The Effect of Bovine Lactoferrin and Probiotic on Performance and Health Status of Ghezel Lambs in Preweaning Phase

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ABSTRACT

Due to concern about antibacterial substances in animal nutrition; bioactive components such as lactoferrin and probiotic with health effects, can be used in some species that are more sensitive to pathogens. Thirty six suckling male Ghezel lambs in a completely randomized design employing a 2×3 factorial arrangement were used to study the effects of bovine lactoferrin (BLF) and probiotic on performance, blood and immune system parameters in the pre-weaning phase. Experimental treatments were as follow: 1) control (without BLF and probiotic), 2) 1 g/d probiotic, 3) 0.25 g/d BLF, 4) 0.25 g/d BLF and 1 g/d probiotic, 5) 0.5 g/d BLF, 6) 0.5 g/d BLF and 1 g/d probiotic. Final body weight, weight gain, feed intake and feed efficiency (FE) were significantly affected by the treatments (P<0.05). Diet supplementation with BLF plus probiotic improved performance parameters more than diets without probiotics; however there is no difference between BLF levels. No significant differences was found among health status indices except medicated days (P<0.05). Moreover, no differences were observed in erythrocyte, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Conversly hemoglobin, white blood cells (WBC), segmented neutrophil and lymphocyte percentag were significantly affected by the treatments (P<0.05). Additionally experimental treatments significantly changed plasma concentrations of Fe, non-esterified fatty acids (NEFA) and glucose (P<0.05). According to results of present experiment, it seems that BLF plus probiotic can have synergisc effect on performance and heath status of Ghezel lamb breed in preweaning phase.

KEY WORDS antibacterial properties, immune system, lactoferrin, probiotic, ruminant's neonate.

INTRODUCTION

High mortality rate of lamb (10 to 77%) around the world is one of the most significant traits which affects sheep farms income (Refshauge *et al.* 2016). Therefore, a great deal of effort should be put on the care of lambs before, during, and especially after birth, in order to reduce lamb mortality rate below 10 and 30% for single and twin, respectively. For this purpose, ruminant neonates that are hypogammaglobinemia, must be consuming colostrum as a source of immunoglobulin in the first few days of their life to obtain passive immunity (Turkson, 2003; Refshauge *et al.* 2016).

Passive immunity commonly recognized based on IgG level, however, recent studies showed that there are some neonate animals with low levels of IgG and high resistancy to diseases (Gokce *et al.* 2014). Moreover, Gokce *et al.* (2014) demonstrated that other significant components of passive immunity such as growth factors, cytokines, acute phase proteins, lactoferrin and some undefined factors could be effective on immune system. Nevertheless the bioactive proteins concentrations in colostrum and milk,

fall few hours after parturition, which happen more rapid in colostrum than milk (Sobczuk-Szul *et al.* 2013). Lactoferrin, a multifunctional iron-binding glycoprotein (MW 80000) of the serum transferrin gene family, can be found in the colostrum, milk, mucosal secretions (e.g. saliva, tears and bile), pancreatic and seminal fluids and specific granules of the polymorphonuclear leukocytes in mammals (Gokce *et al.* 2014; Kieckens *et al.* 2018; Wakabayashi, 2018). It's concentrations in ewe milk is 135 mg/L (Qian *et al.* 1995). Lactoferrin has been described to have several roles, such as a novel food supplement (Tetens, 2012), an antimicrobial (Kieckens, 2018), an immune system regulator (Miyauchi *et al.* 1998; Legrand, 2016), an inhibitor of both solid tumor growth and acceleratore in epithelial cell proliferation (Rejman *et al.* 1992; Bezault *et al.* 1994). Considering the fact that colostrum has higher concentration of LF than other fluids, it seems that LF should be an important factor in the host's defense against a wide range of bacteria in the early stages of life of infants (De Vrese and Schrezenmeir, 2002). Lactoferrin antimicrobial function is due to its ability to take up the Fe^{3+} ion, limiting use of this nutrient by bacteria at the infection site and its direct interaction with bacterial surfaces (González-Chávez *et al.* 2009). Lactoferrin is a Fe-binding protein, which could act as a Fe source for newborn calves (Nagasako *et al.* 1993). Additionally Fe-saturated Lf known as anemia preventive agent; because of its ability to increase hematocrit and hemoglobin concentration in pre-ruminants (Kume and Tanabe, 1996). Prgomet *et al.* (2007) reported that feeding BLF to calves could not raise hematocrit and hemoglobin concentration, as well as erythrocyte and white blood cell counts. Therefore, the effects of BLF on hematology in different studies are contradictory and related to iron status (Kume and Tanabe, 1996; Prgomet *et al.* 2007). Feeding BLF to calves improved feed intake (FI) and average daily gain (ADG) (Joslin *et al.* 2002; Robblee *et al.* 2003) and reduced fecal scores as an index of diarrhea (Connelly and Erickson, 2016).

