Effect of Kefir upon the Performance, Intestinal Microflora and Histopathology of Certain Organs in Laying Hens [1][2]

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[1] This project, originally entitled as “The effects of kefir as a probiotic on egg yield, quality and some parameters in laying hens”, was funded by the Commission for Atatürk University Scientific Research Projects (BAP-Project no: 2010/123)
[2] This study has been presented as poster in the “45th Annual Conference of Physiology and Pathology of Reproduction, 37th Mutual Conference on Veterinary and Human Reproductive Medicine and 1st Joint German-Polish Conference on Reproductive Medicine”. Berlin, 29 February - 2 March 2012 and published (as abstract) in Reprod Domest Anim, 47, (Suppl. 2):p.30 (Abstr. 68), 2012

Summary

In the current study, the effect of kefir upon the performance, intestinal microflora and histopathology of certain organs in laying hens was investigated. Totally, 108 Lohmann Brown layers, aged 24 weeks, were allocated randomly into three groups, as; Group C (control, n=36): no treatment, Group A (n=36): 10 cc, and Group B (n=36): 7.5 cc kefir per litre of water. Animals were fed for 10 weeks with basal diets. Livers showed moderate level of hydropic degeneration, some lipidosis and focal haemorrhages with high amounts of kefir (Group A). Fewer active follicles in the ovarium were also observed in this group. The egg yield was significantly (P<0.01) lower in Group A (89.40±0.91) than in Group C (92.50±0.83) and Group B (92.86±0.87). For the pH of large intestines, unlike the small ones, it was significantly changed (P<0.01) from basic to acidic milieu in kefir-treated groups. The titres of coliform (E. coli), aerobic (Lactobacillus spp.), and anaerobic bacteria (Peptostreptococcus spp.) were significantly decreased (P<0.05 to P<0.001) with increased intake for both intestinal tracts. We conclude that; i) high kefir intake could unfavourably impair the digestive organ structures, ii) the supplementations led to a marked decrease in the large intestinal pH and microbiological load of the intestines, and iii) high kefir level markedly decreased the egg yield, unlike the low concentration as leading to a considerable improvement from the 6th weeks onwards.

Keywords: Kefir, Laying hen, Microbiology, Pathology, Probiotic

Yumurtacı Tavuklarda Kefirin Performans, Barsak Mikroflorası ve Bazı Organların Histopatolojisi Üzerine Etkisi

Özet

Mevcut araştırmada, yumurtacı tavuklarda kefirin performans, barsak mikroflorası ve bazı organların histopatolojisi üzerine etkisi araştırıldı. 24 haftalık toplam 108 adet Lohmann Brown yumurtacı tavuk; Grup K (kontrol, n=36), Grup A (n=36): 10 cc ve Grup B (n=36): 7.5 cc kefiri/L su olarak rastgele 3 gruba ayrıldı. Hayvanlar 10 hafta süreyle bazal rasyonla beslendi. Yüksek kefir miktarı (Grup A), karaçigerlerde orta düzey hidropik dejenerasyon, belli düzeyde lipidosis ve fokal hemorajiler oluşturdu. Ayrıca, bu grupta ovariumdaki aktif follikül sayısının daha az olduğu gözlandı. Grup A'daki yumurta verimi (89.40±0.91) Grup C (92.50±0.83) ve Grup B (92.86±0.87) açısından önemli düzeyde (P<0.01) daha düşük bulundu. Kefir uygulanan gruplardaki kalın bağırsak pH'si, ince bağırsakların aksine, bazı aktivasyon orana doğru önemli düzeyde (P<0.01) değişti. Koliform (E. coli), aerobic (Lactobacillus spp.), ve anaerobik bakteri (Peptostreptococcus spp.) titreleri arttırdı ve probiyotik alımı ile önemli düzeyde (P<0.05 - P<0.001) azaldı. Sonuç olarak; yüksek kefir alımının sindirim organı yapısını olumsuz yönde etkileyebildiği, i) kefirden kalın bağırsak pH'si ve bağırsak mikroflorasının yükünü önemli düzeyde azalttığı, ve iii) yüksek kefir kefire, yumurta verimini önemli düzeyde azaltmasına karşın, düşük konsantrasyonun 6. haftadan sonra belli oranda arttırdığı kanısına varıldı.

