

Effects of kefir as a probiotic source on the performance of goat kids

C. Ataşoğlu[#], H.I. Akbağ, C. Tölü, G. Daş, T. Savaş and İ.Y. Yurtman
Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Animal Science,
17020, Çanakkale, Turkey

Abstract

Kefir is a sour, viscous, slightly carbonated and alcoholic milk beverage, which is traditionally fermented using a culture of bacteria and yeasts. The influence of kefir on health has been well studied in mice and rats. However, research on kefir use in ruminants is rather limited. The aim of this study was to investigate the effect of kefir as a probiotic on the performance of goat kids during the pre- (45 days) and post-weaning (45 days) periods. Forty eight kids were randomly allocated to four treatment groups: Control, Kefir, Auto-Kefir (autoclaved) and Probiotic (a commercial probiotic). The kids were weaned at 45 days of age. The supplementation of different probiotics did not have any significant effect throughout the study on live weight and weight gain of the kids as compared to the Control group. Milk intake or milk-based nutrient intake of kids did not differ significantly among treatments in the pre-weaning period. Similarly, the intake of concentrate feed and nutrients from the concentrate was not affected by the treatments during post-weaning. No significant differences in faecal consistency of kids were found among the treatments. The results of the study indicated that supplementation of kefir as a natural probiotic or a commercial probiotic source does not improve performance of goat kids under the conditions in the present study and suggest that new approaches are required for studying the efficacy of this probiotic.

Keywords: Suckling period, weaning, performance, diarrhoea incidence, ruminant

[#] Corresponding author. E-mail: catasoglu@comu.edu.tr

Introduction

Most of the changes in microbial composition and population in the digestive tract can lead to health and performance problems in animals (Jonsson, 1985). Probiotics are defined as dietary supplements containing potentially beneficial bacteria and yeast, which are believed to provide health benefits to the host (Fuller, 1989). In addition to commercial probiotic products, which differ in microbial composition and are produced on industrial scale, there are natural products which are also regarded as probiotic sources, such as curds (Anandan *et al.*, 1999), yoghurt and kefir (Farnworth, 2005). Kefir is a sour, viscous, slightly carbonated and alcoholic dairy beverage with bioactive components such as peptides and exopolysaccharides. It is produced from the fermentation of milk through the actions of bacteria and yeast present in kefir grains, and is less known than yoghurt (Farnworth, 2005).

The microbial and chemical properties of kefir can be affected by various factors such as starter culture and fermentation conditions (Wszolek *et al.*, 2001). The study by Santos *et al.* (2003) with 58 strains of *Lactobacillus* spp. isolated from kefir revealed that *L. acidophilus* CYC 10051 and *L. kefiranofaciens* CYC 10058 possess the best probiotic properties. The beneficial effects of probiotics on the intestinal ecosystem are claimed to be related with their influence on intestinal microorganisms. Ota (1999) reported that kefir induces the colonization of *Bifidobacterium bifidum* and lactic acid bacteria in the intestines, thereby protecting humans against infections caused by pathogens, such as *E. coli* O-157.

Kefir also contains bioactive compounds such as peptides and exopolysaccharides. Farnworth (2005) pointed out that kefir and its bioactive compounds have crucial effects not only on digestion but also on metabolism and the immune system. Due to health benefits, kefir is nowadays produced on an industrial scale for use by humans.

The effects of kefir on human health have been investigated mainly in animal trials; mice and rats being used in most of the studies (Çevikbaş *et al.*, 1994; Thoreux & Schucker, 2001). As far as our knowledge is concerned, the only study on the potential of kefir in ruminants has been carried out by Daş *et al.* (2007), who demonstrated that supplementation of kefir shortly after weaning reduces coccidial oocyst

output in goat kids. The present study aimed at investigating the efficacy of kefir as a probiotic source in pre- and post-weaning periods in goat kids.

