ABSTRACT

A growing body of evidence suggests health-promoting effects of kefir consumption on different nonruminant species, leading to the speculation that kefir may act as a probiotic and benefit calf performance and health. Our objectives were to determine effects of feeding kefir on performance and health of calves in the first 70 d of life. Thirty 3-d-old female Holstein calves (body weight = 38.2 ± 3.1 kg) were blocked by initial body weight and assigned randomly to 1 of 3 treatments (1 calf per pen; 10 pens per treatment). Kefir was added to whole milk (vol/vol) at 0:1 (control; KF0), 1:3 (KF1), or 1:1 (KF2) and fed twice per day (0800 and 1600 h) from d 3 through 45 and then once per day until weaning, which occurred on d 50. Pre- and postweaning intake of starter, daily body weight gain, and gain-to-feed ratio exhibited no difference among treatments. Adding kefir to whole milk fed directly to calves had no effect on concentration of blood metabolites collected on d 20, 40, and 70. Body length on d 50 (weaning) and 70 was greater in kefir-fed calves. Kefir intake improved fecal scores and reduced days with diarrhea during the first 2 wk of life. Apparent digestibility of organic matter, ether extract, crude protein, and neutral detergent fiber remained unaffected by treatment. Overall, it appears that directly feeding kefir to calves during the preweaning period did not improve the performance of calves under the conditions of the current study; however, its consumption marginally improved body length and fecal consistency in the first weeks of life, which is an important concern in intensive calf-rearing systems. Feed- ing kefir to neonatal calves may be a viable approach to improve the health of calves in commercial calf-rearing operations, although to validate its health-promoting effects additional research is needed to investigate its effects under different calf-rearing conditions.

Key words: calf, health, kefir, performance

INTRODUCTION

An efficient calf-feeding management would contribute significantly to calf growth, health, and productivity and thus guarantee the future profitability and sustainability of a dairy farm (Tahmasbi et al., 2014). However, the first week or two after birth is when neonatal calves are at the greatest risk for enteric disease (Hulbert and Moisá, 2016), which is the most important impedance for welfare and the long-term productivity of calves (Donovan et al., 2002; Signorini et al., 2012; Bayatkouhsar et al., 2013). This highlights the importance of appropriate feeding and management strategies to minimize enteric disease in dairy calves.

Antibiotics have routinely been used to promote growth, health, and prevent disease in livestock (Hammer et al., 2016). However, owing to the emergence of antibiotic-resistant bacteria, an increasing scientific and commercial interest in the use of probiotics in calf-feeding systems is occurring (Donovan et al., 2002; Timmerman et al., 2005).

Probiotics are defined as beneficial microorganisms that have anti-infective or antibacterial activity against pathogenic microbes and can beneficially modify the gastrointestinal microflora and, thus, mitigate the incidence of diarrhea and improve performance (Gilliland et al., 2001; Schrezenmeir and de Vrese, 2001). Roo- poshti and Dabiri (2012) reported that adding a multi-strain probiotic to milk directly fed to calves improved growth performance and reduced fecal Escherichia coli count compared with control calves. Timmerman et al. (2005) also reported that probiotic treatment (Lactoba- cillus spp.) increased weight gain and reduced diarrhea incidence in neonatal calves.

Kefir is a functional fermented dairy product that has been produced through lactic or alcoholic fermentation of milk (Wszolek et al., 2001), soy (McCue and Shetty, 2005), coconut, and rice milks (Otles and
Cagindi, 2003). Kefir grain contains a symbiotic association of lactic acid bacteria (10⁸ cfu/g; 30 species or more), yeasts (10⁶–7 cfu/g), and acetic acid bacteria (10⁵ cfu/g), which are embedded into a polysaccharide matrix known as kefiran (Garrote et al., 2010; Guzel-Seydim et al., 2011). During the fermentation (usually between 18–24 h), kefir grains are propagated and increased in size and weight, which are generally recoverable for reuse in new fermentations after kefir is harvested (Garrote et al., 2010). Kefir has proven probiotic properties (Santos et al., 2003; Sabir et al., 2010; Zheng et al., 2013) and contains large amounts of AA, proteins, phosphorus, and calcium (Nielsen et al., 2014). Past studies indicated that feeding mice with kefir is associated with health benefits (Vinderola et al., 2005; Vinderola et al., 2006; Franco et al., 2013), possibly due to antimicrobial and antagonistic activities against pathogens, which is mainly attributed to the presence of hydrogen peroxide, peptides (bacteriocins), ethanol, carbon dioxide, diacetyl, and lactic and acetic acids (Nielsen et al., 2014; Likotrafiti et al., 2015). Despite a large number of studies on kefir as a nutraceutical product with murine models, to our knowledge no data are available on the responses to kefir consumption in neonatal calves. Because the digestive system of calves functions more similarly to monogastric digestion during the first weeks of life (Baldwin et al., 2004), we speculated that kefir consumption would improve the growth performance and health of neonatal calves.