Anahtar sözcükler: Kefir, Yumurtacı tavuk, Mikrobiyoloji, Patoloji, Probiyotik

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Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

Kafkas Univ Vet Fak Derg
20 (3): 363-370, 2014
DOI: 10.9775/kvfd.2013.10173
INTRODUCTION

Animal performance and feed efficiency are linked closely with the microbial load of digestive tract, structure of intestinal wall and immune system activity [3]. In recent years, supplements known as probiotics have gained considerable popularity.

Probiotics are live microorganisms and they improve the host health at adequate concentration [2]. The major microbes used as probiotics include Lactobacillus, Saccharomyces, Streptococcus, Aspergillus spp. and Bacillus [1,3]. The live bacteria in probiotics affect the host animal beneficially via improving the intestinal microbial balance [4]. Likewise, they inhibit the growth of pathogenic microorganisms by colonial formation [3]. Probiotics might enhance the permeability of epithelium, increase the phagocytosis and strengthen the non-specific immunity [8], and increase the feed efficiency by changing the intestinal microflora [7].

Several reports in poultry indicate valuable results obtained by various probiotics [3,8-10]. Of them, Lactobacillus sporogenes led to a greater egg yield [10]. Further, BioPlus 2B resulted in a higher egg yield but along with a decrease in egg yolk and serum cholesterol [11]. B. subtilis culture improved the egg production and eggshell thickness [12]. In broiler chickens, it was evidenced that high levels of intake may not always lead to the greatest performance [13]. Favourable effects of probiotics depend upon their abilities to tolerate heat, osmotic stress and oxygen stressors [14]. The probiotic bacteria have to survive in stomach (at low pH) and intestinal tract. Lactobacillus strains isolated from kefir have considerable probiotic properties at reasonable quantities [10]. Previously, we observed that kefir (7.5 ml/L) improved the feed conversion ratio [3], increased the live weights and reduced the total cholesterol and lipid levels of sera in broilers [9].

Kefir is a milk-based product and has long been used as soft drink in Northern Caucasia. It involves various bacteria and yeasts [16-18]. It ensures the enhancement and development of beneficial bacteria. These bacteria inhabit at the intestinal mucosa and provide an easier clearance of pathogen microorganisms [3,5,15,17,18].

Despite the numerous studies with probiotics in poultry, little research has been conducted on kefir to investigate their histopathological and microbiological effects in layers. Thus, we aimed to investigate the effect of kefir on the structures of digestive and reproductive organs, microbiological load of intestines and their consequences on the performance in laying hens.

MATERIAL and METHODS

Experimental Animals and Dietary Composition

One hundred and eight Lohmann Brown layers, aged 24-weeks-old were randomly allocated into three trial groups, as each group subdivided into 12 subgroups, comprising of 3 hens, as follows: Groups C (control, n=36): no treatment, A (n=36): 10 cc, and B (n=36): 7.5 cc kefir per litre of drinking water. All groups were fed with basal diets complying with the NRC recommendations for 10 weeks. Water and feed were available ad libitum. Nutrient levels of diet given are illustrated in Table 1. Feed given were analysed according to the methods of AOAC [21]. For this study, a report of ethics has already been obtained from the Local Board of Ethics for Animal Experiments at Atatürk University (Decision No: 26.03.2010/8).

Sampling, Testing and Observations

Egg production and mortality (for calculation of egg yield per live animal) were recorded daily for 10 wks. Egg weights (by weekly calculation) and eggshell thickness (on the onset, during and at the end of experimental period) were determined.

Six birds selected randomly from each treatment group were sacrificed at the end of experiments to determine the organs weights (liver, heart, spleen, gizzard), histopathology of organ structures (liver, gizzard, intestine, ovarium), the populations of intestinal microflora and the pH.