Materials and Methods

Forty eight Saanen kids (25 females and 23 males; 35 twins and 13 singles), born within a period of 13 days, were used in the present study. The kids were housed in individual pens equipped with a combined feed trough for the feeding of a concentrate and hay, and a plastic container for water. Straw was used as bedding material in the pens. Temperature and humidity in the barn ranged between 3.6 - 21.4 °C and 48 - 92%, respectively. The experimental animals were kept, maintained and treated in adherence to accepted standards for the humane treatment of animals.

Following colostrum consumption kids were randomly allocated based on birth type, sex and live weight to four treatment groups (12 kids in each group): Control, Kefir, Auto-Kefir and Probiotic. The Control group received no probiotic supplementation during the study. Kefir in a liquid form with a microbial composition of *Lactococcus* spp. (3.2×10^8 cfu/mL), *Lactobacillus* spp. (1.1×10^8 cfu/mL) and yeast (5.9×10^3 cfu/mL) was given to the kids in the Kefir group throughout the study (Anonymous, 2004). The kids in the Kefir group received 20 mL of kefir per kid per day in the pre-weaning period and 40 mL of kefir per kid per day in the post-weaning period. Kefir was given orally using a sterile syringe before suckling or feeding each morning. Kids in the Auto-Kefir group were given autoclaved kefir, dosed in the same way as in the Kefir group. Kefir was autoclaved at 110 °C for 3 min the day before supplementation, and kept at 4 °C (Mainville *et al.*, 2001). Kids in the Probiotic group received a commercial probiotic source (Biyoteksin™ L, Novartis). According to the supplier the probiotic contains a variety of microbial species: *Lactobacillus plantarum* (1.26×10^7 cfu/kg), *L. delbrueckii* (2.06×10^7 cfu/kg), *L. acidophilus* (2.06×10^7 cfu/kg), *L. rhamnosus* (2.06×10^7 cfu/kg), *Bifidobacterium bifidum* (2.00×10^7 cfu/kg), *Streptococcus salivarius* (4.10×10^7 cfu/kg), *S. facium* (5.90×10^7 cfu/kg), *Aspergillus oryza* (5.32×10^7 cfu/kg) and *Candida pintolopesii* (5.32×10^7 cfu/kg), with lactose as the carrier compound. During the pre-weaning stage five grams of the probiotic was resuspended in 20 mL distilled water and given daily in the same way as the other supplements. In the post-weaning period the amount of the probiotic was increased to 10 grams in 40 mL distilled water per kid daily.

The study was carried out for 103 days, which included 13 days for the introduction of the kids to their respective treatments. Each kid was weaned at 45 days of age. During the suckling period the kids were allowed to stay with their mothers for 90 min (45 min, morning/evening) in the first five weeks of the study, and for 45 min (morning) during the last week of the pre-weaning period. Throughout the study a grower concentrate in pelleted form (886.2 g DM/kg; 201.1 g CP/kg DM; 11.80 MJ ME/kg DM) and lucerne hay (911.0 g DM/kg; 199.0 g CP/kg DM; 10.05 MJ ME/kg DM) were offered to the kids *ad libitum*.

Individual intakes of the grower concentrate were recorded, but due to high spillage, the intake of lucerne hay could not be recorded. Daily individual intakes of the grower concentrate and nutrients in the grower concentrate were determined during pre- and post-weaning periods. In addition, daily milk intakes by kids were recorded once a week during the suckling period by measuring the difference in weight of a kid before and after suckling (Ayıışıǵı *et al.*, 2005). Daily individual milk intakes and milk nutrient composition were used in the prediction of the nutrient intake that the kids received from the milk. On the test days during the suckling period, the analysis for dry matter, solids-not-fat, fat, protein and lactose of the milk samples was carried out using an auto-analyzer (LACTOSCAN® Milk Analyzer). In the calculation of the energy content of the milk samples, the following equation: S_E (MJ/kg) = $(0.41 \times \text{fat}\% + 1.5)$, was used (GfE, 2003). Individual water intakes of kids were also recorded daily. The chemical composition of feeds was determined using analytical methods described by the AOAC (1990).

