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Original research article

Evaluation of kefir as a potential probiotic on growth performance, serum biochemistry and immune responses in broiler chicks

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ABSTRACT

This experiment was conducted to evaluate the effect of milk or molasses kefir as a probiotic on growth performance, carcass traits, serum biochemistry and humoral immune responses in broiler chickens. A total of 192 one-d-old as hatched broiler chicks (Ross 308) were randomly allotted to 4 treatments, each with 4 replicate pens of 12 chicks. The following treatments were applied: 1) a basal diet (C) and normal drinking water, 2) 2% milk kefir in drinking water, 3) 2% molasses kefir in drinking water, and 4) the diet C supplemented with commercial probiotic. At d 42, eight birds per treatment were killed for determination of carcass traits. Broilers at 28 days of age were bled for measuring antibody titers against Newcastle disease virus (NDV) and avian influenza virus (AIV), at 30 days of age for antibody titers against sheep red blood cell (SRBC), and at 42 days of age for biochemical analysis. Supplementing 2% milk kefir increased body weight of broilers at 28 and 42 days of age ($P < 0.05$). Supplementing 2% molasses kefir improved feed conversion ratio (FCR) of broilers during growth period ($P < 0.05$), but FCR of broilers in other periods was not affected. Daily feed intake, internal organ weights, and carcass traits were not influenced by the treatments except for small intestine and ceca length. Small intestinal length significantly decreased in broilers supplemented with milk and molasses kefir ($P < 0.05$). Molasses kefir supplementation significantly ($P < 0.05$) increased antibody titer against SRBC at 31 days of age but other immune related parameters were not statistically different among treatments. Biochemical parameters including serum protein, albumin, and triglyceride concentrations were not statistically ($P > 0.05$) influenced. Broilers supplemented with molasses kefir, had a significantly lower concentration of serum total cholesterol, low density lipoprotein cholesterol and elevated high density lipoprotein cholesterol at 42 days of age ($P < 0.05$). In conclusion, the results indicated that inclusion of 2% milk kefir in drinking water would improve growth performance of broiler chickens.

1. Introduction

Antibiotic growth promoters have been successfully used in subtherapeutic dosage to promote growth and protect health of chickens in poultry industry since 1940 (Landy et al., 2011a,b; Landy et al., 2012; Fekri Yazdi et al., 2014a,b; Nanekarani et al., 2012; Goodarzi et al., 2014). Antibiotic growth promoters were supposed to promote muscle growth in the poultry as a result of improved gut health, resulting in better digestion of feed (Visek, 1978). However, there is a fear that wider use of antibiotics as feed additives can lead to the development of antibiotic resistant bacteria, which poses a potential risk for humans if it is transferred (Nasir and Grashorn, 2006; Toghyani et al., 2010). Thus, efforts have been made in different parts of the world to limit the use of antibiotics in livestock production. Because of the ban on the use of antibiotics, there is growing demand for natural alternative substances, which can sustain or promote growth performance and prevent disease. Consequently, probiotics and prebiotics,

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phytogenic and herbal products have received increased attention as possible antibiotic growth promoter substitutions (Landy and Kavyani, 2014; Gibson and Roberfroid, 1995; Landy et al., 2012). Probiotics have been defined as micro-organisms which favor intestinal microflora balance (Fuller, 1989). Our previous study indicated that supplementing broiler reared under heat stress condition with a multi-strain probiotic (Primalac) could induce favorable influences on performance, immune responses and cecal microflora (Landy and Kavyani, 2014).