For histopathological examinations, tissue samples were obtained from the visceral and genital organs and
fixed in 10% neutral buffered formalin solution. After the routine processing, tissue samples were embedded in paraffin wax and sectioned at 5 µ. The sections were stained with haematoxylin and eosin (H-E). The changes were semi-quantitatively assessed under the light microscope with an ocular grid and 4x, 10x, and 40x objectives, respectively. A total of 10 high-power fields were randomly chosen for evaluations. Changes in the histopathological parameters of different tissues were given in Table 2.

For microbiological examination, the entire intestinal tracts were removed aseptically from the body and sections of the duodenum, lower small intestine and both caeca were ligated with a nylon string. An approximately 1 g of intestinal content was mixed with 9 ml of pre-reduced sterile dilution blank solution [22] and homogenised (for 3 min) using a homogeniser (Hettich Rotina 380 R, UK). From the initial 10⁻¹ dilution, subsequent 10-fold serial dilutions were made in a sterile pre-reduced dilution blank solution for anaerobic bacteria, while using 0.1% peptone for aerobics. The samples from duodenum, lower small intestine and caecum were diluted to 10⁻⁵, 10⁻⁷ and 10⁻⁹, respectively.

For each dilution, a volume of 0.1 ml was inoculated in agar roll-tube for anaerobes and on agar plate for aerobic ones. The medium (of 6 ml) roll-tube used for both culturing and counting the total anaerobes was FM 98-5 [23]. The plate media used were: MRS agar for Lactobacilli (Oxoid, England), Bifidobacteria agar for Bifidobacteri [24], Brain Heart Infusion agar (BHIA) for total aerobic bacterial count, MacConkey agar (BBL) for Coliforms, and KF Streptococcus agar (DIFCO, USA) for Streptococci. All the inoculated roll-tubes and plates were incubated at 39°C. The roll-tubes were incubated for 6 days to determine the total numbers of anaerobes, while the MRS and Bifidobacteria agar plates were incubated anaerobically for 2 days in a Gas-Pak container (Oxoid, England). The plates of total aerobes and Coliforms were incubated (aerobically) for 1 day, while those of Streptococci were incubated for 2 days.

For the pH values in the ileum and caecum, the caeca and 10 cm section of ileum (around the Meckel’s diverticulum ± 5 cm) were ligated and removed following decapitation of six birds. Intestinal contents were collected and their pH determined immediately using an electronic pH meter (WTW Inolab, Germany).

Probiotic kefir used was prepared daily as needed during the experimental period [3]. For culturing the kefir samples taken, Sabouraud’s dextrose agar and enriched culture media were used. Following the incubation period for 18-72 h, both gram (-) and lactophenol cotton blue staining methods were employed. For microscopic evaluations, microorganism identifications of the samples were then made using the conventional methods routinely.

**Statistical Analysis**

Data were presented as mean ± SEM. The values of microbiological findings (Coliform, aerobic and anaerobic bacteria) and pH of both small and large intestines as well as those of egg yields (number, weight and eggshell thickness) from the experimental groups were subjected to one-way analysis of variance (ANOVA) and Duncan multiple comparison test [24]. Differences between the experimental groups were considered significant, using the least significant differences (when P<0.05).

**RESULTS**

Microbial counts of kefir samples used are given in Table 3. It can be seen clearly that the major microorganisms available were Lactobacillus spp.

The appearances of histopathological changes in the liver, intestine, gizzard and ovarium are given according to the groups (A, B and C) in Fig. 1-3, respectively. There were no apparent pathological lesions in the liver and ovaries.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Groups</th>
<th>A (10 ml/L) (n=6)</th>
<th>B (7.5 ml/L) (n=6)</th>
<th>Control (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemorrhage</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydropic degeneration</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ovarium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicle loss</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villous atrophy</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gizzard</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilatation of glands</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cellular infiltration</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) No change, (+) mild change, (++) moderate change and (+++) severe change

<table>
<thead>
<tr>
<th>Microbial Group (n=6)</th>
<th>Microbial Counts (Log cfu ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus spp.</td>
<td>3.62±0.29*</td>
</tr>
<tr>
<td>Lactobacillus spp.</td>
<td>6.50±0.50</td>
</tr>
<tr>
<td>Candida spp.</td>
<td>4.17±0.31</td>
</tr>
</tbody>
</table>

* ±SE
of the Groups C and B. Mild or moderate haemorrhage, hydropic degeneration in some hepatocytes were observed in liver tissues of Group A (Fig. 1a). In intestinal sections, shortening intestinal villi or villous atrophy was detected (Fig. 1b). Besides, an increase for glandules of gizzard (Fig. 1c) was detected and there was a lack of prominent follicles in ovarian tissues (Fig. 1d).

