Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

Effects of probiotic supplementation on milk production, blood metabolite profile and enzyme activities of ewes during lactation

Farokh Kafilzadeh, Shahab Payandeh, Pilar Gómez-Cortés, Darab Ghadimi, Achille Schiavone & Andrés L. Martínez Marín

To cite this article: Farokh Kafilzadeh, Shahab Payandeh, Pilar Gómez-Cortés, Darab Ghadimi, Achille Schiavone & Andrés L. Martínez Marín (2019) Effects of probiotic supplementation on milk production, blood metabolite profile and enzyme activities of ewes during lactation, Italian Journal of Animal Science, 18:1, 134-139, DOI: 10.1080/1828051X.2018.1496040

To link to this article: https://doi.org/10.1080/1828051X.2018.1496040

Published online: 11 Sep 2018.

ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 134–139
https://doi.org/10.1080/1828051X.2018.1496040

Taylor & Francis
Taylor & Francis Group

PAPER

Effects of probiotic supplementation on milk production, blood metabolite profile and enzyme activities of ewes during lactation

Farokh Kafilzadeh[a], Shahab Payandeh[a], Pilar Gómez-Cortés[b], Darab Ghadimi[c], Achille Schiavone[d] and Andrés L. Martínez Marín[e]

[a]Department of Animal Science, Razi University, Kermanshah, Iran; [b]Departamento de Bioactividad y Análisis de Alimentos, Instituto de Investigación en Ciencias de la Alimentación (CSIC-UAM), Madrid, Spain; [c]Institut für Mikrobiologie und Biotechnologie, Max Rubner-Institut, Kiel, Germany; [d]Dipartimento di Scienze Veterinarie, Universitàdegli Studi di Torino, Grugliasco, Italy; [e]Departamento de Producción Animal, Universidad de Córdoba, Córdoba, Spain

ABSTRACT

There is scarce information regarding the effects of probiotics in the diet of lactating ewes on milk yield and composition as well as on metabolite concentrations and enzyme activities in blood. Sixteen Sanjabi ewes, kept under the same feeding and management practices, were divided in two equal groups. The ewes in the first group were fed a diet without probiotics, while the ewes of the second group were fed the same diet supplemented with two grams of commercial probiotics (PrimaLac® 454 Feed grade, containing *Lactobacillus acidophilus*, 2.5×10^7 CFU/g; *Lactobacillus casei*, 2.5×10^7 CFU/g; *Bifidobacterium thermophilum*, 2.5×10^7 CFU/g; *Enterococcus faecium*, 2.5×10^7 CFU/g). Milk yield measurements, milk composition and blood plasma analyses were carried out at the fourth, eighth and twelfth week of lactation. Probiotics had no effects ($p>.05$) on any of the examined variables during the first two-thirds of lactation. In the last third of lactation, probiotic supplemented ewes showed an increase ($p<.05$) of aspartate aminotransferase activity in blood plasma (209 versus 98 U/L) as well as higher ($p<.05$) yields of milk (503 versus 312 g/d), fat (34.8 versus 22.2 g/d), protein (33.1 versus 20.3 g/d) and lactose (22.4 versus 13.9). In conclusion, probiotics barely affected blood plasma metabolite contents and enzyme activities, but positive effects on milk yield and its components were observed during the last third of lactation. Further research would be needed to determine whether the supplementation of ewe diet with probiotics is advisable for sustainable livestock farming systems.

ARTICLE HISTORY
Received 28 August 2017
Revised 4 May 2018
Accepted 22 June 2018

KEYWORDS
Ewes; milk yield; milk composition; blood biochemical parameters

Introduction

Probiotics are single or mixed cultures of live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO 2001). Due to general public and scientific concern about residues in foods from animal origin, probiotics have become an appealing alternative to chemical and hormonal growth promoters (Gaggia et al. 2010). Although exact mechanisms of action have not been fully elucidated, probiotic supplementation effects seem to be related to both a more favourable environment and a more beneficial microbial population within the gastrointestinal tract, which in turn may improve digestive function and immune system (Krehbiel et al. 2003; McAllister et al. 2011). Therefore, probiotic supplementation could be a valuable biotechnological tool to contribute to sustainable ruminant production (Pulina et al. 2017).