Kefir belongs to the probiotic group and it's a popular Middle Eastern drink. It's milk or molasses production fermented by the action of *Lactobacillus* (*Lactobacillus lactis*, *Lactobacillus helveticus*, *Lactobacillus casei*), *Streptococcus* (*S. cremoris*, *S. lactis*) and yeasts (Otles and Cagindi, 2003). Kefir possesses proteins, polysaccharides, ethyl alcohol, lactic acid, fat, minerals and vitamins (Magalhães et al., 2011). Cho et al. (2013) showed that orally administration of milk kefir would improve growth performance and benefit meat quality in broiler chickens. Thoreux and Schmucker (2001) observed the beneficial influence of milk kefir on specific mucosal immune response against cholera holotoxin in rats. Furthermore, Cenesiz et al. (2008) observed the positive effects of milk kefir on performance and serum biochemistry of broiler chicks.

Despite these findings, most of researches in this field have focused on the growth promoting effects of milk kefir and less attention has been given on kefir effects on humoral immune responses of broiler chicks. Also, so far there has not been any comparison between milk and molasses kefir in broilers. The aim of the present study was to examine the effect of milk and molasses kefir as a probiotic on growth performance, carcass traits, serum biochemistry and humoral immune responses in broilers and to ascertain the importance of fermentations culture on these responses.

2. Materials and methods

2.1. Kefir preparation

Bovine milk was purchased and heated to 80°C for 30 min in water bath, before cooling to inoculation temperature. The heat-treated milk and molasses were fermented separately by the addition of kefir grains at 20°C for 2 d. After fermentation, kefir was filtrated to remove the kefir grains.

2.2. Animals and dietary treatments

Procedures performed in this trial were reviewed and approved by the Animal Care Committee of University of Isfahan. A total of 240 one-d-old Ross 308 broiler chickens of mixed sex were obtained from a local hatchery and randomly allotted to 4 treatments, each with 4 replicate pens of 12 chicks. The following treatments were applied: 1) basal broiler diet (C) and normal drinking water (pH: 7; Nitrate: 12 mg/L; hardness: 100 mg/L; sodium: 40 mg/L), 2) the diet C + 2% milk kefir in drinking water, 3) the diet C + 2% molasses kefir in drinking water, and 4) the diet C supplemented with probiotic at 0.15 g/kg of Protexin (Probiotics International Ltd., Somerset, UK) and normal drinking water. Protexin is a multistrain probiotic comprising 7 bacterial and 2 yeast strains: *Lactobacillus plantarum* 1.89×10^{10} cfu/kg; *Lactobacillus delbrueckii* ssp. *Bulgaricus* 3.09×10^{10} cfu/kg; *Lactobacillus acidophilus* 3.09×10^{10} cfu/kg; *Lactobacillus rhamnosus* 3.09×10^{10} cfu/kg; *Bifidobacterium bifidum* 3.00×10^{10} cfu/kg; *Streptococcus salivarius* ssp. *Thermophilus* 6.15×10^{10} cfu/kg; *Enterococcus faecium* 8.85×10^{10} cfu/kg; *Aspergillus oryzae* 7.98×10^{9} cfu/kg; and *Candida pintolopesii* 7.98×10^{9} cfu/kg.

Table 1 lists starter, grower and finisher basal diets used in the study. Nutrient concentrations met the nutrient requirements for Ross 308 (Aviagen, 2009). The growing periods included 3 phases: starter period from 1 to 14 days of age, grower period from 15 to 28 days of age and finisher period from 29 to 42 days of age. The trial was carried out in pens ($120 \times 120 \times 80$ cm) for 6 wk and feed and water were provided for ad libitum intake throughout the entire experimental period. The lighting regimen consisted of a period of 23 h light and 1 h of darkness. The temperature in experimental house was maintained at 32°C from d 1 to 7 and gradually reduced at a rate of 3°C per week, and finally fixed at 22°C until the end of trial.

2.3. Performance and carcass components

On 1, 14, 28, and 42 days of age, body weights (BW) of broilers were determined. Growth performance parameters such as daily weight gain (DWG), daily feed intake (DFI), daily water consumption (DWC) and feed conversion ratio (FCR) defined as DFI/DWG (g:g) were recorded in different periods. Mortality was recorded as it occurred.

Daily weight gain, DFI and DWC were recorded in different growth periods, and FCR was calculated.