Probiotics as a feed supplement consist of living microorganisms which can play several roles in animal rearing. Probiotics could assist to establishment of intestinal microbial population, act as a rival for harmful microbes (Cruywagen *et al.* 1996), a growth promoter, immune system enhancer and have protective effects against many diseases (Gibson and Fuller, 2000). Lema *et al.* (2001), Frizzo *et al.* (2010) and Vosooghi-Poostindoz *et al.* (2014) investigated that probiotics could increase dry matter intake (DMI) and body weight gain (BWG), improve feed conversion ratio (FCR) and prevent diarrhea with replacement beneficial microbial populations in the lamb intestine.

To our knowledge LF has not been fed to lambs especially for an extended period (>10 d). Since LF and probiotics individually have beneficial effect on livestock immune system, it was hypothesized that supplementation of lamb's diet with both BLF and probiotic would be more effective on performance, blood parameters and immune system in pre-weaning phase.

MATERIALS AND METHODS

Animal, experimental design and husbandry

Thirty six Ghezel suckling male lambs (3.9±0.65 kg body weight (BW)) from the Experimental Farm of the Agriculture Faculty at the Tabriz University (38˚ 05´N, 46˚ 28´W) of East Azerbaijan, Iran, were selected for the experiment from the 3rd day of age and housed in individual pens. Lambs were assigned randomly to one of the following 6 groups including: 1) control (without Bovine lactoferrin (BLF) and probiotic), 2) 1 g probiotic, 3) 0.25 g BLF, 4) 0.25 g BLF and 1 g probiotic, 5) 0.5 g BLF, 6) 0.5 g BLF and 1 g probiotic. Bovine lactoferrin (Shangqiu Kangmeida Bio-Technology Co. Ltd) and probiotic (PrimalacTM) were given orally every day (0900). Lambs in the control group received equal amounts of normal saline as placebo. Suckling lambs were fed fresh milk from ewes (Table 1) by nipple bottle three times per day (06:00, 14:00 and 22:00) up to 58th days. The starter diet (Table 2) that's formulated based on BW, were offered triple (06:00, 14:00 and 22:00) daily directly and water was available after 2nd week of experiment. Diet formulated to meet suckling lamb requirements according to NRC (2007).

Feed intakes and BW were recorded daily and weekly, respectively. Also, feed efficiency (FE) calculated by (weight gain per week) / (dry matter (DM) of milk and stater per week). Starter, milk and orts were sampled weekly, composited, and frozen at -20 ˚C. Forages and other feeds analysed according to the association of official analytical chemists (AOAC, 2005) method. The samples of diets were packaged and sent to the laboratory for analysis of dry matter, crude protein, crude fibre, ether extract, ash and neutral detergent fibre. Additionally, milk fat and protein content analysed 3 times during the experimental period. The Lacti-Check ultrasound milk analyser was used to measure the fat and protein contents.