Several types of microorganisms can been included in probiotic supplements for ruminants, being *Lactobacillus*, *Enterococcus*, *Bacillus* and *Saccharomyces* the most widely used (Gaggia et al. 2010). Compared with dairy cows, few studies have dealt with the effects of probiotics on milk production and blood biochemical parameters of dairy ewes, and the most studied one has been *Saccharomyces cerevisiae* (Mašek et al. 2008a, 2008b; Milewski and Sobiech 2009; Macedo et al. 2012; Mousa et al. 2012). Much less is known about the effects of bacterial probiotics (Lubbadeh et al. 1999; Kritas et al. 2006), and the information about their effects during lactation is scant. It has been observed in wethers that, under induced subacute rumen acidosis, bacterial probiotics cause some changes in the microbial populations and

CONTACT Prof. Andrés L. Martínez Marín pa1martm@uco.es Departamento de Producción Animal, Universidad de Córdoba, Córdoba, Spain

fermentation patterns (Lettat et al. 2012). If the incorporation of probiotics to the diet of clinically healthy dairy ewes affected the ruminal ecosystem in any way, available substrates for body metabolism could be altered and milk yield and composition might also change. Therefore, a simultaneous assessment of milk production and blood biochemical parameters may lead to a better understanding of ewe's metabolism changes, if any, after probiotic supplementation. Moreover, some studies have shown that *Lactobacilli* supplementation to ewes, lambs and goat kids may alter blood cholesterol levels, which has been related either to cholesterol deconjugation in the intestines or to an improvement in the intestinal absorption of nutrients (Lubbadeh et al. 1999; Chiofalo et al. 2004; Vosooghi-Poostindoz et al. 2014).

Sanjabi is a fat-tailed sheep breed that is widely kept in the province of Kermanshah, located in the western region of Iran. Milk of Sanjabi ewes, besides used for lamb rearing, is consumed fresh or as cheese, yoghurt and other dairy products by the nomadic shepherds.

Payandeh et al. (2017) reported the effects of bacterial probiotics on milk fatty acid composition of Sanjabi ewes during lactation. The aim of the present work was to report the effects observed in the same experiment on milk yield, milk composition and blood plasma metabolite profile and enzyme activities.

Material and methods

This study was carried out in the Animal Research Station of Razi University Agriculture College (Kermanshah, Iran). The research protocol was approved by the Animal Care and Use Committee of Razi University. Sixteen Sanjabi ewes, balanced in parity (~third lambing), were selected 1 month before lambing from a synchronised pure flock. All ewes carried one foetus and were clinically healthy and free from internal and external parasites. In order to eliminate the confounding influences of changes in pasture composition, the animals were kept in individual straw-bedded pens provided with individual water and feeding troughs, under the same management practices, and were fed the same diet, without grazing at all. After lambing, the animals were divided in two equal groups ($n = 8$) of similar body weight (59.9 ± 6.08 kg and 58.7 ± 6.41 kg, p=.82). Each group was allocated to one of the following treatments: diet without probiotics (CON) or diet supplemented with two grams of PrimaLac® probiotics (PRO). PrimaLac® 454 Feed grade (Star-Labs, Clarksdale, M.O., U.S.A.) is a multi-strain commercial preparation in powder form (1×10^8 CFU/g) that consists of *Lactobacillus acidophilus* (2.5×10^7 CFU/g), *Lactobacillus casei* (2.5×10^7 CFU/g), *Bifidobacterium thermophilum* (2.5×10^7 CFU/g) and *Enterococcus faecium* (2.5×10^7 CFU/g). PrimaLac® 454 was chosen because its wide variety of bacterial strains, including Lactobacillus *spp*., and its availability in the local market.

Diet was based on alfalfa hay (60% DM) and a concentrate mix (40% DM) consisting of maize grain (26% DM), soybean meal (9% DM), wheat bran (3.5% DM), and minerals and vitamins (1.5% DM). The ration was fed in two equal amounts at approximately 07:30 and 17:30 h. Primalac® supplement was thoroughly mixed everyday with about 10 g of concentrate feed and fed individually to ewes before morning feeding. Daily feed amounts were calculated according to the recommendations of National Research Council (1985). Thus, 2.25 and 1.66 kg/d (as fed basis) were offered from parturition until week six and from week seven onwards. Water was provided *ad libitum* for the entire experimental period.