On 42 days of age, 8 birds per treatment were randomly selected, weighed, and killed by a manual neck cutter. Carcass yields were calculated by dividing eviscerated carcasses that were free from the head, feet, abdominal fat pad, and viscera by live weight. Proventriculus, gizzard, liver, pancreas, abdominal fat, small intestine, and cecum weights were determined and expressed as a percentage of live weight. Small intestine and ceca length was measured.

2.4. Immunity

On 9 days of age, broiler chicks were vaccinated with Newcastle disease virus (NDV) and avian influenza (AI; subtype H9) inactivated vaccine subcutaneously and NDV (Lasota) at 21 days of age (orally). Antibody titers against NDV, avian influenza virus (AIV), and sheep red blood cells (SRBC), and heterophil to lymphocyte (H:L) and albumin to globulin (A:G) ratios were measured as immune responses. At 25 days of age, chicks were sexed and 2 male broilers within each replicate were inoculated i.v. with 1 mL of 1% SRBC. At 6 d after inoculation, chicks were bled and plasma was collected. Total SRBC antibody was measured by the procedure described by Wegmann and Smithies (1966). Antibody titers were expressed as the $\log_2$ of the reciprocal of the last dilution which agglutination was observed. At 28 days of age, serum sample collected from 2 male broilers from each pen, and were used to analysis of antibody antigen NDV and AIV, via the hemagglutination inhibition methods (HI), HI antibodies were then converted to $\log_2$.

At 42 days of age, 2 broilers per pen were tested for H:L ratio. Chicks were bled and their blood samples were collected using syringes containing heparin to avoid blood clot formation. Blood smears were stained using May–Greenwald–Giemsa (Lucas and Jamroz, 1961). One hundred leukocytes, including granular (heterophils, eosinophils, and basophils) and nongranular (lymphocytes and monocytes), were counted, and the H:L ratio was calculated (Gross and Siegel, 1983).

To determine A:G ratio, at 42 days of age, 2 broilers per pen were bled and serum sample collected by the method described previously, albumin and protein concentrations were determined using spectrophotometer and the kit thereafter (Pars Azmoon Company; Tehran, Iran). Serum concentration of globulin was computed by subtracting albumin concentration from proteins.

Table 1
The ingredient and calculated composition of basal starter, grower, and finisher diets.

Item	Starter (1 to 14 d)	Grower (15 to 28 d)	Finisher (29 to 42 d)
Ingredient (as fed), g/kg			
Corn	545.5	540	567
Soybean meal 44% CP	400	390	366
Soybean oil	11	34	39
Dicalcium phosphate 22%	19.3	17	10
$CaCO_3$	10.6	8.9	8.8
NaCl	3	3	3
Trace mineral premix[1]	2.5	2.5	2.5
Vitamin premix[2]	2.5	2.5	2.5
DL-methionine	3	2.1	1.6
L-lysine	1.4	–	–
Calculated composition, g/kg			
Metabolizable energy, kcal/kg	2,810	2,980	3,050
Crude protein	215	210	200
Calcium	9.7	8.6	8.1
Available phosphorus	4.6	4.3	4
Methionine + Cysteine	10	9	8.2
Lysine	13.2	11.9	11.1
Threonine	8.3	8.3	6.3

[1] Provided the following per kg of diet: Mg, 56 mg; Fe, 20 mg; Cu, 10 mg; Zn, 50 mg; Co, 125 mg; I, 0.8 mg.
[2] Provided the following per kg of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 5 IU; vitamin K, 2 mg; riboflavin, 4.20 mg; vitamin B_{12}, 0.01 mg; pantothenic acid, 5 mg; nicotinic acid, 20 mg; folic acid, 0.5 mg; choline, 3 mg.