The microbial loads and pH of small and large intestines with different levels of kefir are given in Table 4. The titres of Coliform (E. coli, for small intestines only), aerobic (Lactobacillus spp.), and anaerobic bacteria (Peptostreptococcus spp.) significantly (ranging from P<0.05 to 0.001) decreased as the amount of intake increased for both intestinal tracts.

For the pH of large intestines, unlike the small ones, it was significantly changed (P<0.01) from basic to acidic milieu in both kefir-treated groups.

The effects of kefir intake upon the parameters of egg yield in laying hens are given in Table 5. The weekly egg yields of groups concerned are illustrated in Fig. 4. For the egg yield, the decline observed during
the 6th-8th weeks was minimised in Group B (7.5 ml/L) as compared to those in other groups. Moreover, the greatest values from the 6th weeks onwards were also obtained in this group.

The effects of kefir on some organ weights in laying hens are given in Table 6.

**DISCUSSION**

In the current study, the effects of kefir upon the relationships between digestive, microbiological and pathological traits, and their consequences on reproduction in laying hens were investigated.
Effect of Kefir upon the...

Probiotics affect the host animal beneficially by both improving its intestinal balance and creating gut micro-ecological conditions that suppress harmful microorganisms like Campylobacters, Clostridium, Salmonella and Coliforms, and by favouring the beneficial ones like Lactobacillus and Bifidobacterium. Kefir is claimed to act against the pathogens and to have some anti-inflammatory activities. As with the previous findings, the increasing levels markedly lowered Coliform counts in small intestines. Lactobacillus can be classified as ‘colonising’ species, but its amount was markedly decreased in kefir-treated layers herein. The potential of probiotics to improve the beneficial bacteria while possibly suppressing the pathogenic ones in the intestines have been shown previously. Further, an increase of beneficial microorganisms in the intestine affected the performance favourably. The pH value is one of the main factors for flora competition and suppression of pathogenic bacteria. Probiotics tend the cause favourable impact on nutrient absorption by reducing the pH. Herein, we observed that kefir had no marked effect on the small intestinal pH, while it markedly decreased the pH of large intestine. The lowest pH value was observed in Group B. Similarly, Lactobacillus culture in broiler ration had no effect on the pH of the small intestine, but it decreased the caecal one. Also, the live yeast supplementation reduced the ileal pH in layers. However, the probiotic had no effect on the caecal pH in quails. These may presumably indicate the profound effects of the type of production and the species of birds.

Additionally, it was observed that although the supplementation of 7.5 ml/L kefir had no marked effect on the egg yield, but high concentration led to markedly lower yields. Besides, when weekly egg yield was considered, there was some increase (from the 6th week onwards) in the kefir group as compared to other groups. This was assumed to be the adaptation period for kefir intake during the earlier weeks. Our findings are somewhat similar to a previous study in that the 2.5 and 5.0 cfu.t⁻¹ Enterococcus lactiferm both led to a relative decline in egg yield. In another study, although the probiotic had no effect on the yield, but a marked decline was noted in egg weight. Herein, the kefir at 7.5 ml/L did not affect the egg yield, while there was a marked decline in egg weight. The 100 mg/kg probiotic markedly improved daily egg yield and shell thickness. Likewise, there were proportional

<p>| Table 6. The effects of kefir on some organs weights of hens |</p>
<table>
<thead>
<tr>
<th>Tablo 6. Yumurtacı tavuklarda kefirin bazı organ ağırlıkları üzerine etkileri</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organs Studied (g/100 g CA)</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Liver</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Spleen</td>
</tr>
<tr>
<td>Gizzard</td>
</tr>
</tbody>
</table>

