Clostridium perfringens-induced necrotic enteritis. *Poultry Science,* 92(2): 370–374.
Jianbiao Luo; Chaminda Senaka Ranadheera; Stuartking; Craig Evans and Surinder Baines. (2017). *In vitro* investigation of the effect of dairy propionibacteria on rumen pH, lactic acid and volatile fatty acids. *Journal of Integrative Agriculture*. Volume 16, Issue 7, July 2017, Pages 1566-1575
Kadir Emre BUGDAYCI1; Mustafa Numan OGUZ1; Fatma KARAKAS OGUZ1; Metin Koray ALBAY2 and Jale ONER3. (2016). Effects of live yeast culture addition into sucrose supplemented diet on fattening performance, some blood and histological parameters in Saanen male kids fed without forage. Ankara *Üniv Vet* Fak Derg, 63, 163-170,

Khafipour E, Li S; Plaizier JC and Krause DO. (2009). Rumen microbiome composition determined using two nutritional models of subacute ruminal acidosis. *Appl Environ Microbiol.* 2009 Nov; 75(22):7115-24.
Kowalik B.; Michałowski T.; Pająk J.J.; Taciak M. and Zalewska M. (2011). The effect of live yeast, Saccharomyces cerevisiae, and their metabolites on ciliate fauna, fibrolytic and amylolytic activity, carbohydrate digestion and fermentation in the rumen of goats. *Journal of Animal Feed Science* 20, 526-536
Lodemann, U. (2010). Effects of Probiotics on Intestinal Transport and Epithelial Barrier Function. pp. 303 et seq. in: Bioactive Foods in Promoting Health: Probiotics and Prebiotics. Academic Press, Waltham, USA.
Mountzouris, K. C.; Balaskas, C.; Xanthakos, I.; Tzivinikou, A. and Fegeros, K. (2009). Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with Salmonella enteritidis. *British Poultry Science*, 50(4): 467–478.
Mountzouris, K. C.; Tsirtsikos, P.; Kalamara, E.; Nitsch, S.; Schatzmayr, G. and Fegeros, K. (2007). Evaluation of the efficacy of a probiotic containing Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poultry Science*, 86(2): 309–317.
Niba, A.; Beal, J.; Kudi, A. and Brooks, P. (2009). Bacterial fermentation in the gastro-intestinal tract of non-ruminants: influence of fermented feeds and fermentable carbohydrates. *Tropical Animal Health and Production*, 41(7): 1393–1407
Nierenberg and Danielle, (2011). "Global meat production and consumption continue to rise." *Worldwatch Institute* (2011).
Nocek JE; Kautz WP; Leedle JA and Allman JG, (2002). Ruminal supplementation of direct-fed microbials on diurnal pH variation and in situ digestion in dairy cattle. *Journal of Diary Science* Feb; 85(2):429-33.
Peng Sun; Jia Qi Wang and Hai Tao Zhang. (2011). Effect of supplementation of Bacillus subtilis natto Na and N1 strains on rumen development in diary calves. *Animal feed Science and Technology*. 164, issues 3 -4, 20 March 2011, pages 154 – 160.
Qadis AQ; Goya S; Ikuta K; Yatsu M; Kimura A; Nakanishi S and Sato S. (2014). Effects of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of Holstein calves. *Journal of Veterinary Medicine Science* 2014 Jun; 76(6):877-85.
Roselli, M.; Finamore, A.; Britti, M.S.; Konstantinov, S.R.; Smidt, H.; de Vos, W.M. and Mengheri, E. (2007). The novel porcine Lactobacillus sobrius strain protects intestinal cells from enterotoxigenic Escherichia coli K88 infection and prevents membrane barrier damage. *Journal of Nutrition*, 137(12): 2709–2716.
Russell JB nad Wilson DB. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low PH. *Journal of Diary Science.* 1996 Aug; 79(8):1503-9.
Sadiek A and Boehm J. (2001). Influence of pronifer (R) as a probiotic on the rumen fluid and blood parameter of sheep feed different roughage concentrate based diet. Wien Tierarztl Monatsschr 88: 4:10
SAS (2009). Institute Inc, Cary, North Carolina, The United States. SAS users guide statistics.
Steele MA; Dionissopoulos L; AlZahal O; Doelman J. and McBride BW. (2012). Rumen epithelial adaptation to ruminal acidosis in lactating cattle involves the coordinated expression of insulin-like growth factor-binding proteins and a cholesterolgenic enzyme. *Journal of Diary Science* 2012; 95: 318–327. pmid:22192211 •

Stobo, I. J. F.; Roy, J. H. B. and Gaston, H. J. (1966). Rumen development in the calf. *British Journal of Nutrition*, 20, 189-215.

Timmerman HM, Mulder L; Everts H; van Espen DC; van der Wal E; Klaassen G; Rouwers SM; Hartemink R; Rombouts FM and Beynen AC. (2005). Health and growth of veal calves fed milk replacers with or without probiotics. *Journal of Diary Science* 2005 Jun; 88(6):2154-65.

Tripathi, M. K., and Karim, S. A. (2011). Effect of yeast cultures supplementation on live weight change, rumen fermentation, ciliate protozoa population, microbial hydrolytic enzymes status and slaughtering performance of growing lamb. *Livestock Science*, *135*(1), 17-25.

Vyas D; Uwizeye A; Mohammed R; Yang WZ; Walker ND. and Beauchemin KA. (2014). The effects of active dried and killed dried yeast on sub-acute ruminal acidosis, ruminal fermentation, and nutrient digestibility in beef heifers. *Journal of Animal Science.* 2014; 92: 724–732. pmid:24398831

Warner R. G.; Flatt W. P. and Loosli J. K. (1956). Dietary factors influencing the development of the ruminant stomach. *Agric. Food Chem.* 4 1956788792

Weinberg ZG; Muck RE and Weimer PJ, (2003). he survival of silage inoculant lactic acid bacteria in rumen fluid. *J Applied Microbiology 2003*; 94(6):1066-71.

Weinberg ZG; Muck RE; Weimer PJ; Chen Y and Gamburg M. (2004) Lactic acid bacteria used in inoculants for silage as probiotics for ruminants. *Applied Microbiology and Biotechnology*. Jul-Sep; 118(1-3):1-9.

Zaunmüller T; Eichert M; Richter H and Unden G (2006). "Variations in the energy metabolism of biotechnologically relevant heterofermentative lactic acid bacteria during growth on sugars and organic acids". *Applied Microbiology and Biotechnology*. 72 (3): 421

Zintan R.; Kuhla S. and Sanftleben P. (2005). Diet induced ruminal papillae development in neonatal calves not correlating with rumen butyrate. *Veterinary Medicine Czech* 2005; 50: 472-479.