MATERIALS AND METHODS

Kefir Preparation

Kefir grain, provided by the Department of Food Science and Technology (Isfahan University of Technology), was inoculated into milk at a ratio of 5% (wt/vol), incubated at 30°C for 22 h in the dark, and then aseptically filtered through a plastic sieve and cold-stored for the next culture incubation. Prior to being assigned to treatments, samples of kefir were collected weekly throughout the preweaning period and analyzed for pH and concentrations of TS and fat (Foss Electric, Hillerød, Denmark) at the Animal Nutrition Laboratory, Isfahan University of Technology. Kefir was characterized as having a pH of 3.9, 2.30% fat, and 12.5% DM. Only on d 0 and 41 of the experiment were the microbiological analyses carried out to determine the microflora of kefir (Lactobacillus spp., Lactococcus spp., Acetobacter, and yeasts) according to the procedures described by Habibi et al. (2011). The data are presented in Supplemental Table S1 (http://dx.doi.org/10.3168/jds.2016-10921).

Calves, Treatments, and Management

The experiment was implemented at a local dairy farm (FKA Agriculture and Animal Husbandry, Isfahan, Iran), and all procedures involving calves were in compliance with guidelines set by the Iranian Council of Animal Care (1995). The experiment was conducted from July through October 2014, when the average temperature (outside the barn) was 29°C and ranged from 19 to 40°C. Average values for wind speed, minimum relative humidity, and maximum relative humidity were 1.94 m/s, 10.2%, and 34.2%, respectively, throughout the experiment.

Thirty female Holstein calves born at the calf-rearing facility of the dairy farm were immediately separated from their dam after birth, weighed, fed their dam’s colostrum (within 2 to 6 h after birth), and moved to individual pens (1.5 m wide × 2.5 m long), all as a part of the farm’s protocol. Calves were entered to the study within 5 d; each day following parturition in the herd, the calves were checked for general health and initial BW and then enrolled for the study. Bedding was renewed every 24 or 48 h with fresh sawdust. Each pen was provided with natural light as well as artificial lighting (fluorescent light), which provided enough light at the eye level of calves to simulate the lighting outside. Artificial lighting was automatically switched on at night.

Calves were blocked by initial BW, ranging from 30 to 40 kg (mean = 38.2 ± 3.1 kg), individually housed in each pen, and received either the pasteurized waste milk (3.40 ± 0.11% fat, 2.70 ± 0.07% CP, and 4.92 ± 0.05% lactose; based on bulk tank samples), hereafter referred to as whole milk, at the rate of 4 kg/calf per day, with no kefir (control; KF0), kefir mixed with milk at 1:3 (vol/vol; KF1), or kefir mixed with milk at 1:1 (vol/vol; KF2). All calves were fed equal amounts of milk in steel buckets twice daily at 0800 and 1600 h. On d 45, the milk fed to all calves was reduced to once daily (morning only; 2 kg/calf per day), and then on d 50 calves were weaned. Calves remained in the study until d 70 to monitor their postweaning performance. From d 3 onwards calves had free access to starter diet (in meal form; particle size ≤2 mm) with 10% alfalfa hay (DM basis) included. Chemical analysis of calf starter met or exceeded the recommendations of the NRC (2001), and its ingredients and nutrient composition are presented in Table 1.