2.5. Serum biochemistry

At 42 days of age, after 12 h of fasting, blood samples were collected from the brachial vein into non-heparinised tubes from 2 birds in each replicate and incubated at 37°C for 2 h, centrifuged at 2,000 × *g* for 10 min to obtain serum (SIGMA 4–15 Lab Centrifuge, Germany). Albumin, total protein, triglyceride, total cholesterol, high density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, were measured using the kit package (Pars Azmoon Co; Tehran, Iran).

2.6. Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (SAS Inst. Inc., Cary, NC). Means were compared using Tukey test. Statements of statistical significance were based on $P < 0.05$.

3. Results

3.1. Performance and carcass traits

Data on performance indices are summarized in Table 2. Broiler BW did not differ between the experimental treatments on d 14, though it tended to increase in broilers supplemented with potexin or milk kefir. At 28 d of age, broilers supplemented with milk kefir (1,271.8 g) or probiotic (1,283.1 g) had higher BW compared with broilers supplemented with molasses kefir (1,144.3 g), but did not differ from the control (1,189.6 g) that was intermediate. At 42 d of age, broilers supplemented with milk kefir had higher BW (2,509.6 g) compared with control (2,440.9 g), and broilers supplemented with probiotic (2,487.7 g) or molasses kefir (2,327.4 g) that were not different between them. There were no significant differences in DFI between treatments, during starter, grower, finisher periods and entire experimental period. During the finisher period, broilers receiving molasses kefir lower FCR (1.86) compared with control (1.92), and broilers supplemented with probiotic (2.12) or milk kefir (1.99) that were not different between them. Feed conversion ratio of broilers in other periods was not affected, though it tended to improve in broilers supplemented with potexin or milk kefir. No differences because of treatment effects were observed on mortality. Experimental treatments had not any significant effect on DWC.

Table 2
Effect of experimental treatments on performance indices of broilers at different ages.

Item	Experimental treatments				SEM
	Control	Probiotic	Milk kefir	Molasses kefir	
Body weight, g					
14 days of age	385.4	404.0	407.2	391.5	11.5
28 days of age	1,189.6^{ab}	1,283.1^{a}	1,271.8^{a}	1,144.3^{b}	48.4
42 days of age	2,440.9^{ab}	2,487.7^{ab}	2,509.6^{a}	2,327.4^{b}	82.2
Daily feed intake, g/d					
1 to 14 days of age	32.0	32.4	32.2	33.3	1.2
15 to 28 days of age	93.2	95.3	94.2	87.2	21.0
29 to 42 days of age	170.7	178.0	176.8	166.8	6.3
1 to 42 days of age	98.6	101.8	101.0	95.7	3.5
Feed: gain, g:g					
1 to 14 days of age	1.37	1.31	1.29	1.40	0.1
15 to 28 days of age	1.63	1.53	1.53	1.64	0.4
29 to 42 days of age	1.92^{ab}	2.12^{a}	1.99^{ab}	1.86^{b}	0.1
1 to 42 days of age	1.74	1.78	1.72	1.72	0.03

SEM = standard error of mean.
[a,b] Values in the same row not sharing a common superscript differ ($P < 0.05$).

Table 3 shows relative weight means of organs as a percentage of live weight, and absolute small intestine and cecum lengths. Carcass yield and relative weight of digestive and non-digestive organs were not markedly affected by dietary treatments, although small intestinal weight tended to decrease in broilers supplemented with probiotic, milk or molasses kefir ($P > 0.05$). Cecum length increased in broilers supplemented with protexin compared with other groups. Small intestinal length decreased in broilers supplemented with milk (217 cm) and molasses kefir (210 cm) compared with control (247 cm), but did not differ from the broilers supplemented with probiotic (232 cm).

3.2. Immune responses

The effect of experimental treatments on humoral immune responses is presented in Table 4. The treatments had no effect on antibody titers against NDV and AIV. Antibody titer against SRBC increased in the group treated with molasses kefir (9) compared with control (7.75), and broilers supplemented with probiotic

Table 3
Effect of experimental treatments on carcass yield and internal relative organ weight of broilers at 42 days of age.