The ewes and their lambs were weighed at the beginning and the end of the experimental period. Milk yield measurements, as well as milk and blood sampling were carried out at the fourth, eighth and twelfth week of lactation (WOL), representing the three thirds of lactation. Milk yield was individually determined throughout 24-h periods. Lambs were separated from their dams at 16:30 h on the day before each test day and bottle-fed. On the test day, ewes were milked twice by hand at 06:30 and 16:30 h, and milk from each ewe was individually sampled at each milking. The morning samples were kept refrigerated at 4 °C. Then, they were mixed with the corresponding evening samples, and the resulting individual subsamples were immediately taken to the laboratory for analysis. Milk fat, protein and lactose contents were determined for each animal using a Milko-Scan (Foss Electric, Hillerød, Denmark). For measuring blood plasma parameters, individual jugular blood samples were collected in heparinised tubes before morning and afternoon feedings. Plasma was separated by centrifugation (750*g* for 15 min at 4 °C) and preserved at −80 °C until analysis. Commercial kits (ELITech France and Pars Azmoon Co., Tehran, Iran) and a Hitachi 902 Chemistry Analyzer (Hitachi, Tokyo, Japan) were used to determine (in parenthesis catalogue numbers): Glucose (117500), urea (129400), creatinine (109400), uric acid (135400), total cholesterol (CHSL-0707), high density lipoprotein (HDL; HDLL-0390), low density lipoprotein (LDL; LDLL-0380) and triglyceride concentrations (132500), as well as aspartate aminotransferase (AST; 118400), alanine aminotransferase (ALT; 119400), alkaline phosphatase (ALP; 102400) and lactate dehydrogenase (LDH; 122050) activities. For each

Table 1. Effects of supplementing ewes with probiotics on milk yield and composition.

		Week of lactation (WOL)				Probability		
Parameter	Treatment (T)	4	8	12	SEM	T	WOL	T × WOL
Yield, g/d	CON	453^{A}	$366^{A,B}$	$312^{B,b}$	20.1	0.14	0.22	<0.01
	PRO	448	449	503^{a}				
FPCM,[1] g/d	CON	374	350	348^{b}	21.2	0.12	<0.05	<0.01
	PRO	347^{C}	453^{B}	$550^{A,a}$				
Fat								
%	CON	4.00^{B}	5.43^{B}	7.39^{A}	0.330	0.98	<0.001	0.25
	PRO	3.25^{B}	6.22^{A}	7.40^{A}				
g/d	CON	17.8	19.2	22.2^{b}	1.61	0.16	<0.001	<0.01
	PRO	14.2^{C}	26.6^{B}	$34.8^{A,a}$				
Protein								
%	CON	$6.59^{B,b}$	7.03^{A}	6.85^{A}	0.061	0.49	0.30	<0.01
	PRO	7.09^{a}	6.90	6.87				
g/d	CON	29.9^{A}	$24.9^{A,B}$	$20.3^{B,b}$	1.28	0.10	0.18	<0.05
	PRO	30.8	29.9	33.1^{a}				
Lactose								
%	CON	$4.62^{A,b}$	4.63^{A}	4.58^{B}	0.007	0.17	<0.001	<0.01
	PRO	$4.67^{A,a}$	4.61^{A}	4.58^{B}				
g/d	CON	20.3^{A}	$16.4^{A,B}$	$13.9^{B,b}$	0.90	0.13	0.15	<0.01
	PRO	20.3	20.1	22.4^{a}				

[1]Fat and protein corrected milk according to Pulina and Nudda (2004).
[A,B,C]For each parameter, within each treatment, least squares means without a common superscript are significantly different ($p<.05$) between weeks of lactation.
[a,b]For each parameter, within each week of lactation, least squares means without a common superscript are significantly different ($p<.05$) between treatments.
SEM: standard error of the mean; CON: diet without probiotics; PRO: diet supplemented with two grams of PrimaLac® probiotics.

parameter, the values of the morning and afternoon sampling times within each animal were averaged.

SAS UE 3.5 (SAS Institute Inc., Cary, NC) was used to perform the statistical analyses. Prior to carrying out the experiment, a power analysis was conducted utilising the data generated in the study of Payandeh et al. (2016). All variables to be studied were used to calculate power. An estimate of 1.5 standard deviations was used for power calculation. It was determined that a sample size of eight animals per treatment would be enough to give 80% power (at a significance level of .05) to detect differences between treatments. Data of initial and final BW of ewes and average daily gain of lambs were analysed with the ANOVA procedure, using the treatment as fixed effect. A repeated measurements analysis of milk yield, milk component contents and yields, and blood parameters data was carried out with the MIXED procedure. The statistical model included the fixed effects of treatment, WOL, and their interaction, and the random effect of ewe within treatment, assuming a compound symmetry structure on the basis of Schwarz's Bayesian information model fit criteria. When statistically significant interactions were found, orthogonal contrast tests were used to test for differences within the levels of the interaction. Statistical significance was declared at $p<.05$.