None of the calves died or were removed from the study throughout the experiment. Calves were checked on a daily basis by animal caretakers and treated (Supplemental Table S2; http://dx.doi.org/10.3168/jds.2016-10921) as necessary according to protocols
established by the herd veterinarian. Injections were given subcutaneously or intramuscularly, and the dosage administered was based on the directions on the bottle and BW of calves.

**Data Collection and Sampling**

Starter DMI was recorded daily on an individual basis and BW was recorded on d 3 of age and then every 10 d throughout the experiment (7 times). Gain-to-feed ratio was calculated as kilograms of BW gain per kilogram of total DMI. Blood samples were taken from the jugular vein in the neck of each calf on d 2 (immediately before treatment administration), 20, 40, and 70 after birth after the morning feeding. Blood samples were collected into evacuated tubes containing EDTA as an anticoagulant. Samples were placed immediately on ice, and then centrifuged at 2,000 × g for 20 min at 4°C. The harvested plasma was preserved at −20°C awaiting analysis. Body length (distance between the points of shoulder and rump), hip width (distance between hip bones), and heart girth (circumference of the chest) were measured on d 3 (initial), 50 (weaning), and 70 (end of study). These variables are hereafter referred to as skeletal growth variables. Fecal consistency was scored according to a 5-point scale as 1 = normal, 2 = soft to loose, 3 = loose to watery, 4 = watery, mucous, slightly bloody, and 5 = watery, mucous, and bloody (Heinrichs et al., 2003).

**Laboratory Analyses**

Representative samples of starter, ort and feces were dried at 55°C for 72 h and then ground through a 1-mm screen for subsequent analysis of ash, CP, and ether extract (EE) according to standard procedures described by AOAC (1990). Neutral and acid detergent fiber (inclusive of ash) contents were determined according to the method of Van Soest et al. (1991). Apparent digestibility of OM, CP, EE, and NDF was measured using total fecal collection during a 3-d period, from d 68 to 70, using acid-insoluble ash as an internal marker (Van Keulen and Young, 1977). Concentration of blood metabolites was determined using commercial kits [Pars Azmoon Kits, Tehran, Iran; catalog numbers: glucose (1–500–017)], total protein (1–500–028), albumin (1–500–001), globulin (1–500–011), calcium (1–100–008), phosphorus (1–500–027), and BUN (1–400–029)] according to the manufacturer’s instructions using an automated analyzer (Technicon-RA 1000 Auto analyzer; DRG Instruments GmbH, Marburg, Germany). Plasma concentration of globulin was calculated as (total protein – albumin).

**Statistical Analysis**

The experimental design was according to 10 pens per treatment, with 1 calf per pen, resulting in a total of 30 pens. Starter DMI, daily BW gain, and gain-to-feed ratio were analyzed separately for preweaning period (d 3 to 50), postweaning period (d 51 to 70), and over the entire experiment (d 3 to 70) as a complete randomized block design. Data on DMI of starter, daily BW gain, gain-to-feed ratio, and blood metabolites were analyzed using repeated measures over time by Proc Mixed in SAS (SAS Institute, 2002). Treatment, time of measurement (day or week), and treatment-by-time interactions were included in the model as the fixed components. Calf, time, and calf-by-time interaction were included in the model as the random components. Three different variance-covariance structures (compound symmetry, autoregressive, and Toeplitz) were tested; the autoregressive order 1 structure had the smallest Akaike and Bayesian information criterion and was used to model the error term for the within-subject effects of repeatedly-measured variables (Littell et al., 2000). Data on skeletal growth variables and nutrients digestibility were analyzed as described previously, with the exception that time and its interaction...
with treatment were excluded from the model. Initial BW at study enrollment was considered a covariate for preweaning starter intake, daily BW gain, and gain-to-feed ratio; weaning weight was considered a covariate for starter intake, daily BW gain, and gain-to-feed ratio during the postweaning period. Initial skeletal growth variables were considered as covariates in the model for skeletal growth analyses, and concentration of each blood metabolite on d 2 of age (baseline) was considered as a covariate for each corresponding metabolite. Data are presented as least squares means and standard errors of the means. The differences among treatments with \( P \leq 0.05 \) were considered significant. Separation of least squares means was accomplished using the Tukey’s test. For the analysis of fecal score, the daily recorded fecal scores, on a scale from 1 to 5, were first binary coded as 1 if the fecal score was 3 or greater (diarrhea) and as 0 if the score was 1 or 2 (healthy). Then, the differences in the fecal score data among the 3 treatment groups (KF0, KF1, and KF2) were compared using the PROC GENMOD with a binomial distribution and logit link function (SAS version 9.2). Calf was included in the model as random variable component to adjust for repeated measurements on the same calf. The working correlation structure was selected based on a first-order autoregressive [AR(1)], as this had the lower Akaike’s information criterion compared with the exchangeable correlation matrix structure.