Health status

The health status was evaluated based on the some clinical traits such as fecal score, rectal temperature and days medicated. In order to receive more signals from the health status, we observed some parameters as feeding behavior like as appetite, nasal and eye discharge, respiratory sounds and cough in along experiment.

Table 1 Ewes milk composition

Composition	Value
Total dry matter (%)	20.12
Crude protein (% of dry matter)	29.63
Fat (% of dry matter)	34.15
Metabolizable energy[1] (Mcal/kg)	5.58

[1] Based on sheep NRC (2007).

Table 2 Ingredient and chemical composition of starter

Ingredient	%
Alfalfa hay-M. bloom-fine chop	17.54
Soybean meal-finely ground	17.54
Barley grain-finely ground	29.24
Wheat bran-finely ground	30.86
Sodium bicarbonate	1.00
Mineral and vitamin premix[1]	1.75
Calcium carbonate	1.17
Salt	0.90
Chemical compounds	
Dry matter	90
Crude protein[2]	20.41
Crude fibre[2]	10.47
neutral detergent fibre[2]	32.96
Ether estract[2]	1.65
Ash[2]	8.38
Calcium[2]	0.83
Phosphorus[2]	0.67
Metabolizable energy (Mcal/kg)[3]	2.37

[1] Composition of minerals and vitamins premix (per kg feed): Zn: 4.9 mg; Mn: 4.05 mg; Cu: 0.45 mg; I: 0.075 mg; Se: 0.1 mg; vitamin A: 2500 IU; vitamin D: 400 IU and vitamin E: 2.5 IU.
[2] Based on AOAC (2005).
[3] Based on sheep NRC (2007).

Feeding behavior was studied by measuring the total time lambs spent eating each day. These activities were monitored and recorded for individual lambs at 5 minutes (5') intervals up to 2 hours after each feeding meal using a chronometer. Feces were scored 3 days in week on a scale of 1 through 5, with 1= firm pellets, 2= normal pellets, 3= soft pellets, 4= soft (no pellets) but not running, 5= soft and running according to Lema *et al*. (2001). Scores of 4 and 5 were considered to be diarrhea. Rectal temperatures (≤37.50 and 39.50≤) were determined in lambs that appeared languid, listless to eat and had diarrhea. Days medicated were recorded as each days that a lamb received drug. Feeding behavior was studied by measuring the total time lambs spent eating each day. These activities were monitored and recorded for individual lambs at 5 minutes (5') intervals up to 2 hours after each feeding meal using a chronometer.

Blood parameters

A quantity of 6 ml (tubes containing Na-heparin) and 3 mL (tubes containing ethylenediaminetetraacetic acid (EDTA)) blood were collected from jugular veins at 2 hours prior feeding for complete blood cell count (CBC) and at 3 hours post feeding for metabolites on days 1, 28 and 56 of expriment. Tubes were kept on crushed ice (0-4 ˚C) for 1 hour before centrifugation. Blood samples were centrifuged at 3000 rpm for 15 min. to separated plasma which was stored at -20 ˚C for later analysis.

Blood samples for CBC were analyzed by an automated cell counter machine (Diatron Abacus-Austria). Plasma Fe was determined by atomic absorption spectrophotometry after dilution with distilled water.

Glucose, cholesterol, non estrified fatty acid (NEFA), beta hydroxy butyric acid (BHBA), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) concentrations were determined by an automated biochemical analyzer (Biotecnica, Targa 3000, Rome, Italy) using commercial kits (Pars Azmoon Company, Tehran, Iran) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed as repeated measures data using the MIXED PROC model of SAS software 9.2 (SAS, 2004). The model included the fixed effects of bLF, probiotic, and their interaction.

The covariance structure that yielded the smallest Akaike's information criterion was used according to a description of Littell *et al.* (1996). Initial BW served as a covariate for growth and FI characteristics. Covariates were not used for statistical analysis of blood parameters and health status index. The effects of diets and times of the measured variables were considered as the fixed effect. The lambs' effects were considered random and the experimental data analyzed as a completely randomized design ANOVA. The GLM PROC model was also used when was necessary. Comparisons were conducted on weekly and preweaning, postweaning, and overall least-squares means.