Results

Visual observation of the troughs revealed that feed offered daily was almost completely eaten from day to day. Ewes' body weight at the end of the experimental period did not differ ($p>.05$) between treatments (53.9 ± 7.19 kg and 53.5 ± 4.92 kg in the CON and PRO treatments, respectively), with an average body weight change of −5.6 kg. Average daily gain of lambs did not reach statistical significance (0.103 ± 0.035 kg/d and 0.124 ± 0.015 in the CON and PRO treatments, respectively; $p=.13$). No differences ($p>.05$) were observed in average milk yield and milk fat, protein and lactose contents (Table 1). However, except for fat content, several interactions were found between treatment and WOL. In the CON treatment, milk yield showed a decreasing linear trend, with a ∼20% drop ($p<.001$) from the eighth WOL onwards, while no changes ($p>.05$) were observed in the PRO treatment between measurement times. Thus, at the twelfth WOL milk yield was 61% higher ($p<.05$) in the probiotic supplemented ewes. Milk protein content was higher ($p<.05$) in the PRO treatment at the fourth WOL and remained stable thereafter ($p>.05$), while an increase ($p<.05$) was observed between the fourth and eighth WOL, without further changes ($p>.05$), in the CON treatment. On the other hand, the PRO treatment showed a higher milk lactose content at the fourth WOL, but no differences ($p>.05$) between treatments were observed thereafter. At the twelfth WOL, the yields of milk components and the fat and protein corrected milk (FPCM) levels were higher ($p<.05$) in the PRO treatment due to the higher milk yield and the absence of differences ($p>.05$) in the fat, protein and lactose contents between treatments at that time.

Table 2. Effects of supplementing ewes with probiotics on blood plasma metabolite concentration (mg/dL) and enzyme activities (U/L).

		Week of lactation (WOL)				Probability		
Parameter	Treatment (T)	4	8	12	SEM	T	WOL	T × WOL
Glucose	CON	$60.7^{A,B}$	65.6^{A}	59.2^{B}	1.15	0.81	<0.001	0.95
	PRO	61.5^{B}	67.0^{A}	59.6^{B}				
Urea	CON	23.1^{B}	36.5^{A}	27.1^{B}	1.36	0.93	<0.001	0.09
	PRO	18.8^{C}	36.5^{A}	30.9^{B}				
Creatinine	CON	0.59^{B}	0.77^{A}	0.80^{A}	0.023	0.63	<0.001	0.43
	PRO	0.58^{B}	0.81^{A}	0.84^{A}				
Uric acid	CON	0.15^{B}	0.14^{B}	0.18^{A}	0.006	0.93	<0.05	0.10
	PRO	0.13	0.16	0.16				
Cholesterol	CON	56.8^{B}	63.8^{A}	64.4^{A}	1.65	0.88	<0.001	0.27
	PRO	54.6^{B}	65.2^{A}	68.3^{A}				
HDL	CON	36.2^{B}	39.9^{A}	$37.7^{A,B}$	1.09	0.78	<0.01	0.26
	PRO	35.2^{B}	41.2^{A}	40.5^{A}				
LDL	CON	14.7^{B}	19.3^{A}	20.4^{A}	0.72	0.96	<0.001	0.62
	PRO	13.8^{B}	19.7^{A}	21.1^{A}				
Triglycerides	CON	25.8^{A}	25.6^{A}	17.3^{B}	0.82	0.35	<0.001	0.81
	PRO	23.8^{A}	24.7^{A}	16.3^{B}				
AST	CON	109.0	99.8	98.0^{b}	9.44	0.06	<0.01	<0.01
	PRO	94.0^{B}	105^{B}	$209.0^{A,a}$				
ALT	CON	14.7	16.3	13.2	0.75	0.31	0.07	0.91
	PRO	16.8	18.5	16.2				
ALP	CON	221^{B}	211^{B}	261^{A}	15.1	0.43	<0.01	0.61
	PRO	166^{B}	171^{B}	234^{A}				
LDH	CON	533	539	516	16.6	0.78	<0.01	0.48
	PRO	524^{A}	524^{A}	488^{B}				

[A,B,C]For each parameter, within each treatment, least squares means without a common superscript are significantly different ($p<.05$) between weeks of lactation.