Faeces were observed daily and consistency was scored by using a faecal consistency scaling system ranging from 1 to 4 (1 = watery; 2 = runny; 3 = soft; 4 = normal; Ayıışıǵı *et al.*, 2005). In the statistical analysis of data of all the traits except for faecal consistency, treatment (Control, Kefir, Auto-Kefir or Probiotic), sex (male or female), birth type (single or twin), age (covariant) and interactions were used as fixed factors in a repeated measurement variance analysis. The Generalized Estimating Equations Logistic Regression Method, which took all the factors and interactions into account, was used to analyze diarrhoea scores. All the analyses were carried out in the SAS (1999) Package Programme.

Results

Live weights on a weekly basis are presented in Figure 1. The treatments had no significant effects on live weight or live weight gain of kids in pre- and post-weaning periods of the study (Figure 1). Live weight was significantly affected by sex ($P = 0.002$) and age ($P = 0.001$), whereas live weight gain was affected by sex ($P = 0.006$), birth type ($P = 0.001$) and age ($P = 0.001$) in the pre-weaning period. In this regard, male and single kids were heavier than others in terms of live weight. Weaning live weight values at 45 days of age were 9.3 ± 0.42 , 9.1 ± 0.48 , 9.4 ± 0.48 and 9.0 ± 0.48 kg for Control, Kefir, Auto-Kefir and Probiotic groups, respectively ($P = 0.897$). No effect of factors or interactions except for sex ($P = 0.019$) and birth type ($P = 0.025$) on weaning weight was recorded ($P > 0.05$). At weaning the male kids (9.8 ± 0.41 kg) were heavier than the females (8.6 ± 0.34 kg). Likewise, weights of the single kids at weaning (9.8 ± 0.41 kg) were higher than those of the individual twins (8.7 ± 0.24 kg). In the post-weaning period, live weight was not affected by any of the factors or interactions ($P > 0.05$) except for age ($P = 0.001$). On the other hand, live weight gain was significantly affected by age ($P = 0.001$) and group x birth type interaction ($P = 0.006$).

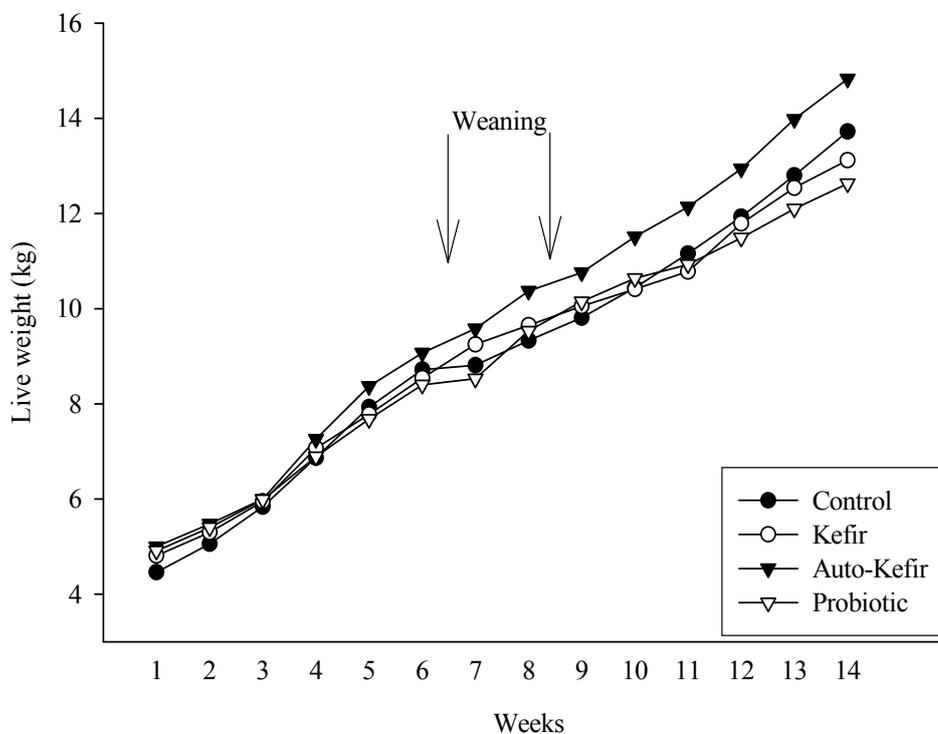


Figure 1 Live weight changes of kids that received different probiotics throughout the study ($P > 0.05$).