RESULTS AND DISCUSSION

This paper describes for the first time the effect of kefir intake on performance and health of dairy calves. In this study, we hypothesized that adding kefir to the whole milk may beneficially improve calf performance and health.

Least squares means of starter DMI, daily BW gain, and gain-to-feed ratio calculated for preweaning (d 3 to 50), postweaning (d 51 to 70), and overall (d 3 to 70) periods are presented in Table 2. The average BW of calves at the start of the experiment (d 3) was 38.4, 37.8, and 38.3 kg for control (KF0), KF1, and KF2 calves, respectively. Average BW at weaning was 62.3 ± 1.3 kg (\( P = 0.50 \)). The experiment continued until d 70 when calves weighed 84.2 ± 2.28 kg (\( P = 0.71 \)). Over the experiment, feed intake and daily BW gain did not differ between treatments (\( P > 0.05 \)). This finding was expected because BW gain is usually reflected by change in DMI, which was not affected by treatment (Table 2).

The observations on growth performance of calves tended to contrast previous studies reporting the growth-promoting effect of probiotics in neonatal calves (Cruywagen et al., 1996; Timmerman et al., 2005; Bayatkouhsar et al., 2013). Recently, a meta-analysis study (Frizzo et al., 2011) found that the lactic acid bacteria supplementation increased BW gain (228 g/d) and improved gain-to-feed ratio (814 g less feed consumed/kg of daily weight gain), especially during the first 60 d of age, compared with calves receiving a diet without probiotic. Similarly in a recent study in chicken (Cho et al., 2013), it was shown that inclusion of 0.1% kefir improved growth performance. However, studies with goat kids (Ataşoğlu et al., 2010; Daş et al., 2012) showed that kefir did not improve the growth performance or nutrient intake. Likewise, overall mean of daily starter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KF0</th>
<th>KF1</th>
<th>KF2</th>
<th>SEM</th>
<th>Trt</th>
<th>Period</th>
<th>Trt × Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI of starter, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Preweaning (d 3 to 50)</td>
<td>0.47</td>
<td>0.56</td>
<td>0.53</td>
<td>0.06</td>
<td>0.55</td>
<td>&lt;0.01</td>
<td>0.38</td>
</tr>
<tr>
<td>Postweaning (d 51 to 70)</td>
<td>2.34</td>
<td>2.38</td>
<td>2.37</td>
<td>0.04</td>
<td>0.23</td>
<td>&lt;0.01</td>
<td>0.71</td>
</tr>
<tr>
<td>Overall (d 3 to 70)</td>
<td>1.00</td>
<td>1.14</td>
<td>1.06</td>
<td>0.08</td>
<td>0.74</td>
<td>&lt;0.01</td>
<td>0.43</td>
</tr>
<tr>
<td>Daily BW gain, kg/d</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preweaning (d 3 to 50)</td>
<td>0.50</td>
<td>0.53</td>
<td>0.56</td>
<td>0.05</td>
<td>0.37</td>
<td>&lt;0.01</td>
<td>0.72</td>
</tr>
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<td>Postweaning (d 51 to 70)</td>
<td>1.12</td>
<td>1.08</td>
<td>1.07</td>
<td>0.08</td>
<td>0.13</td>
<td>&lt;0.01</td>
<td>0.77</td>
</tr>
<tr>
<td>Overall (d 3 to 70)</td>
<td>0.68</td>
<td>0.68</td>
<td>0.71</td>
<td>0.06</td>
<td>0.78</td>
<td>&lt;0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>Gain-to-feed ratio, kg/kg</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preweaning (d 3 to 50)</td>
<td>0.53</td>
<td>0.51</td>
<td>0.56</td>
<td>0.07</td>
<td>0.43</td>
<td>&lt;0.01</td>
<td>0.92</td>
</tr>
<tr>
<td>Postweaning (d 51 to 70)</td>
<td>0.48</td>
<td>0.45</td>
<td>0.45</td>
<td>0.02</td>
<td>0.70</td>
<td>0.10</td>
<td>0.96</td>
</tr>
<tr>
<td>Overall (d 3 to 70)</td>
<td>0.47</td>
<td>0.46</td>
<td>0.47</td>
<td>0.09</td>
<td>0.61</td>
<td>&lt;0.01</td>
<td>0.86</td>
</tr>
</tbody>
</table>