[a,b]For each parameter, within each week of lactation, least squares means without a common superscript are significantly different ($p<.05$) between treatments.

HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; SEM: standard error of the mean; CON: diet without probiotics; PRO: diet supplemented with two grams of PrimaLac® probiotics.

Probiotic supplementation did not have any effects ($p>.05$) on average metabolite contents and enzyme activities in blood plasma (Table 2). Except for uric acid, the pattern of change in blood plasma metabolite contents during lactation was similar between treatments. In both treatments, blood plasma contents of cholesterol, HDL and LDL were higher ($p<.05$) at the eighth and twelfth WOL than at fourth WOL, while triglycerides contents showed the opposite trend. Blood plasma activity of AST did not show any trend ($p>.05$) in the CON treatment during lactation, while it showed an interaction between treatment and WOL and markedly increased ($p<.05$) between the eighth and twelfth WOL (~100%) in the PRO treatment, reaching a value that was higher ($p<.05$) than in the CON treatment. Activity of ALP in both treatments and that of LDH in the PRO treatment reached values at the twelfth WOL that were different ($p<.05$) to those observed at the fourth and eighth WOL.

Discussion

The present study is part of a trial conducted to investigate the use of probiotics in lactating Sanjabi ewes. In a previous article, we reported the effects of probiotics on milk fatty acid composition (Payandeh et al. 2017). In the current article, we focused on the effects of probiotics on productive results and blood biochemical indicators in an attempt to add information to the little published research on the matter.

The non-significant increase (~24%) of milk yield observed in the PRO treatment (Table 1) agreed with the findings of Mousa et al. (2012), who supplemented a live culture of *Saccharomyces cerevisiae* to ewes. On the contrary, previous research have reported significant increases of milk yield in ewes after supplementation with *Bacillus spp.* (Kritas et al. 2006) or with a live culture of *Saccharomyces cerevisiae* (Mašek et al. 2008a, 2008b; Milewski and Sobiech 2009). With regard to milk fat and protein contents, the values found in the present work were within the ranges reported formerly in sheep milk (Raynal-Ljutovac et al. 2008). Our results contrast with several studies that have linked the use of probiotics with a positive response of milk composition parameters (Kritas et al. 2006; Mašek et al. 2008a, 2008b; Mousa et al. 2012). However, it should be highlighted that none of those authors reported the effects of the interaction between probiotic supplementation and stage of lactation on milk yield and composition, which had a

clear and significant effect on most of the parameters analysed in the present work (Table 1). The higher output of nutrients observed in the milk from the PRO treatment in the last third of lactation should be considered favourable, since sheep milk is a valuable food product in countries where climatic conditions are not favourable for cattle raising (Boyazoglu and Morand-Fehr 2001) and it has traditionally enriched the nomadic shepherd's diet (Degen 2007).

Blood biochemical parameters are commonly used to assess the nutritional and physiological status of lactating animals (Caldeira et al. 1999; Piccione et al. 2009). The data obtained in the present work (Table 2) were similar to those previously reported in other fat-tailed sheep (Nazifi et al. 2005; Eshratkhah et al. 2008). The observed trends in the blood plasma metabolite contents and enzyme activities as lactation progressed were in agreement for the most part with the patterns of change with time previously found by Payandeh et al. (2016) in Sanjabi ewes. Again, the few reported effects of probiotic supplementation on a diversity of blood indices of dairy ewes are controversial. Mašek et al. (2008a, 2008b) did not find differences on the urea content, lipid profile and AST, ALT and ALP activities in blood plasma between supplemented and non-supplemented ewes. In contrast, Mousa et al. (2012) observed significantly higher glucose and urea concentrations and AST activity, without changes on creatinine concentration or ALT activity, in the blood plasma of ewes fed probiotics compared with the controls. Milewski and Sobiech (2009) reported higher glucose and lower creatinine contents with no changes of triglycerides, cholesterol and urea concentrations nor AST, ALT and ALP activities in the blood plasma of probiotic-supplemented ewes compared with the unsupplemented ones, whereas Lubbadeh et al. (1999) found non-significant lower levels of blood plasma cholesterol in ewes fed *Lactobacillus acidophilus*. It is worth mentioning that those studies, except for Lubbadeh et al. (1999), used live yeast cultures as probiotics. Thus, regarding blood metabolites, our results are in total (Mašek et al. 2008a, 2008b) and partial (Milewski and Sobiech 2009) agreement with previous findings. The absence of probiotic effects on ALT and ALP activities find in the present work agreed with Milewski and Sobiech (2009), while the higher AST activity in the last third of lactation observed in the PRO treatment would be in coincidence with the results presented by Mousa et al. (2012). On the contrary to Lubbadeh et al. (1999), we did not found any indication of probiotic effect on blood cholesterol level. The inconsistency of the results reported in the literature with regard to the effects of probiotic supplementation to ewes on blood parameters might be related to the type and composition of the probiotic tested, nature of the diet, sheep breed, and animal physiological status and level of performance.