RESULTS AND DISCUSSION

Performance

Performance parameters of the experimental lambs are shown in Table 3. Lambs had the same initial BW at the start of experiment, but final BW was significantly different among the treatments (P<0.05). Both bLF and probiotic improved final BW (P<0.05), however no improvement was found for their interactions. Weight gain, FI and FE were affected by the treatments (P<0.05). Supplementation of BLF to the diets increased DMI (P<0.05), though there were no difference between high and low levels of bLF. Both BLF and probiotic supplementation improved feed efficiency during the total experimental period (P<0.05), but added probiotic had no benefit on days 1 to 28 of experiment. The changes in FE during the trial periods were comparable with DMI fluctuation which had consistency with weight gain.

Health status

The results of Table 4 show that the use of BLF and probiotics did not have a significant effect on fecal scores, although there was an expected improvement. Rectal temperature showed a tendency to reduction by probiotic and BLF supplementation (P<0.1), but lactoferrin supplementation reduced medicated days of the lambs significantly (P<0.05), (Table 4). The results indicated that the changes in feces score and rectal temperature during the trial periods were related to medicated days fluctuation. The results in this study indicate that there was unusual difference in health status including parameters of nasal and eye discharge, respiratory sounds, cough and even nutritional behavior such as appetite.

Hematology

Hematological values are shown in the Table 5. No differences were observed in red blood cell (RBC), MCV, MCH, MCHC and erythrocytes (i.e. eosinophils, basophils and monocytes) among the treatments, however the hemo-

globin concentration increased by bLF supplementation (P<0.05). Experimental treatments had no effect on the hematocrit concentration. Bovine LF supplementation negatively affected white blood cells (WBC) concentration (P<0.05).

Meanwhile, a reduced percentage of segmented neutrophil and increased percentage of lymphocyte were observed when BLF fed to the lambs (P<0.05). By the way BLF supplementation increased plasma iron concentration and higher level of BLF resulted to higher concentration of plasma iron (P<0.05, Table 6). Similarly glucose concentration positively related to the BLF levels (P<0.05), whereas bLF supplementation had a convers effect on NEFA level (P<0.05, Table 6). Cholesterol, beta-hydroxybutyric acid (BHBA), alanine transaminase (ALT), aspartate transaminase (AST) and gamma-glutamyl transferase (GGT) did not affected by the experimental treatments (Table 6).

To our knowledge, there is no study with the use bLF plus probiotic in ruminant neonates. Joslin *et al.* (2002) fed lactoferin to the calves in the preweaning phase and found that it was effective on final BW, weight gain and feed effiency. They hypothesized that healthier calves can consume more feed and higher DMI can improve weight gain and other performance properties. Aditionally, Prgomet *et al.* (2007) fed lactoferin to the growing calves and observed enlarged peyer's patches size and modulated gastrointestinal tract (GIT) morphology which have ability to improve animal performance. Yeoman and White (2014) fed probiotic to the ruminant and reported an improvement in the immune function and GIT morphology. Moreover, growth factor activity which is considered as one of the most important peroperties of BLF (Zhang *et al.* 2001), led to increased intestinal growth and nutrient absorption (Robblee *et al.* 2003), which can improve ADG and FE. We observed positive effect of either lactoferin or probiotic supplementation on weight gain of lambs (Table 3). Based on the hypothesis, we were expected more ADG and improved FE in our study. Interestingly, higher weight gain was observed when probiotic and lactoferin were used together (Figure 1). It confirms our hypothesis about synergistic effects of lactoferrin and probitic on lambs performance. Joslin *et al.* (2002) reported that relationship between ADG and FE with medicated days is significantly reverse. Diarrhea as one of the main causes of mortality in neonate lambs, usually resulted from E. coli, rotavirus and other enterotoxigenic microorganisms (Tzipori *et al.* 1981). Superti *et al.* (1997) fed BLF to the calves and reported a reduction in the establishment of *E. coli* in the gut, which led to reduced diarrhea. Lower incidence of diarrhea and total number of medicated days followed a similar response pattern in the study of (Robblee *et al.* 2003).