1 KF0 = calves fed milk without kefir; KF1 = calves fed milk with kefir at 3:1 (vol/vol); KF2 = calves fed milk with kefir at 1:1 (vol/vol).
2 Trt = treatment effect; Period = time effect; Trt × Period = treatment × period interaction.
3 Gain-to-feed ratio was calculated as kg of BW gain/kg of total DMI.
intake was not affected by probiotics supplementation in neonatal Holstein dairy calves (Laborde, 2008).

When calves are under stress, the presence of unfavorable microbial populations may dominate and compromise the health status of the animal, where the health-promoting effects of probiotics on growth performance may be present (Timmerman et al., 2005). Because the current experiment was completed in well-managed, high-hygienic farming conditions, the favorable conditions and minimized environmental stressors resulted in the calves remaining healthy, which may explain the lack of significant difference on the growth performance of young calves fed different amounts of kefir.

**Blood Metabolites**

Least squares means for plasma concentrations of calves receiving whole milk with or without kefir, analyzed at 4 time points, including d 2 (baseline), 20, 40, and 70, are presented in Table 3. No treatment or day of sampling effects were detected for plasma globulin, albumin-to-globulin ratio, calcium, and calcium-to-phosphorus ratio. Albumin, total protein, and BUN were not affected by kefir treatment, but increases with sampling time ($P < 0.05$; Table 3). Plasma glucose and phosphorus were not affected by treatment or time of sampling; however, significant treatment-by-time interactions were observed for glucose and phosphorus concentrations ($Table 3; P < 0.01$ and $P = 0.02$, respectively).

The increase in blood albumin and BUN with advancing age (postweaning) is possibly due to the function of increased DM and CP intake (Khan et al., 2007). Knowles et al. (2000) studied the age-related changes in blood composition of dairy calves and reported that BUN decreased from birth to 6 d, but after weaning tended to increase linearly up to 83 d, which suggests the normal physiological shift with initiation of a functional rumen fermentation (Khan et al., 2008).

The serum constituents might be markers for protein (total protein, BUN, and albumin) and energy status (glucose; Hill et al., 2010), which may indicate that protein and energy status of calves was not affected by kefir intake. In agreement, Adams et al. (2008) investigated the effects of a probiotic bacterial strain in calves and reported that glucose, BUN, and total protein concentration in plasma remained unaffected.

**Skeletal Growth**

Least squares means for initial (d 3), weaning (d 50), and final (d 70) measurements of hip width, heart girth, and body length are presented in Table 4. On both d 50 (weaning) and 70, kefir-fed calves had a greater body length than did KF0 (control) calves. Heart girth was not affected by treatment. Kefir-fed calves tended to have greater hip width at weaning compared with KF0 calves ($Table 4; P = 0.06$).

A study by Riddell et al. (2010) showed that changes in hip width and heart girth remained unaffected by adding probiotic to the milk replacer. However, our observations tend to be consistent with the studies of Bayatkouhsar et al. (2013) and Lesmeister et al. (2004), which reported that probiotic-fed calves had greater hip height than control calves and attributed the difference to greater DMI and, thus, daily BW gain in probiotic-fed calves. However, in our experiment neither starter intake nor BW gain was affected by kefir consumption (Table 2). Other factors, therefore, might have contributed to the improvements in some skeletal growth variables. One possible explanation for this observation might be partitioning of intake nutrients to skeletal or fat deposition in response to kefir consumption, which may be caused by differences in postruminal availability of nutrient between the treatments. However, this hypothesis is not certain because a probiotic influence on skeletal growth of calves has not frequently been indicated in the literature.