The fact that milk yield did not show any decreasing trend as lactation progressed in the PRO treatment, which did occur in the CON treatment, suggests a higher availability of nutrients for sustaining milk synthesis during the last third of lactation. This point would be supported by both the higher AST activity during the last third of lactation and the relatively higher increase of total cholesterol, HDL and LDL levels from the second third of lactation onwards that were observed in the blood plasma of the probiotic-supplemented ewes. The AST activity has been related to increased metabolism of amino acids, as energy source or glucose precursors (Caldeira et al. 1999), whereas increased lipoprotein levels would indicate a greater absorption of nutrients in the intestines (Chiofalo et al. 2004). Due to the scarcity of published papers, no straightforward conclusions can be drawn with regard to the relationship between blood biochemical parameters and milk yield and composition after probiotic supplementation in ewes, and more research would be advisable on the subject. Nevertheless, our results together with those of Milewski and Sobiech (2009) and Mousa et al. (2012) would suggest that probiotics might improve the digestion processes, increasing the nutrients absorbed, processed in the liver, and available to the mammary gland.

Conclusion

To the best of our knowledge, this article presents for the first time a comprehensive study of the effects of bacterial probiotics on milk yield and composition and blood biochemical indicators in ewes during lactation. Under the conditions assayed, probiotics barely affected blood plasma metabolite contents and enzyme activities, but positive effects were observed on milk yield and its components during the last third of lactation. However, due to limited literature on the matter, further research is needed to evaluate the potential benefits of probiotic supplementation in lactating ewe diets.

Acknowledgements

The authors are grateful to the Razi University Agriculture College and the Agriculture Organisation of Kermanshah province for giving us permission to use their animals and

facilities for this study. The assistance of the Animal Production Department of the University of Cordoba is also gratefully acknowledged.

Ethical approval

The research protocol was approved by the Animal Care and Use Committee of Razi University.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Achille Schiavone http://orcid.org/0000-0002-8011-6999
Andrés L. Martínez Marín http://orcid.org/0000-0003-0052-9724

References

Boyazoglu J, Morand-Fehr P. 2001. Mediterranean dairy sheep and goat products and their quality - a critical review. Small Ruminant Res. 40:1–11.

Caldeira RM, Almeida MA, Santos CC, Vasques MI, Portugal AV. 1999. Daily variation in blood enzymes and metabolites in ewes under three levels of feed intake. Can J Anim Sci. 79:157–164.

Chiofalo V, Liotta L, Chiofalo B. 2004. Effects of the administration of *Lactobacilli* on body growth and on the metabolic profile in growing Maltese goat kids. Reprod Nutr Dev. 44:449–457.

Degen AA. 2007. Sheep and goat milk in pastoral societies. Small Ruminant Res. 68:7–19.

Eshratkhah B, Sadaghian M, Khajeye M, Ahmadi H, Mostafavi H. 2008. Evalution of non-electrolytes normal values in blood of Makuei sheep breed. J Anim Vet Adv. 7:316–318.

FAO/WHO. 2001. Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Food and Agriculture Organization of the United Nations and World Health Organization. FAO Food and Nutrition Paper n° 85 url: http://www.fao.org/3/a-a0512e.pdf

Gaggia F, Mattarelli P, Biavati B. 2010. Probiotics and prebiotics in animal feeding for safe food production. Int J Food Microbiol. 141:S15–S28.

Krehbiel CR, Rust SR, Zhang G, Gilliland SE. 2003. Bacterial direct-fed microbials in ruminant diets: performance response and mode of action. J Anim Sci. 81:E120–E132.