Table 3 Effect of probiotic and bovine lactoferrin (BLF) supplementation on performance parameters of experimental lambs

Measurement	Without probiotic[1]			With probiotic			SEM	P-value		
	0	0.25	0.50	0	0.25	0.50		Probiotic	BLF	Probiotic × BLF
Initial body weight (kg)	3.96	3.98	3.98	3.95	3.95	4.05	0.15	NS	NS	NS
Final body weight (kg)	11.89[b]	13.73[a]	13.86[a]	12.24[b]	14.66[a]	14.75[a]	0.37	*	**	NS
Weight gain (kg/day)										
3-30 days	0.173[c]	0.181[bc]	0.186[ac]	0.151[b]	0.215[a]	0.216[a]	0.012	NS	**	†
31-58 days	0.109[c]	0.166[a]	0.166[a]	0.144[b]	0.167[a]	0.167[a]	0.010	*	**	*
3-58 days	0.141[b]	0.174[a]	0.176[a]	0.148[b]	0.191[a]	0.191[a]	0.010	*	**	NS
Feed intake (kg/day)										
3-30 days	0.235[b]	0.253[b]	0.259[a]	0.249[b]	0.266[a]	0.270[a]	0.010	†	*	NS
31-58 days	0.435[c]	0.509[c]	0.540[b]	0.458[d]	0.552[ab]	0.565[a]	0.011	**	**	NS
3-58 days	0.335[d]	0.381[b]	0.400[ab]	0.351[c]	0.409[a]	0.418[a]	0.010	*	**	NS
Feed efficiency										
3-30 days	0.97[b]	0.94[b]	0.97[b]	0.95[b]	1.10[a]	1.11[a]	0.04	*	NS	NS
31-58 days	0.220[c]	0.294[a]	0.297[a]	0.244[bc]	0.287[a]	0.284[b]	0.02	NS	**	NS
3-58 days	0.597[b]	0.621[b]	0.630[b]	0.600[b]	0.693[a]	0.697[a]	0.02	*	*	NS

[1] 0, 0.25 and 0.50 g/day/head of BLF.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
† P < 0.10; * P < 0.05 and ** P < 0.001.
SEM: standard error of the means.
NS: non significant.

Table 4 Effect of probiotic and bovine lactoferrin (BLF) supplementation on health status indices of the experimental lambs

Measurement	Whithout probiotic[1]			Whith probiotic			SEM	P-value		
	0	0.25	0.50	0	0.25	0.50		Probiotic	BLF	Probiotic × BLF
Feces score[2]	2.62	2.48	2.34	2.55	2.51	2.34	0.30	NS	NS	NS
Rectal temperature	39.30	39.11	39.67	39.10	38.81	38.68	0.25	NS	NS	NS
Medicated days	2.21[a]	1.76[b]	1.24[c]	1.85[a]	1.65[b]	1.05[c]	0.15	*	**	†

[1] 0, 0.25 and 0.50 g/day/head of BLF.
[2] Feces score; 1: separate hard lumps and 4: liquid consistency without solid space; diarrhea.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
† P < 0.10; * P < 0.05 and ** P < 0.001.
SEM: standard error of the means.
NS: non significant.