Item	Experimental treatments				SEM
	Control	Probiotic	Milk kefir	Molasses kefir	
Carcass, %	67.3	68.5	67.6	67.5	0.60
Abdominal fat, %	0.97	1.07	1.21	1.37	0.60
Liver, %	2.37	2.51	2.56	2.58	0.20
Gizzard, %	1.94	1.71	1.85	1.94	0.03
Proventriculus, %	0.43	0.50	0.49	0.53	0.07
Pancreas, %	0.285	0.275	0.282	0.266	0.04
Small intestine, %	6.44	5.83	5.79	5.66	2.10
Cecum, %	0.812	0.952	0.844	0.893	0.10
Small intestine, cm	247.0^{a}	232^{abc}	217^{bc}	210^{c}	11.1
Cecum, cm	41^{ab}	44^{a}	40^{b}	40^{b}	1.7

SEM = standard error of mean.
[a–c] Values in the same row not sharing a common superscript differ ($P < 0.05$).

Table 4
Effect of experimental treatments on antibody titers against Newcastle disease virus (NDV) and avian influenza virus (AIV) at 28 days of age and sheep red blood cells (SRBC) at 31 days of age, and heterophil to lymphocyte and albumin to globulin ratios at 42 days of age.

Item	Experimental treatments				SEM
	Control	Probiotic	Milk kefir	Molasses kefir	
NDV (log_2)	2.75	3.12	3.00	3.14	0.20
AIV (log_2)	2.37	2.75	2.62	2.66	2.20
SRBC (log_2)	7.75^{ab}	8.25^{ab}	7.28^{b}	9^{a}	0.50
H:L	0.30	0.30	0.35	0.35	0.03
A:G	0.89	0.92	0.85	0.90	0.10

SEM = standard error of mean; H:L = heterophil to lymphocyte ratio; A:G = albumin to globulin ratio.
^{a,b} Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

(8.25) or milk kefir (7.28) that were not different between them. Heterophil to lymphocyte and A:G ratios at d 42 were not markedly affected by the experimental treatments, though A:G ratio tended to decrease in broilers supplemented with milk kefir.

3.3. Serum biochemistry

Table 5 summarizes the impact of treatments on serum constituents at day 42 of age. Treatments did not induce any significant effect on the serum concentration of protein, albumin, and triglyceride. Broilers supplemented with milk kefir (95) or molasses kefir (96.85) had lower total cholesterol compared with broilers supplemented with probiotic (116.25), but did not differ from the control (106.37) that was intermediate. Broilers receiving molasses kefir had higher HDL and lower LDL cholesterol concentrations compared with other groups that were not different between them.

4. Discussion

In the present study, performance of broilers was improved by addition of milk kefir in drinking water; on the other hand supplementation of molasses kefir in drinking water could not induce any beneficial effect on performance of broilers. Similar to our results, Cenesiz et al. (2008) reported a higher body weight for broilers supplemented with 7.5% milk kefir in drinking water compared with control group, which is in agreement with the findings of Cho et al. (2013) who reported that broiler supplemented with 0.1% milk kefir in diets had significantly higher BW in the whole 5-week period compared with the control. Kefir is a natural product containing complex mixture of lactic acid bacteria and yeasts which have been reported to be probiotics (Fuller, 1989).

Table 5
Effect of experimental treatments on serum biochemical parameters of broilers at 42 days of age.

Item	Experimental treatments				SEM
	Control	Probiotic	Milk kefir	Molasses kefir	
Protein, g/mL	3.88	3.76	3.48	3.40	0.25
Albumin, g/mL	1.82	1.80	1.58	1.58	0.13
Triglyceride, mg/100 mL	56	56	49	57	4.90
Total cholesterol, mg/100 mL	106^{ab}	116^{a}	95^{b}	96^{b}	7.03
LDL-cholesterol, mg/100 mL	29^{ab}	34^{a}	29^{ab}	25^{b}	2.60
HDL-cholesterol, mg/100 mL	64^{b}	80^{ab}	65^{b}	95^{a}	8.20

SEM = standard error of mean; LDL = low density lipoprotein; HDL = high density lipoprotein.
^{a,b} Values in the same row not sharing a common superscript differ significantly ($P < 0.05$).