Kritas SK, Govaris A, Christodoulopoulos G, Burriel AR. 2006. Effect of *Bacillus licheniformis* and *Bacillus subtilis* supplementation of ewe's feed on sheep milk production and young lamb mortality. J Vet Med Series A. 53:170–173.

Lettat A, Noziere P, Silberberg M, Morgavi DP, Berger C, Martin C. 2012. Rumen microbial and fermentation characteristics are affected differently by bacterial probiotic supplementation during induced lactic and subacute acidosis in sheep. BMC Microbiol. 12:142.

Lubbadeh W, Haddadin MSY, Al-Tamimi MA, Robinson RK. 1999. Effect on the cholesterol content of fresh lamb of supplementing the feed of Awassi ewes and lambs with *Lactobacillus acidophilus*. Meat Sci. 52:381–385.

Macedo RJ, Arredondo V, Garcia F, Aguilar M, Prado O, Rodriguez R. 2012. Effect of supplemental yeast culture and physiological factors on colostrum and milk composition of Pelibuey ewes. Trop Anim Health Prod. 44:349–354.

Mašek T, Mikulec Z, Valpotic H, Antunac N, Mikulec N, Stojevic Z, Filipovic N, Pahovic S. 2008a. Influence of live yeast culture (*Saccharomyces cerevisiae*) on milk production and composition, and blood biochemical of grazing dairy ewes during the milking period. Acta Vet Brno. 77:547–554.

Mašek T, Mikulec Z, Valpotic H, Kušće L, Mikulec N, Antunac N. 2008b. The influence of live yeast cells (*Saccharomyces cerevisiae*) on the performance of grazing dairy sheep in late lactation. Vet Arhiv. 78:95–104.

McAllister TA, Beauchemin KA, Alazzeh AY, Baah J, Teather RM, Stanford K. 2011. Review: the use of direct fed microbials to mitigate pathogens and enhance production in cattle. Can J Anim Sci. 91:193–211.

Milewski S, Sobiech P. 2009. Effect of dietary supplementation with *Saccharomyces cerevisiae* dried yeast on milk yield, blood biochemical and haematological indices in ewes. Bull Vet Inst Pulawy. 53:753–758.

Mousa KH, El-Malky OM, Komonna OF, Rashwan SE. 2012. Effect of some yeast and minerals on the productive and reproductive performance in ruminants. J American Sci. 8:291–303.

National Research Council (NRC). 1985. Nutrient requirements of sheep, sixth revised edition. Washington, DC: National Academy Press.

Nazifi S, Saeb M, Karimi T, Ghanbari S. 2005. Diurnal variation of serum biochemical parameters in the Iranian fat-tailed sheep. Comp Clin Path. 14:1–4.

Payandeh S, Kafilzadeh F, Angel de la Fuente M, Ghadimi D, Martinez Marin AL. 2016. Patterns of milk production, blood metabolite profile and enzyme activities of two fat-tailed sheep breeds. Anim Prod Sci. 56:1469–1474.

Payandeh S, Kafilzadeh F, Juárez M, de la Fuente MA, Ghadimi D, Martinez Marin AL. 2017. Probiotic supplementation effects on milk fatty acid profile in ewes. J Dairy Res. 84:128–131.

Piccione G, Caola G, Giannetto C, Grasso F, Runzo SC, Zumbo A, Pennisi P. 2009. Selected biochemical serum parameters in ewes during pregnancy, post-parturition, lactation and dry period. Anim Sci Pap Rep. 27:321–330.

Pulina G, Francesconi AHD, Stefanon B, Sevi A, Calamari L, Lacetera N, Dell'Orto V, Pilla F, Marsan PA, Mele M, et al. 2017. Sustainable ruminant production to help feed the planet. Ital J Anim Sci. 16:140–171.

Pulina G, Nudda A. 2004. Milk production. In: Pulina G, Bencini R, editors. Dairy sheep nutrition. Wallingford, UK: CABI Publishing; p. 1–12.

Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I, Chilliard Y. 2008. Composition of goat and sheep milk products: an update. Small Ruminant Res. 79:57–72.

Vosooghi-Poostindoz V, Foroughi AR, Delkhoroshan A, Ghaffari MH, Vakili R, Soleimani AK. 2014. Effects of different levels of protein with or without probiotics on growth performance and blood metabolite responses during pre- and post-weaning phases in male Kurdi lambs. Small Ruminant Res. 117:1–9.