Table 5 Effect of probiotic and bovine lactoferrin (BLF) supplementation on complete blood cells and plasma Fe concentration of the experimental lambs

Measurement	Without probiotic			With probiotic			SEM	P-value		
	0[1]	0.25	0.50	0	0.25	0.50		Probiotic	BLF	Probiotic × BLF
RBC (10^6 cells/mL)	13.95	14.14	13.94	14.21	14.15	13.72	0.27	NS	NS	NS
HGB (g/dL)	11.23[c]	11.80[b]	12.19[ab]	11.26[c]	11.92[b]	12.44[a]	0.23	NS	**	NS
HCT (%)	35.56	35.60	35.93	35.23	35.94	36.18	0.42	NS	NS	NS
MCV (fL)	25.68	25.27	25.88	24.94	25.47	26.65	0.58	NS	NS	NS
MCH (pg)	8.34	7.92	8.06	8.05	8.08	8.28	0.30	NS	NS	NS
MCHC (%)	32.48	31.46	31.34	32.18	31.79	31.07	0.98	NS	NS	NS
WBC (10^3 cells/mL)	9.25[a]	8.23[b]	7.81[b]	8.99[a]	7.88[b]	7.51[c]	0.24	NS	**	NS
Eosinophils (%)	1.00	0.83	1.00	1.11	1.22	1.00	0.18	NS	NS	NS
Basophils (%)	1.67	1.50	1.39	1.17	1.61	1.17	0.19	NS	NS	NS
Monocytes (%)	5.00	2.66	4.00	3.33	4.17	3.83	0.55	NS	NS	NS
Neutrophils (%)	44.94[a]	41.38[b]	40.38[b]	43.38	40.94[b]	40.27[b]	0.79	NS	**	NS
Lymphocytes (%)	48.50[b]	52.50[ab]	53.17[a]	50.94[b]	52.61[ab]	53.94[a]	0.84	NS	**	NS

[1] 0, 0.25 and 0.50 g/day/head of BLF.
HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration and WBC: white blood cells.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
** P < 0.001.
SEM: standard error of the means.
NS: non significant.

Table 6 Effect of probiotic and bovine lactoferrin (BLF) supplementation on some plasma parameters of the experimental lambs

Measurement	Without probiotic[1]			With probiotic			SEM	P-value		
	0	0.25	0.50	0	0.25	0.50		Probiotic	BLF	Probiotic × BLF
Fe (mg/dL)	154.56[c]	158.06[bc]	162.56[ab]	156.72[c]	164.06[ab]	167.61[a]	3.26	NS	*	NS
Cholesterol (mg/dL)	61.39[a]	58.66[c]	57.50[cd]	59.78[b]	58.61[c]	56.33[a]	0.77	NS	**	NS
Glucose (mmol/L)	79.94[c]	81.61[b]	81.66[b]	80.33[bc]	81.16[b]	83.72[a]	0.92	NS	*	NS
NEFA (mmol/L)	0.63[ab]	0.65[ab]	0.60[bc]	0.68[a]	0.61[bc]	0.55[c]	0.03	NS	*	NS
BHBA (mmol/L)	0.36	0.37	0.39	0.35	0.38	0.36	0.02	NS	NS	NS
GGT (U/g Hb)	20.11	19.20	19.06	19.39	18.89	19.14	0.47	NS	NS	NS
ALT (U/L)	10.84	10.54	10.50	10.02	10.20	10.38	0.33	NS	NS	NS
AST (U/L)	37.00	36.06	36.00	35.89	35.83	35.89	0.80	NS	NS	NS

