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Effects of Kefir as a Probiotic Source on the Performance and Health of Young Dairy Calves

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ABSTRACT

The aim of this study was to investigate the effect of kefir as a probiotic on the performance and health status of calves. Thirty Holstein female calves with 3-day-old were randomly allocated to three treatment groups: Control (without any probiotic), probiotic (a commercial probiotic mixture-3 g/d/calf bacteria-based and 2 g/d/calf yeast-based) and kefir (20 ml/d/calf). The calves were weaned at 56 days of age. The experiment was performed in 70 days. Treatment had no effect (P>0.05) on weaning and final body weight and starter intake. Although differences in weight gain were not significant (P>0.05), there were trend to increase by probiotic treatments during 0-14 days**.** Probiotic treatments tended to have a positive effect on the population of the fecal lactic acid bacteria at 14 days. The results of the study indicated that kefir as a natural probiotic in calf nutrition may be beneficial during the first weeks of life.

Introduction

In intensive rearing systems, calves are susceptible to enteric bacterial imbalance and usually suffer from diarrhea and respiratory diseases, leading to inefficient digestion and absorption of nutrients and consequently retarded growth (Radostits, 1975). Antibiotics have been successfully used in reducing these problems also to obtain economic benefits in terms of improved calves performance and reduced medication costs. However, the use of antibiotics in animal production has been queried due to the potential of appearance of residues in animal products (Russell and Houlihan, 2003). Recently, some additives have been increasingly evaluated to replace or facilitate reductions in the use of antibiotics. Probiotics are examples of these additives (Frizzo et al., 2010). Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its microbial balance (Fuller, 1989). Probiotics have been shown to have many function, including protecting young animal against enteropathic disorders (Timmerman et al., 2005), improving feed efficiency and weight gain (Cruywagen et al., 1996; Lesmiester et al., 2004) and improve immune system (Timmerman.et al., 2005; Sun et al., 2010; Novak et al., 2012).

Kefir is a sour, viscous, slightly carbonated and alcoholic milk beverage, which is traditionally fermented with bacteria and yeasts. Kefir is prepared by inoculating cows, sheep's or goat's milk with the kefir grains (Farnworth, 2005). It contains proteins, polysaccharides, ethyl alcohol, lactic acid, fat, minerals and vitamins (Farnworth, 2005). Kefir grains consist of lactic acid bacteria, acetic acid bacteria such as *Lactobacillus* species, *Lactobacillus acidophilus*, *Leuconostoc*, *Acetobacter* species and *Streptococcus* species, yeasts as *Saccharomyces* and *Torula* and other microorganisms (Toba et al., 1990). The influence of kefir on health has been well studied in mice, rats,poultry and goat kids (Çevikbaş et al., 1994; Thoreux and Schucker, 2001; Atasoglu et al., 2010; Toghyani et al., 2015). However, researches on usage of kefir in calves are rather limited. The aim of this study was to investigate the effect of kefir as a probiotic on the performance of young dairy calves. The present study aimed at investigating the efficacy of kefir as a probiotic source in female calves.

Materials and Methods

The experiment was carried out in August-September. The experiment was conducted in a commercial dairy farm (İtimat Süt ve Süt Ürünleri Çiftliği-Bursa, Turkey).

The management of calves and all procedures in the present study were performed according to the Animal Experimental Guidelines for Uludağ University Local Ethical Committee.

Animals, Diets, and Experimental Design

Thirty Holstein female calves (initial body weight=40.53 kg) were assigned randomly at 3-day-old to one of three treatments. Treatments included: no probiotic supplementation (control), commercial probiotic (3

