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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.

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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.

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