It is accepted that probiotics can promote gut health by inhibiting the growth of pathogens and favor beneficial microorganisms, resulting in better digestion and absorption of nutrients (Line et al., 1998; Pascual et al., 1999; Jin et al., 2000). Yaman et al. (2006) reported that milk kefir supplementation in drinking water significantly increased *Lactobacilli* spp. and total aerobic bacteria populations and decreased the populations of *Enterobacteriaceae* and coliforms in geese faecal. It has been previously reported the positive effect of single or mixture of *Lactobacilli* cultures on performance criteria of broilers (Zukifli et al., 2000; Salarmoini and Fooladi, 2011).

It has been previously reported that the efficacy of probiotics on performance depends on many factor such as microorganism composition and viability, application level, administration method, frequency of favor microbial, diets, age of the bird and environmental status (Patterson and Burkholder, 2003; Wang and Gu, 2010). Muna Hashim Ghazzay (2014) compared kefir fermentation in three media, including Minimum Essential Media (MEM), MEM plus molasses, and MEM plus lactose. Minimum Essential Media plus lactose showed the highest fermentation rate and microbial richness. It seems that in the present study molasses kefir could not improve broilers performance as a result of low rate of fermentation and ultimately lower microbial richness. Unfortunately, no other reports are available on the effects of molasses kefir on bird growth performance.

In the current study, experimental treatments failed to have any significant impact on carcass traits except for small intestinal length, which decreased in broilers supplemented with milk or molasses kefir. It may have caused poor FCR in the chicks, but it did not occur. Despite having shorter small intestinal length, the broilers utilized feed efficiently similarly to control group did. This might be a result of improved intestinal microbiota and morphology criteria. Furthermore, the decreasing effect of treatments on small intestinal weight can be attributed to beneficial effects of treatments on epithelial tissue of intestine. Broderick et al. (2014) reported that the microbiota affected gut morphology through their impacts on epithelial renewal rate, cellular spacing, and the composition of different cell types in the epithelium.

Fermented milks administered to mice resulted in significant effects on various immune responses such as increased immunoglobulin A (IgA) -producing cells (Perdigón et al., 1999, 2001), increased macrophage activity (Perdigón et al., 1994), and increased specific antibody responses (Cano and Perdigon, 2003). Can et al. (2012) reported a significant increase in immunoglobulin M (IgM) level of Çoruh trout which were supplemented with milk kefir. In the current study, immune related parameters neither positively nor negatively were affected. It is likely that a higher dosage of kefir may be needed to stimulate humoral immune responses.

Probiotics have been reported to possess hypolipidemic and hypocholesteremic properties in animal studies. Mohan et al. (1995) reported that supplementation of laying hen with probiotic reduced plasma and egg cholesterol. Cenesiz et al. (2008) reported that broilers received milk kefir had a significant lower total lipid and cholesterol. The lower plasma cholesterol concentration in broilers received molasses or milk kefir might be due cholesterol digestion by lactobacilli bacteria (Buck and Gilliland, 1994). The reduction of serum LDL and increasing HDL cholesterol by addition of molasses kefir in drinking water observed in the current trial might be due to the reduction of synthetic enzyme activities. Sanders (2000) reported that probiotics have an inhibition effect on hepatic β-hydroxy-β-methylglutaryl coenzyme A reductase which is an intermediate of mevalonate during the synthesis of cholesterol from acetyl-Co A.

5. Conclusion

In conclusion, the results indicated that inclusion of 2% milk kefir in drinking water would improve growth performance, and it can be used as a probiotic in broilers diet.

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