g/d/calf bacteria-based and 2 g/d/calf yeast-based), kefir (20 ml/d/calf). The bacteria-based probiotic powder contains a mixture of *Lactobacillus plantarum, L. delbrueckii, L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus salivarius, Enterococcus faecium* ($2x10^{11}$ cfu/g) and the yeast-based probiotic powder contains *Saccharomyces cerevisiae* strain NCYC Sc47 ($1x10^{10}$ cfu/g). Kefir was in a liquid form with a microbial composition of *Lactococcus spp.* ($3.2x10^{8}$ cfu/mL), *Lactobacillus spp.* ($1.1x10^{8}$ cfu/mL) and yeast ($5.9x10^{3}$ cfu/mL) (Anonymous, 2004). Calves were housed individually in calf house with separated pens of 1.5 m (length) x 1.2 m (width), each of which was equipped with feeding and watering trough as required for calves. All pens were located in the same calf house and the calves were randomly allocated. There were two empty pens left between the groups to minimize the possible group effect (contamination by spores). The calf house was equipped with controlled ventilation and the bedding in the pens was chopped straw. Manure was removed daily and chopped straw was given to all pens again. Calves fed 2 L of fresh colostrum by nipple bottle at birth, and again after 4 h, and every 12 h thereafter. Calves were fed colostrums for 3 d then switched to milk until weaning (8 weeks). All calves received 4.5 kg whole milk which divided into two equal portions and fed at 0800 and 1600 h. Doses of kefir and commercial probiotic were chosen on the basis of results of other studies (Cruywagen et al., 1996; Timmerman et al., 2005; Atasoglu et al. 2010). Kefir was given orally using a sterile syringe before feeding each morning. Commercial probiotic (3 g/d/calf bacteria-based and 2 g/d/calf yeast-based) was resuspended in 20 mL distilled water and given daily in the same way as kefir. A calf starter was individually offered for *ad libitum* from 14 d of age. Calf starter was pelleted (diameter of 3 mm) and contained no growth promoters. The composition and the analyzed nutrient content of offered feeds are provided in Table 1. Calves were weaned when they started to consume 0.8 kg of calf starter per day for three consecutive days (at 56 days of age). The experiment was performed in 70 days. Starter intake as air-dry matter and fecal consistency scoring were measured daily. Fecal samples were collected and analysed on days of 14 and 28. Calves were weighted every two weeks.

Sampling, Measurement and Analyses

The dry matter and N analysis were determined in the calf starter (AOAC, 1995), as well as neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991). The NDF was analysed with addition of a heat stable alpha-amylase and without sodium sulphite. The NDF and ADF contents are expressed inclusive of residual ash. 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.

Fecal scoring of fecal fluidity, consistency, odor, and days scoured was conducted daily in the morning (9.00 AM). Fecal scores based on a four-point scale were recorded using the procedure of Larson et al. (1977). Scoring was as follows: for fecal fluidity, 1= normal, 2 = soft, 3 = runny or 4 = watery.

Fecal samples were collected from rectum with sterile rubber gloves and place in sterile 50 mL plastic tubes. The samples were transported to the laboratory. The samples were stored in a freezer at -20°C until analysis for counts of lactobacilli and coliforms. A subsample (1 g) of the feces was placed in a 50 mL falcon tube and mixed with 9 mL of distilled water. The mixture was vortexed. Bacterial enumeration was carried out using selective growth media and growth conditions. Fecal subsamples (1 g) were serially diluted with 9 mL of sterilized saline water dilution from 10^{-1} to 10^{-8}. From each dilution, 100 µl of suspension was plated out, in triplicate on the MRS agar (Merck, Darmstadt, Germany) and eosin methylene blue agar (Oxoid) for the determination of the total cell count of Lactobacillus spp. and coliforms, respectively. The MRS broth agar plates were incubated anaerobically at 37°C for 24 h. Eosin methylene blue agar plates were incubated an anaerobic condition at 37°C for 48 h. After incubation, viable bacterial colonies on each medium were counted. The total cell counts of lactobacilli and coliforms per gram of fecal material were calculated. Numbers of colony forming unit (CFU) were expressed as $\log_{10}$ CFU/g feces.