**Fecal Scoring**

During the first 2 wk of life calves receiving kefir had a lower incidence of diarrhea ($P < 0.05$; Figure 1), which may suggest a potential health-promoting effect of kefir as a probiotic product. Very few fecal scores of 4 or 5 were detected in calves on any treatment (data not shown).

In contrast to the results of this experiment, a study with goat kids (Ataşoğlu et al., 2010) showed that kefir consumption did not affect fecal consistency. However, our findings may partially be supported by findings of a recent meta-analysis by Signorini et al. (2012), who reported a lower incidence of diarrhea in lactic acid bacteria-fed calves and attributed the response to the indirect or direct modulation of the endogenous microbiota or the intestinal immune system by probiotics. Moreover, Czamanski et al. (2004) proposed the bacteriostatic effect of kefir against both gram-positive and gram-negative bacteria, leading to the improved ecosystem of gastrointestinal microflora. The favorable effects of probiotic supplementation on gastrointestinal health, especially during the first weeks of life when the susceptibility to early-life enteric diseases is highest, is of paramount importance because of costs associated with high mortality and reduced productivity, and thus
long-term financial consequences (Quigley et al., 2005; Hulbert and Moisá, 2016). Increased survivability from enteric disease will, therefore, contribute significantly to the improvement of well-being and profitability of calf industry (McGuirk, 2008). This beneficial effect is especially highlighted when calves are under stress, such as when calves are kept in intensive-rearing systems or when they are under preventive antibiotic treatments where beneficial microorganisms, such as lactobacilli and bifidobacteria populations, may decrease and instead those pathogenic may increase, thus resulting in microbiota imbalance (Signorini et al., 2012).

**Total-Tract Apparent Digestibility of Nutrients**

Least squares means for apparent digestibility of OM, CP, EE, NDF, and ADF are presented in Table 5. These values are comparable with those reported in the literature for the weaned calves (Hill et al., 2010; Montoro et al., 2013). No differences were detected for

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment 1</th>
<th>P-value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>KF0 KF1 KF2</td>
<td>SEM</td>
</tr>
<tr>
<td>d 2 (baseline)</td>
<td>99.1 96.5 99.1</td>
<td>5.09</td>
</tr>
<tr>
<td>d 20</td>
<td>81.9 83.0 86.5</td>
<td>3.14</td>
</tr>
<tr>
<td>d 40</td>
<td>80.5 91.6 83.8</td>
<td>3.69</td>
</tr>
<tr>
<td>d 70</td>
<td>84.2 81.6 87.9</td>
<td>2.07</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 2</td>
<td>15.5 18.0 20.3</td>
<td>0.99</td>
</tr>
<tr>
<td>d 20</td>
<td>11.2 12.5 11.5</td>
<td>0.81</td>
</tr>
<tr>
<td>d 40</td>
<td>11.3 12.7 12.7</td>
<td>1.17</td>
</tr>
<tr>
<td>d 70</td>
<td>15.1 16.8 16.8</td>
<td>2.96</td>
</tr>
<tr>
<td>Total protein, g/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 2</td>
<td>6.50 6.93 6.51</td>
<td>0.27</td>
</tr>
<tr>
<td>d 20</td>
<td>5.91 5.70 5.90</td>
<td>0.18</td>
</tr>
<tr>
<td>d 40</td>
<td>6.12 5.91 6.13</td>
<td>0.08</td>
</tr>
<tr>
<td>d 70</td>
<td>3.15 3.05 3.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td></td>
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</tr>
<tr>
<td>d 2</td>
<td>2.73 2.63 2.73</td>
<td>0.06</td>
</tr>
<tr>
<td>d 20</td>
<td>2.93 2.88 3.01</td>
<td>0.08</td>
</tr>
<tr>
<td>d 40</td>
<td>3.15 3.05 3.12</td>
<td>0.05</td>
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<tr>
<td>Albumin:globulin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 2</td>
<td>0.72 0.62 0.72</td>
<td>0.07</td>
</tr>
<tr>
<td>d 20</td>
<td>1.07 1.06 1.09</td>
<td>0.06</td>
</tr>
<tr>
<td>d 40</td>
<td>0.98 1.01 1.04</td>
<td>0.07</td>
</tr>
<tr>
<td>d 70</td>
<td>1.04 1.06 1.06</td>
<td>0.04</td>
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<tr>
<td>Ca, mg/dL</td>
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</tr>
<tr>
<td>d 2</td>
<td>11.2 11.3 10.8</td>
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</tr>
<tr>
<td>d 20</td>
<td>10.4 10.4 10.4</td>
<td>0.15</td>
</tr>
<tr>
<td>d 40</td>
<td>10.7 10.1 10.7</td>
<td>0.16</td>
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<tr>
<td>P, mg/dL</td>
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</tr>
<tr>
<td>d 2</td>
<td>5.81 6.50 5.62</td>
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</tr>
<tr>
<td>d 20</td>
<td>7.03 6.94 7.10</td>
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</tr>
<tr>
<td>d 40</td>
<td>6.70 7.04 6.93</td>
<td>0.24</td>
</tr>
<tr>
<td>d 70</td>
<td>7.53 6.21 6.80</td>
<td>0.27</td>
</tr>
</tbody>
</table>