The analysis of milk samples on test days indicated that kids in all treatment groups received milk with a similar chemical composition and energy content. Overall fat and energy content of milk were $4.07 \pm 0.82\%$ and 3.17 ± 0.33 MJ, $4.35 \pm 0.74\%$ and 3.29 ± 0.30 MJ, $4.26 \pm 0.55\%$ and 3.24 ± 0.22 MJ and $4.11 \pm 0.90\%$ and 3.18 ± 0.37 MJ in the Control, Kefir, Auto-Kefir and Probiotic groups, respectively. Daily milk intake of kids was not affected by the treatments ($P = 0.864$). The highest daily milk intake (1.36 ± 0.089 kg) was found in the Auto-Kefir treatment (Table 1). Sex ($P = 0.094$) and birth type ($P = 0.051$) did not have any significant effect on milk intake. Daily intakes of milk by female and male kids were 1.23 ± 0.063 and 1.38 ± 0.060 kg, respectively. Age ($P = 0.040$) but not interactions among factors ($P > 0.05$) had a significant effect on milk intake.

In the pre-weaning period of the present study the kids in all treatment groups took in negligible amounts of the concentrate (Figure 2). The highest daily concentrate feed intakes were 0.026, 0.019, 0.026 and 0.033 kg/kid for the Control, Kefir, Auto-Kefir and Probiotic groups, respectively. Lucerne hay intakes

were not taken into account in calculations of daily nutrient intakes of the kids owing to the high spillage of the hay. Similarly, calculations on daily nutrient intakes of kids during pre-weaning period were based on daily milk intakes of kids.

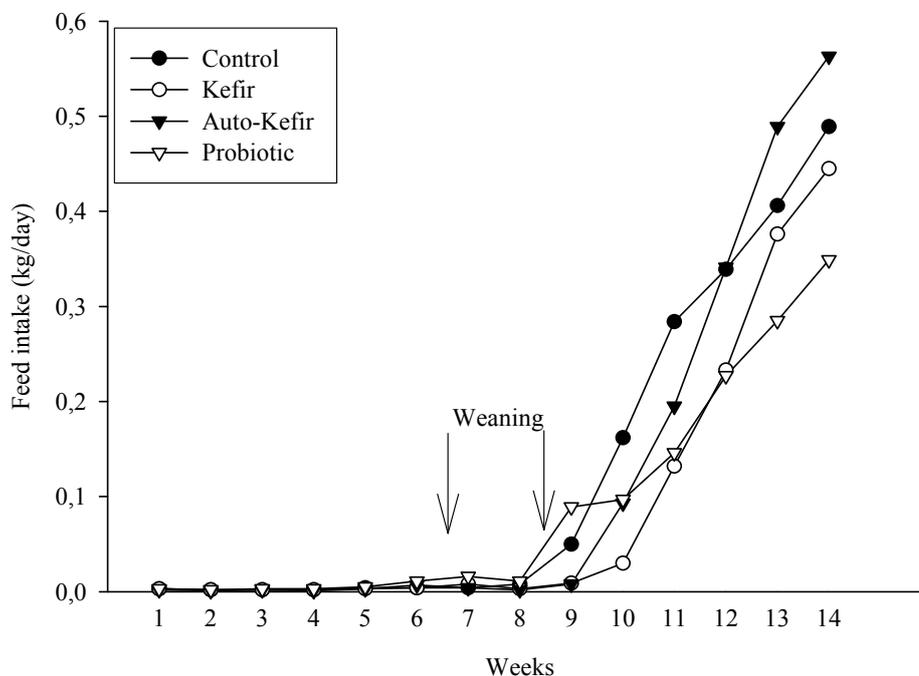


Figure 2 Changes in concentrate feed intakes of kids that received different probiotics throughout the study.