*Means (±SEM) within the same row having different superscripts are significantly different from each other, NS: Non significant (P>0.05), *P<0.05

**Fig 4.** Weekly egg yields of kefir-treated groups* in laying hens

Groups; Group A: 10, Group B: 7.5, Control: zero ml/L kefir

**Şekil 4.** Kefir uygulanan gruplardaki* yumurtacı tavuklarda haftalık yumurta verimleri
increases in the same parameters in layers fed with 0.4% and 0.8% live yeast [29]. Further, various levels of probiotic (10 and 20 g of probiotic/kg ration) led to a marked increase in egg weight, but with only a slight increase in egg yield [30]. It can be seen clearly that the effects of probiotic on the egg yield, egg weight and eggshell thickness were variable in different studies. This may be due to differences of animal species and/or their age as well as the type/dose of probiotic used.

We observed no effect of kefir on heart and spleen weights, while a marked increase in liver and gizzard weights in kefir-treated groups. This may indicate that the increase of liver weight could be associated with the lipidoses occurred. By contrast, kefir was reported to have no marked effect on the organ weights in geese [31] and broilers [32].

In broilers, the probiotic led to a marked increase in the serum levels of LH, FSH and T3 hormones [33]. So, we presume that these could collectively lead to an improvement in the egg weights and egg yields following the supplementation at optimal level. The T3 is responsible for the gonadotropic hormone secretion. In poultry, the FSH is responsible mainly from the follicular growth, while the LH is responsible from the ovulation [34]. We observed broadly that the 10 ml/L kefir led to a small-size of follicles and low follicular numbers in the ovarium, while the 7.5 ml/L had no such adverse effects. Clearly, the ultimate results might vary due to both the type of supplement and its concentration used at rather narrow limits.

In Group A, common hyperplasic epithelial cells with desquamation, hyperplasia and dilatation in the intestinal lumens were observed. There were also hyperkeratotic areas in the lamina epithelialis and mononuclear cell infiltration in the intestinal propria. Further, the cell infiltration was observed in the gizzard propria. Likewise, the feeding with 1-2 kg/ton of Bioplas 2B caused a marked proliferation of lymphatic system in the lamina propria layer, along with hyperplasia in intestines of layers [35].

On the other hand, the probiotic had no deleterious effect on the morphology of gastrointestinal tract, liver and pancreas [36]. In the 10 ml/L kefir group, a shortening in the intestinal villi or villous atrophy was detected. By contrast, the higher levels of B. subtilis LS 1-2 led to an increase in the villus height in duodenum and ileum [37]. Moreover, the yeast derivatives improved the numbers of intestinal goblet cells, while reducing those of enterocytes undergoing apoptosis in broilers [38].

Finally, the higher amounts of kefir led to hepatic haemorrhage, lipidosis and hydropic degeneration. The undesirable pathological organ changes were thought to affect, more or less, the yield unfavourably. Phospholipoproteins of the yolk are synthesised in the liver, so we may presume that the pathological changes might have led to a decrease in the egg yield.

Conclusively, we suggest that; i) the high amount of kefir intake per se impaired the organ structures of digestive system, large intestinal pH and microbiological load of the intestinal tracts, as collectively leading to markedly lower egg numbers only, ii) the lower amount of intake impaired the microbiological load of both intestinal tracts and resulted in marked change in the pH of large intestines, as collectively leading to markedly lower egg weight only. Nevertheless, the low concentration resulted in considerable improvement in egg yield from the 6th weeks onwards as compared to other groups. Furthermore, we could presume that the improvement in egg yield with 7.5 ml/L kefir may well enhance the yield, if the duration of intake would have been prolonged well beyond the 10th weeks (up to the entire laying period). However, further studies comprising various concentrations of kefir upon the layer performance during longer durations in different bird species are warranted in future to confirm the present findings.

ACKNOWLEDGEMENTS

We gratefully acknowledge Assoc.Prof. Ertan Oruç (DVM, PhD) of Atatürk University for his kind help with pathological assessment of samples.

REFERENCES


25. SPSS 19 (for MacOS X): SPSS Inc, New York, USA.