Table 1 The composition of fresh milk and calf starter

Ingredients	Calf starter	Fresh milk
Maize	410	
Dried distillers grains with solubles	100	
Wheat shorts	158	
Molasses	50	
Soyabean meal	237	
Salt	5	
Vitamin-mineral premix*	25	
Limestone	15	
Analysed content (g/kg)		
Dry matter	897.2	83.0
Crude protein	184.4	30.3
Fat	49.6	35.0
Neutral detergent fiber	156.4	-
Acid detergent fiber	43.4	-

*Vitamin-mineral premix per kg includes: 15.000.000 IU Vit A; 3.000.000 IU Vit D, 30.000 mg Vit.E; 50.000 mg Mn; 50.000 mg Zn; 50.000 mg Fe; 10.000 mg Cu; 800 mg I;200 mg Co; 300 mg Se; 100 mg Mg; 143.272 mg Ca

Statistical Analysis

Results were subjected to analyses of variance, and the means were separated by Duncan's (1955) multiple range test. Means were considered significantly different at P<0.05 (Minitab, 2000). The mathematical model used was as the following:

$$Yij = \mu + ai + bj + eij$$

where *Yij* is the individual observation, μ is experimental mean, *ai* is treatment effect, *bj* is replication effect, and *eij* is the error term.

Results and Discussion

Effects of dietary treatments on growth performance in female calves were presented in Table 2. Supplementation of different probiotic sources did not influence (P>0.05) weaning and final body weight. Supplementation of different probiotic sources did not influence weaning and final body weight (P>0.05). Reports on probiotic supplementation for calves have different results with respect to growing performance. Previous some papers reported beneficial effects of probiotics on animal growth (Abe et al., 1995; Timmerman et al., 2005; Frizzo et al., 2010), while others (Jenny et al., 1991; Higginbotham and Bath, 1993; Abu-Tarboush et al., 1996; Cruywagen et al., 1996) reported no effects. This discrepancy among the different studies may be related to several factors such as age at supplementation (Cruywagen et al., 1996) and environmental factors (Krehbiel et al., 2003). In the present study, probiotic sources during 0–14 days tended to increase daily weight gain compared the control group suggesting that commercial probiotic and kefir could have beneficial effect on daily weight gain during the first weeks of life. This result could be related to the health beneficial effect of these probiotics. Additionally, this explanation is further supported by tended to the improvement in fecal scores and *Lactobacillus* count in the feces in calves fed different probiotics. Cruywagen et al. (1996) reported that average daily gain during week 2 was affected by *L. acidophilus* supplementation. Timmerman et al. (2005) report a clear increase in weight gain in 1 week old veal calves supplemented with probiotics but limited beneficial effects during the first 2 weeks of life. Supplementation of different probiotic sources did not affect on starter intake of calves throughout the study (P>0.05). Similarly, Quigly et al. (1992) found no significant effect of yeast probiotic on intake of starter in dairy calves. Abney (2001) reported no significant difference in dry matter intake between calves received probiotic and control group. In contrast, Higginbotham and Bath (1993) and Abe et al. (1995) reported positive effects on dry matter intake by feeding probiotics. Rust et al. (2000) reported increased dry matter intake in beef steers which received lactic acid based probiotic. The lack of response to probiotics in the present study was probably because of the calves were not stressed. According to Ruppert et al. (1994), when the diet was supplemented with a probiotic and when calves were kept under stressful conditions, feed intake of calves (2 to 28 d) was higher than intake for calves in the negative control group. In the present study, probiotic sources during 0–14 days tended to increase weight gain without changes in starter intake suggesting that probiotic sources may have positive effects on feed efficiency. Previous some studies (Cruywagen et al., 1996; Lesmeister et al., 2004; Frizzo et al., 2008) also reported improvement in utilization of feed with probiotics.

Table 2 Growth performance of calves fed different probiotics.

Parameter	Control	Commercial Probiotic *	Kefir**	SEM	P
Body weight, kg					
Initial	40.29	40.36	40.23	0.96	0.99
Weaning	67.96	69.16	68.40	1.16	0.76
Final	78.14	79.74	78.87	1.25	0.66
Daily weight gain, g/day					
Pre-Weaning					
0-14 days	272.1	365.0	342.1	29.87	0.09
14-28 days	506.4	471.4	461.4	32.96	0.61
28-42 days	552.9	555.7	573.6	15.55	0.60
42-56 days	645.0	665.0	635.0	15.09	0.37
Post-Weaning					
56-70 days	727.1	755.7	747.8	22.15	0.64
0-70 days	530.7	562.6	552.0	22.51	0.59
Starter intake, g/day					
Pre-Weaning					
14-28 days	257.8	264.7	259.9	3.33	0.34
28-42 days	475.3	479.6	474.3	3.71	0.56
42-56 days	668.7	685.2	665.7	8.47	0.23
Post-Weaning					
56-70 days	907.1	921.7	897.3	12.95	0.42
0-70 days	577.3	587.8	574.3	38.54	0.96