After weaning daily concentrate feed intakes of the kids started to increase in all treatment groups (Figure 2), but were not affected by treatments ($P = 0.843$). Furthermore, concentrate feed intake was not affected by factors such as sex or birth type or interactions ($P > 0.05$) except for age ($P = 0.001$). Average intakes were 0.194 ± 0.025 and 0.245 ± 0.033 kg for female and male kids and 0.208 ± 0.030 and 0.232 ± 0.029 kg for single and twin kids, respectively.

Mean daily water intakes of the kids were 1.36 ± 0.144 , 1.22 ± 0.127 , 1.42 ± 0.126 and 1.28 ± 0.125 L for the Control, Kefir, Auto-Kefir and Probiotic groups, respectively. Treatments did not significantly affect water intakes ($P = 0.707$). Similarly, no effects of other factors or interactions ($P > 0.05$) were recorded on water intake except for age ($P = 0.001$).

In the present study, the incidence of watery and runny faeces was quite low in all treatment groups (Table 2). Faecal consistency was not affected by the treatments ($P = 0.445$). Faecal consistency values were significantly affected by birth type ($P = 0.011$) and age ($P = 0.001$), but not by other factors or interactions ($P > 0.05$).

Discussion

Supplementation of different probiotic sources did not influence live weight of kids throughout the study ($P > 0.05$). However, kids in the Auto-Kefir group appeared to show a better performance than kids in the other groups. This aspect is worth further investigation. Live weight gains of kids were not affected by the treatments ($P > 0.05$). The highest weight gain of kids in the pre-weaning period occurred in the Auto-Kefir group (0.142 kg/day), followed by the Control (0.137 kg/day), Kefir (0.132 kg/day) and Probiotic (0.131 kg/day) groups. Live weight gain followed a similar pattern in the post-weaning period.

Table 1 Least square means (LSM) and standard errors (SE) of daily live weight gain and intake parameters of kids that received different probiotics in pre- and post-weaning periods

Period/Trait	Treatments								P-values
	Control		Kefir		Auto-Kefir		Probiotic		
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	
ILW	3.46	0.13	3.30	0.15	3.27	0.15	3.29	0.15	0.727
FLW	13.72	2.79	13.20	1.93	14.31	2.04	12.68	3.58	0.814
Pre-weaning									
LWG	0.137	0.008	0.132	0.009	0.142	0.009	0.131	0.009	0.839
MI	1.33	0.080	1.27	0.089	1.36	0.089	1.27	0.089	0.864
MDMI	0.117	0.007	0.113	0.008	0.119	0.008	0.111	0.008	0.884
MCPI	0.062	0.004	0.060	0.004	0.063	0.004	0.059	0.004	0.892
MEI	4178	267.9	4119	300.1	4337	300.1	4065	300.1	0.927
Post-weaning									
LWG	0.085	0.011	0.069	0.013	0.094	0.012	0.084	0.012	0.609
FI	0.243	0.039	0.194	0.043	0.211	0.043	0.231	0.042	0.843
FCPI	37.8	5.92	29.0	6.56	31.1	6.55	34.3	6.47	0.771
FEI	2227	349.1	1712	386.8	1833	385.9	2026	382.6	0.771

ILW - initial live weight, kg; FLW - final weight gain, kg; LWG - live weight gain, kg; MI - milk intake, kg; MDMI - milk dry matter intake g; MCPI - milk crude protein intake, g CP; MEI - milk energy intake, MJ ME; FI - feed intake, kg; FCPI - feed crude protein intake, g CP; FEI - feed energy intake, MJ ME.

Table 2 Proportional distribution of faecal consistency scores (based on factors, %) of kids that received different probiotics

Factor	Trait	Faecal consistency score*			
		1	2	3	4
Treatment	Control	0.58	4.04	13.39	81.99
	Kefir	0.24	3.19	7.91	88.67
	Auto-Kefir	0.37	2.48	7.83	89.32
	Probiotic	0.47	3.98	9.12	86.43
Sex	Female	0.35	2.43	8.07	89.15
	Male	0.52	4.95	11.89	82.64
Birth Type	Single	0.18	2.25	6.93	90.64
	Twin	0.53	4.02	10.92	84.53
Overall Mean		0.42	3.44	9.61	86.54

* Faecal consistency 1 - watery; 2 - runny; 3 - soft; 4 - normal.