[1] 0, 0.25 and 0.50 g/day/head of BLF.
NEFA: non estrified fatty acid; BHBA: beta hydroxy butyric acid; GGT: gamma-glutamyl transferase; ALT: alanine aminotransferase and AST: aspartate aminotransferase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
* P < 0.05 and ** P < 0.001.
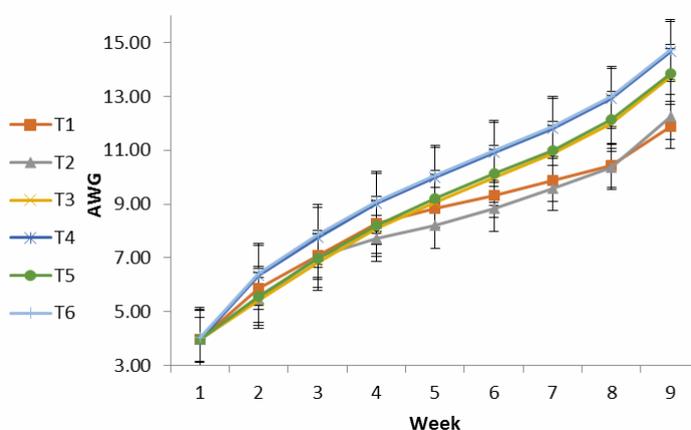SEM: standard error of the means.
NS: non significant.

Figure 1 Average daily gain (AWG) of lambs in treatment groups in along experiment
T1) control (without BLF and probiotic); T2) 1 g probiotic daily; T3) 0.25 g BLF daily; T4) 1 g probiotic and 0.25 g BLF daily; T5) 0.5 g BLF daily and T6) 1 g probiotic and 0.5 g BLF daily

Likewise, De Bortoli *et al*. (2007) and Yekta *et al*. (2011) demonstrated that effective microbial population of probiotic and antibacterial function of BLF, could remove microbial agents which causing diarrhea and help to reach normal feces score along the initial period of life. No sever diarrhea was observed in the present study. It seems that experimental treatments have been effective in the control of GI pathogen agents. Feeding BLF and probiotic to the lambs in the present study improved fecal score of lambs (however nonsignificant) which was in accordanc to their rectal temperature, as well as medicated days reduction (Table 4). The lambs received BLF and probiotic tended to have lower rectal temperature (P<0.10). Moreover these lambs had lower medicated days which was significant for bLF administration (P<0.05). A higher rectal temperature in the control group was in accordance with the results of other researchers (Muri *et al*. 2005; Dwyer and Morgan, 2006) and may be understood as an indication of elevated

metabolic activity and growth of the lambs. Similarley, Dionysius *et al*. (1993) and Teraguchi *et al*. (1994) reported a decreased medicated days with BLF supplementation. As well as, they illustrated that BLF has antibacterial properteis which are effective against pathogenic bacteria such as *E. coli*. Decrease in pathogenic microorganism in digestive tract can cause to reduced diarrhea rate and intestinal damages (Jang *et al*. 2009).

Kume and Tanabe (1993) reported that low level of colostrum Fe could be inadequate for maintain normal levels of blood hematocrit and hemoglobin in newborn ruminants, however hematological values in the present study were in normal range and in accordance with the metaanalysis study of Jones and Allison (2007) (Table 5). Bovine lactoferin supplementation, improved hemoglobin (HGB) concentration and higher bLF level resulted to higher HGB concentration (P<0.05, Table 5). This manner was observed for plasma Fe concentration as well (P<0.05,

Table 5), however no significant improvement was happened in the hematocrit concentration of lambs in the present study. Rosa *et al.* (2017) reported that oral addministration of BLF resulted to higher HGB and hematocrit in human; however more improvemnt was observed for HGB after 30 days consuming of BLF. Similarly Tsuji *et al.* (1990) and Shin *et al.* (1998) revealed that BLf as a source of Fe can elevate hematocrit and hemoglobin in ruminant neonates which are consuming colostrum with low levels of Fe. Several studies in different species such as human (Levay and Viljoen, 1995), mice (Fransson *et al.* 1983), pigs (Fransson *et al.* 1983) and calves (Kume and Tanabe, 1996) demonstrated that BLF would improve Fe absorpion via several mechanisms such as the elevating enterocytes ability to extract iron from lactoferrin, the more lactoferrin uptake by enterocytes (Levay and Viljoen, 1995), the transport of iron across the intestinal brush border by transporting lactoferrin and the accumulation of iron from lactoferrin in brush border membrane vesicles (Davidson and Lonnerdal, 1989). There are few information about the effect of probiotic on plasma Fe levels. Antunović *et al.* (2005) fed probiotic to the lambs and reported an increase in the plasma Fe concentration. We observed an increase in the plasma Fe concentration when probiotic was fed to the lambs as well, however this increase was not significant (Table 5).