* Commercial probiotic (3 g/d/calf bacteria-based and 2 g/d/calf yeast-based) **kefir (20 ml/d/calf)

Table 3 Fecal consistency scores and microbial counts in feces of calves fed different probiotics.

Parameter	Control	Commercial Probiotic *	Kefir**	SEM	P value
Fecal consistency score***					
Pre-Weaning					
0-14 days	2.32	2.20	2.22	0.046	0.14
14-28 days	2.03	1.91	1.96	0.051	0.25
28-56 days	1.66	1.63	1.60	0.034	0.45
Post-Weaning					
56-70 days	1.52	1.54	1.56	0.042	0.77
0-70 days	1.84	1.78	1.79	0.024	0.18
Coliform, (log_{10} of count/g of feces)					
14 days	6.84	6.24	6.35	0.30	0.35
28 days	6.45	6.17	6.09	0.29	0.66
Average	6.64	6.21	6.22	0.20	0.24
Lactobacilli, (log_{10} of count/g of feces)					
14 days	6.83	7.60	7.76	0.30	0.09
28 days	6.55	7.35	7.03	0.31	0.22
Average	6.67^{a}	7.48^{b}	7.40^{ab}	0.22	0.03

* Commercial probiotic (3 g/d/calf bacteria-based and 2 g/d/calf yeast-based) **kefir (20 ml/d/calf), *** Faecal consistency score 1-normal 2- soft; 3-runny; 4- watery, ^{ab}Differences in superscript indicate significance at $P<0.05$.

Effects of dietary treatments on fecal consistency scores and microbial counts in feces of calves fed different probiotics in female calves were presented in Table 3. Different probiotic sources did not affect ($P>0.05$) on faecal consistency scores of calves throughout the study. However, probiotic treatments tended ($P<0.14$) to lower fecal score during 0–14 days compared to the control. Abu-Tarboush et al. (1996) also reported that calves fed *L. acidophilus* 27SC had a significantly lower scour index compare with calves fed the control diet. Magalhaes et al. (2008) used yeast culture and reported improvement in fecal scores in calves. In contrast, Cruywagen et al. (1996) reported no positive effects on general health by feeding probiotics. In the present study, although on 14 and 28 days the fecal populations of coliform bacteria were no different ($P>0.05$) among the treatments; there were a tendency to increase in fecal populations of lactobacilli by probiotic treatments at d 14. Also, commercial probiotic treatment increased the average fecal population of lactobacilli compared to the control. On the other hand, kefir tended to increase the average fecal population of lactobacilli. Increases in fecal lactobacilli with probiotic supplementation have been also reported by many researchers (Ellinger et al., 1980; Jenny et al., 1991; Abu-Tarboush et al., 1996). Gilliland et al. (1980) fed *L.acidophilus* isolated from a human and calf intestinal tract to newborn calves and observed an increase in facultative lactobacilli in the feces during 0-14 days. The mechanism of action of probiotics is still debated, but generally, it is related to function by maintaining the presence of beneficial microorganisms in the gut by the competitive exclusion of pathogenic bacteria adherence (Riddell et al., 2010). In this way probiotics can influence the intestinal microbiota as well as host health, also increasing nutrient utilization, producing bacteriocins and stimulating the immune system (Corcionivoschi et al., 2010).

Conclusion

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. Results from this study showed that although kefir feeding had no effects on weaning and final body weight, starter intake and overall fecal score, it tended to improve daily weight gain, fecal score and in lactobacilli count in the feces during 0-14 days. Kefir as a probiotic may be improving daily weight gain and health status of calves particularly during the early stage of life. A further study is needed to prove the efficacy of using kefir in commercial farms on a larger scale.

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