In some studies the feeding of probiotics to neonatal calves has been reported to improve weight gain (Abu-Tarboush *et al.*, 1996; Herich *et al.*, 1998), but not in others (Morill *et al.*, 1995). Likewise, variable responses to probiotic supplementation in terms of weight gain have been obtained in lambs (Birch *et al.*,

1994) and kids (Anandan *et al.*, 1999; Ayışığlı *et al.*, 2005). Considering digestive and health benefits, positive effects of probiotic supplementation on performance are expected to be accompanied by improvements in feed intake. Indeed, none of the treatments significantly affected intake parameters in the present study.

Changes in the intake capacity of kids for solid feeds can influence growth rate, particularly in the post-weaning period. Adaptation to solid feeds in the post-weaning period can largely be influenced by feed characteristics and herd management as well as milk yield of the dam and milk intake of the kid (Goetsch *et al.*, 2001; Genandoy *et al.*, 2002). Therefore, the intake of concentrate feed by kids at very low levels in all groups should be considered together with milk intake levels in this study. Intake values determined in the present study are similar to values reported by Ayışığlı *et al.* (2005). Protein and energy intakes of kids were very close to the protein and energy requirement recommendations of GfE (2003), suggesting that the milk intakes of kids in all the groups were nearly adequate. Increase in solid feed intake stimulates rumen development. It has been indicated that solid feeds in particular concentrates are crucial for the development of the rumen of neonatal ruminants. Based on various reports Baldwin *et al.* (2004) pointed out that neonatal ruminants fed solely on milk diets, display limited rumen development in terms of rumen weight, capacity and papilla growth during the initial months of life. In this study, late adaptation to concentrate feed and the relation between milk and concentrate feed intake can be explained by a mechanism “reticular groove” proposed by Ørskov *et al.* (1970). Ørskov *et al.* (1970) suggested that direct passage of milk via the reticular groove to the abomasum results in lack of substrates in the rumen for the stimulation of ruminal fermentation and hence rumen development.

Variable performance responses to probiotic supplementations in growing animals have encouraged the investigation of the mechanisms, which determine the efficacy of probiotic use. Yoon & Stern (1995) reviewed the results of studies that focused on the effects of probiotics and concluded that less than 40% of the studies showed positive responses to microbial supplements. A number of factors are involved in the regulation of the efficiency of microbial supplements. Studies have so far focused mainly on age at supplementation (Cruywagen *et al.*, 1996) and environmental factors (Donovan *et al.*, 2002; Krehbiel *et al.*, 2003). On the other hand, the efficacy of probiotics depends on the adaptation of microbial species to the environmental conditions and their ability to survive and compete with pathogens in the gastrointestinal tract, and is closely related with the dose and microbial composition of the probiotic source. Different results from the studies using commercial or natural probiotic supplements with different doses of lactic acid bacteria and yeast have so far been obtained (Cruywagen *et al.*, 1996; Agarwal *et al.*, 2002; Ayışığlı *et al.*, 2005; Timmerman *et al.*, 2005). The doses of kefir (1.2×10^9 cfu/kg LW) and the commercial probiotic source (2.15×10^5 cfu/kg LW) supplemented to kids in the present study were similar to the doses used in other studies reported in the literature.