1KF0 = calves fed milk without kefir; KF1 = calves fed milk with kefir at 3:1 (vol/vol); KF2 = calves fed milk with kefir at 1:1 (vol/vol).

2Trt = treatment effect; Period = sampling time effect; Trt × period = treatment × period interaction.
apparent digestibility of OM, CP, EE, NDF, and ADF (P > 0.05). Zhang et al. (2016) reported that dietary supplementation of dairy calves with *Lactobacillus plantarum* and *Bacillus subtilis* improved digestibility of CP, which was attributed to the development of the probiotic community in the rumen and thus increased utilization of nutrients. Close correlation exists between gain-to-feed ratio and the utilization of nutrients (Zhang et al., 2016), which may explain why gain-to-feed ratio was not affected by treatment (Table 2).

**CONCLUSIONS**

Milk kefir had no detectable effect on BW gain; however, calves receiving kefir were observed to have a slightly greater body length and a lower incidence of diarrhea during the first 2 wk of life. Fermentation of milk with kefir grains therefore seems to be an economical, simple, and viable technique to improve calf health and productivity in intensive calf-rearing operations. However, the effect of different kefir-to-milk ratios on calf health and performance needs to be further investigated.

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**REFERENCES**


Hulbert, L. E., and S. J. Moisá. 2016. Stress, immunity, and the man-


Gilliland, S. E., L. Morelli, and G. Reid. 2001. Health and nutritional

Franco, M. C., M. A. Golowczyc, G. L. De Antoni, P. F. Pérez, M.

Fox, D. G., T. P. Tylutki, K. J. Czymmek, C. N. Rasmussen, and V.


Douro, C. Tölü, Y. Yurtman, and T.

Czamanski, R., D. Greco, and J. West. 2004. Evaluation of anti-

Cruywagen, C. W., I. Jordaan, and L. Venter. 1996. Effect of

Cho, J. H., Z. Zhang, and I. Kim. 2013. Effects of single or com-


Barker, A., F. Tahmasebi, A. Naserian, R. Mokarram, and R.

Baeyens, F. Functional characteristics of infant milk formulae.

Aldrich, J. 2000. Development and application of the Cornell uni-

1990. Evaluation of probiotics in food, including powdered milk with live


1984. Rumen development, intestinal growth and hepatic metabo-

1983. Administration of kefir-fermented milk protects mice against

1979. Development and application of the Cornell university nutri-

1978. Evaluation of potential antimicrobial synbiotics using Lactobacillus


1974. Effect of supplementation of lactic acid bacte-


1963. The antimicrobial properties of different strains of Lactoba-


1960. The antimicrobial properties of different strains of Lactoba-

1959. The antimicrobial properties of different strains of Lactoba-

1958. The antimicrobial properties of different strains of Lactoba-


1954. Development and application of the Cornell university nutri-


1945. Development and application of the Cornell university nutri-


1942. Rumen development, intestinal growth and hepatic metabo-