Lower level of hematological factors such as red blood cells (RBC), HGB and hematocrit (HCT) could lead to higher mortality rates due to disruption of iron transmissions (Nagasako *et al.* 1993) and debilitation of immune system (Ekiz *et al.* 2005). Reznikov (2014) illuministrated that BLF can significantly reduce WBC in piglets. It can be due to reduced levels of pathogens in the digestive tract by lactoferin administration (Weinberg and Des, 2007). Similarly reduced WBC concentration in the present study can be a result of lower pathogens in the GIT of experimental lambs. Among the WBCs only neutrophils reduced with BLF administration and conversely lymphocytes were increased (P<0.05, Table 5). These results can be supported by the findings of Legrand (2016), who suggested that lactoferrin is a component of secondary neutrophil granules. Analougously, (Debbabi *et al.* 1998) demonstrated that neutrophils can suppress invading pathogens in the body by oxidant reaction, which will lastly lead to a reduction in segmented neutrophil percentage. It seems that BLF by providing a safe environment in GIT resulted to a reduction in the WBC compared to control group and higher level of lactoferin has been more efficient.

Lactoferin administration to the lambs increased blood glucose and conversely reduced NEFA level (P<0.05) (Table 6). Muri *et al.* (2005) and Cowles *et al.* (2006) reported an increased level of glucose in neonate calves when lactoferrin was fed. Cowles *et al.* (2006) explained that more absorption of glucose could be caused by BLF roles in increase and promotion of intestinal epithelial size and function. Moreover it seems that starter consumption by the lambs from the 3th week of experiment could be effective on volatile fatty acids (VFA) production in the rumen of lambs which can led to gluconeogenesis and higher blood glucose (Bergman, 1990). Both of glucose and NEFA are considered as energy balance indicators of the body, therefore it seems that BLF has improved energy balance in the lambs and higher glucose and lower NEFA levels in the plasma of experimental lambs confirms this suggestion. Our findings in NEFA concentration were similar to Muri *et al.* (2005), however we observed significantly effect of BLF and BLF plus probiotic on NEFA concentration as an energy balance indicator, that could therefrom long term usage of treatments. Additionally Chiofalo *et al.* (2004) pointed out that probiotic lead to a better metabolic status and a positive energetic balance in goat kids. Probiotics, through rumen pH control, provide suitable environmental condition for increasing microbial activity. With increasing microbial population resulting in increased VFA and other fermentation indexes, the level of energy access will increase (Thomas, 2017; Arowolo and He, 2018). In this line, this study showed that probiotics increase the energy available in the rumen.

Generally, the activity of ALT, AST and GGT enzymes is considered as an indicator of stress-induced tissue damage and infection (Cristaldi *et al.* 2005). These enzymes are naturally intercellular, but released into the blood with damage to the cells and are indicators of tissue damage (Davis *et al.* 2008). A samall and nonsignificant reduction were found in GGT and AST concentration of hepatic enzymes due to BLF and probiotic usage which was in line with Hillal *et al.* (2011) and may be a result of effectiveness of these additives. Although it cannot definitely be stated that both of BLF and probiotic are effective on these enzymes, however our results shown slightand nonsignificant reduction in these.

CONCLUSION

In conclusion, bLF and probiotic supplementation improved young lamb's performance. Although both of bLF and probiotic increased DMI and FE, our results demonstrated that these additives could have a synergistic effect with each other. Overall, bLF and probiotic separately or simultaneously could positively be effective on performance and health status. Further research is necessary to determine more interactions and optimum amounts of bLF and probiotic to add on starter and feed in different ages of lambs.

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