Krehbiel *et al.* (2003) suggested that the efficacy of probiotics should be evaluated more in terms of their health benefits rather than performance gains. Faecal consistency has been examined in many studies. Anandan *et al.* (1999) using a daily prepared natural probiotic “curds” in goat kids, classified diarrhoea incidences based on “level of diarrhoea progression”. Diarrhoea incidences progressed for 1 - 2 days were classified as mild; for 3 - 4 days as moderate and for five days or more as severe in the study. These authors found that kids in the Control group had higher incidences of diarrhoea in all diarrhoea groups than those in groups receiving curd. In the present study, a faecal consistency scoring system (1 = watery; 2 = runny; 3 = soft; 4 = normal) was used to determine the effects of the treatments on diarrhoea incidence. Faecal consistency in all the groups was mainly between 3 and 4 throughout the study. This suggests that kids in all the groups did not have a problem of diarrhoea at a severe level during the entire study except for a fall in faecal consistency scores between 3 - 6 weeks of the study. Low proportions of watery and runny faecal samples presented in Table 2 support the statement above. Lack of benefits of probiotic supplements on diarrhoea incidence might be explained by a very low risk of stress factors and pathogens in studies where environmental factors have been controlled (Cruywagen *et al.*, 1996; Donovan *et al.*, 2002; Krehbiel *et al.*, 2003; Ayışığlı *et al.*, 2005).

Conclusions

Kefir may offer a great potential as a probiotic source for investigation in animal production since it is natural, cheap and easy to be produced on the farm. In this study, neither kefir nor autoclaved kefir exhibited

any significant effect on growth, nutrient intake and faecal consistency of kids, indicating that under circumstances of good nutrition and management kefir does not improve animal performance. Therefore, new approaches would be required for studying the efficacy of kefir under different conditions in future. In this regard, research on kefir should be carried out on farm level or with animals infected with pathogens. Studies on the influence of changes in digestive physiology and microbiology on the efficacy of kefir supplementation in ruminants are also warranted.

Acknowledgements

The authors thank Raziye Işık, Gökçe Gamlı and Coşkun Konyalı for their help during the study. This study was funded by the Scientific Research and Technological Council of Turkey (Project Number: 106O514).

References

- Abu-Tarboush, H.M., Al-Saiady, M.Y. & Keir El-Din A.H., 1996. Evaluation of diet containing *Lactobacilli* on performance, fecal coliform, and *Lactobacilli* of young dairy calves. *Anim. Feed Sci. Technol.* 57, 39-49.
- Agarwal, N., Kamra, D.N., Chaudhary, L.C., Agarwal, I., Sahoo, A. & Pathak, N.N., 2002. Microbial status and rumen enzyme profile of crossbred calves fed on different microbial feed additives. *Lett. Appl. Microbiol.* 34, 329-336.
- Anandan, S., Dey, A., Deb, S.M., Kumar, S. & Harbola, P.C., 1999. Effects of curds as probiotic supplement on performance of Cheghu crossbred kids. *Small Rumin. Res.* 32, 93-96.
- Anonymous, 2004. Report on chemical and microbial composition of kefir. TUBITAK Marmara Res. Center, Adapazarı, Turkey.
- AOAC, 1990. Official Methods for Analysis of the Association of Official Analytical Chemists, 15th edn. AOAC, Washington, D.C., USA.
- Ayışığı, K., Ataşoğlu, C., Yurtman, İ.Y., Mendes, M. & Pala, A., 2005. Effect of probiotic supplementation shortly before and after weaning on growth of Turkish Saanen kids. *Arch. Tierzucht* 48, 601-611.
- Baldwin, R.L., McLeod, K.R., Klotz, J.L. & Heitmann, R.N., 2004. Rumen development, intestinal growth and hepatic metabolisms in the pre- and postweaning ruminant. *J. Dairy Sci.* 87, E55-E65.
- Birch, K.S., Thomas, J.D. & Ross, T.T., 1994. Growth and carcass and carcass characteristics of newly received lambs treated with probiotics and vitamin E. *Sheep Goat Res. J.* 10, 201-206.
- Çevikbaş, A., Yeni, E., Ezzedenn, F.W., Yardımcı, T., Çevikbaş, U. & Stohs, S.J., 1994. Antitumoral antibacterial and antifungal activities of kefir and kefir grain. *Phytother. Res.* 8, 78-82.
- Cruywagen, C.W., Jordaan, I. & Venter, L., 1996. Effect of *Lactobacillus acidophilus* supplementation of milk replacer on preweaning performance of calves. *J. Dairy Sci.* 79, 483-486.
- Daş, G., Ataşoğlu, C., Ülkü, H.I., Tölu, C., Savaş, T. & Yurtman, İ.Y., 2007. Can kefir reduce coccidial oocysts output in goat kids? In: Proc. 58th Annual Meeting of the EAAP, Dublin, Ireland.
- Donovan, D.C., Franklin, S.T., Chase, C.C.L. & Hippen, A.R., 2002. Growth and health of Holstein calves fed milk replacers supplemented with antibiotics or enteroguard. *J. Dairy Sci.* 85, 947-950.
- Farnworth, E.R., 2005. Kefir - a complex probiotic. *Food Sci. Technol. Bull.* 2 (1), 1-17.
- Fuller, R., 1989. A review: Probiotics in man and animals. *J. Appl. Bacteriol.* 66, 365-378.
- Genandoy, H., Sahlu, T., Davis, J., Wang, R.J., Hart, S.P., Puchala, R. & Goetsch, A.L., 2002. Effects of different feeding methods on growth and harvest traits of young Alpine kids. *Small Rumin. Res.* 44, 81-87.
- GfE, 2003. Recommendations for the supply of energy and nutrients to goats. Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie. DLG, Verlag.
- Goetsch, A.L., Detweiler, G., Sahlu, T. & Dawson, L.J., 2001. Effects of different management practices on preweaning and early postweaning growth of Alpine kids. *Small Rumin. Res.* 41, 109-116.
- Herich, R., Bomba, A., Nemocova, R. & Gancarcikova, S., 1998. The influence of application of probiotics on the immune system of 2-3 month old calves. *Arch. Tierzucht* 41, 565-572.
- Jonsson, E., 1985. *Lactobacilli* as probiotics to pigs and calves - a microbiological approach. Swedish University of Agricultural Sciences Department of Animal Nutrition and Management, Uppsala, Sweden, Report No 148.

- Krehbiel, C.R., Rust, S.R., Zhang, G. & Gilliland, S.E., 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *J. Anim. Sci.* 81, E120-E132.
- Mainville, I., Montpetit, D., Durand, N. & Farnworth, E.R., 2001. Deactivating the bacteria and yeast in kefir using heat treatment, irradiation and high pressure. *Int. Dairy J.* 11, 45-49.
- Morrill, J.L., Laster, J.F., Morrill, M.M. & Feyerherm, A.M., 1995. Plasma proteins and probiotics as milk replacer ingredients. *J. Dairy Sci.* 78, 902-907.
- Ørskov, E.R., Benzie, D. & Kay, R.N.B., 1970. The effects of feeding procedure on closure of the oesophageal groove in young sheep. *Br. J. Nutr.* 24, 785-794.
- Ota, A., 1999. Protection against an infectious disease by enterohaemorrhagic *E. coli* 0-157. *Med. Hypotheses* 53 (1), 87-88.
- Santos, A., San Mauro, M., Sanchez, A., Torres, J.M. & Marquina, D., 2003. The antimicrobial properties of different strains of *Lactobacillus* spp. isolated from kefir. *Syst. Appl. Microbiol.* 26, 434-437.
- SAS, 1999. Statistical Analysis Systems, SAS Online Doc®, Version 8.0. SAS Institute Inc, Cary, N.C., USA.
- Thoreux, K. & Schmucker, D.L., 2001. Kefir milk enhances intestinal immunity in young but not old rats. *J. Nutr.* 131, 807-812.
- Timmerman, H.M., Mulder, L., Everts, H., Van Espen, D.C., Van der Wal, E., Klaassen, G., Rouwers, S.M., Hartemink, R., Rombouts, F.M. & Beynen, A.C., 2005. Health and growth of veal calves fed milk replacers with or without probiotics. *J. Dairy Sci.* 88, 2154-2165.
- Wszolek, M., Tamime, A.Y., Muir, D.D. & Barclay, M.N.I., 2001. Properties of kefir made in Scotland and Poland using bovine, caprine and ovine milk with different starter cultures. *Food Sci. Technol-Leb.* 34, 251-261.
- Yoon, I.K. & Stern, M.D., 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. *Asian-Austral. J. Anim. Sci.* 8, 533-555.