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

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

RESEARCH ARTICLE

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

Güler YENİCE [1] Demet ÇELEBİ [2] Mehmet Akif YÖRÜK [1] Ömer UÇAR [3]
Yavuz Selim SAĞLAM [4] Muhammet Ali TUNÇ [5] Serdar ALTUN [4]

[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*

[1] Division of Animal Nutrition and Nutritional Disorders, Faculty of Veterinary Sciences, Atatürk University, TR-25240 Erzurum - TURKEY
[2] Division of Microbiology, Faculty of Veterinary Sciences, Atatürk University, TR-25240 Erzurum - TURKEY
[3] Division of Reproduction & A.I., Faculty of Veterinary Sciences, Atatürk University, TR-25240 Erzurum - TURKEY
[4] Division of Pathology, Faculty of Veterinary Sciences, Atatürk University, TR-25240 Erzurum - TURKEY
[5] Division of Laboratory and Veterinary Sciences, Narman Vocational Collage, Atatürk University, TR-25530 Erzurum - TURKEY

Makale Kodu (Article Code): KVFD-2013-10173

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 kefir/L su olarak rastgele 3 gruba ayrıldı. Hayvanlar 10 hafta süreyle bazal rasyonla beslendi. Yüksek kefir miktarı (Grup A), karaciğerlerde orta düzey hidropik dejenerasyon, belli düzeyde lipidosis ve fokal hemorajiler oluşturdu. Ayrıca, bu grupta ovaryumdaki aktif follikül sayısının daha az olduğu gözlendi. Grup A'daki yumurta verimi (89.40±0.91), Grup C (92.50±0.83) ve Grup B'dekinden (92.86±0.87) önemli düzeyde (P<0.01) daha düşük bulundu. Kefir uygulanan gruplardaki kalın bağırsak pH'sı, ince bağırsakların aksine, bazikten asidik ortama doğru önemli düzeyde (P<0.01) değişti. Koliform *(E. coli)*, aerobik *(Lactobacillus* spp.), ve anaerobik bakteri *(Peptostreptococcus* spp.) titreleri artan probiyotik alımıyla birlikte önemli düzeyde (P<0.05 - P<0.001) azaldı. Sonuç olarak; i) yüksek kefir alımının sindirim organı yapılarını olumsuz yönde etkileyebildiği, ii) katkıların kalın bağırsak pH'sını ve bağırsakların mikrobiyolojik yükünü önemli düzeyde azalttığı, ve iii) yüksek kefir düzeyinin yumurta verimini önemli düzeyde azaltmasına karşın, düşük konsantrasyonun 6. haftadan sonra belli oranda artırdığı kanısına varıldı.

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

İletişim (Correspondence)
+90 442 2315560
gulerata@atauni.edu.tr

INTRODUCTION

Animal performance and feed efficiency are linked closely with the microbial load of digestive tract, structure of intestinal wall and immune system activity [1]. 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 [5]. Probiotics might enhance the permeability of epithelium, increase the phagocytosis and strengthen the non-specific immunity [6], 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 [15]. 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 [3,5,15,17,19].

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 [20] 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

Table 1. *The ingredients and chemical composition of basal diet*
Tablo 1. *Bazal rasyonun içeriği ve kimyasal bileşimi*

Ingredients	Amount %	Calculated Analysis	
Corn 8.53	44.5	ME	2.8 kcal g^{-1}
Soybean meal Brasil-46	17.0	Crude Protein	17.00
Wheat 10%	11.5	Calcium	3.37
Limestone	7.5	Available phosphate	0.38
Sunflower seed meal 36	7.5	Sodium	0.15
Soybean oil	5.0	Chloride	0.15
Corn Gluten-60	4.0	Linolenic acid	1.82
DCP 18	2.4	Lysine	0.79
Salt	0.26	Threonine	0.58
Min[1]-Vit[2] Premix	0.2	Tryptophan	0.19
DL Methionine 98%	0.09	Methionine+ Cysteine	0.73
L-Lysine	0.06		
Total[3]	100		

[1] Premix supplied per kg of diet: 10 mg Cu, 0.99 mg I, 50 mg Fe, 100 mg Mn, 0.08 mg Se, 100 mg Zn, [2] Premix supplied per kg of diet: 9.000 IU vitamin A, 1.78 mg vitamin B_1, 6.6 mg vitamin B_2, 30 mg niacin, 10 mg pantothenic acid, 3 mg vitamin B_6, 0.15 mg biotin, 1.500 mg choline, 0.015 mg vitamin B_{12}, 2.000 IU vitamin D, 18 IU vitamin E, 2 mg vitamin K, [3] All the values given were calculated from the NRC value [20]

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, MacConkay 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 (WTV 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 [25]. Differences between the experimental groups were considered significant, using the least significant differences (when $P<0.05$).

Table 2. *Types of histopathological changes and the level of their severities in control and experimental groups using different doses of kefir*
Tablo 2. *Farklı dozlarda kefir uygulanan deneysel ve kontrol grubunda histopatolojik değişim tipleri ve şiddet dereceleri*

Lesions	Groups		
	A (10 ml/L) (n=6)	B (7.5 ml/L) (n=6)	Control (n=6)
Liver			
Haemorrhage	++	-	-
Hydropic degeneration	+++	+	-
Ovarium			
Follicle loss	+++	-	-
Intestine			
Villous atrophy	++	-	-
Gizzard			
Dilatation of glandules	++	-	-
Cellular infiltration	+	-	-

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

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 3. *Microbial counts in kefir samples*
Tablo 3. *Kefir örneklerinin mikrobiyel bileşimi*

Microbial Group (n=6)	Microbial Counts (Log cfu ml^{-1})
Streptococcus spp.	3.62±0.29*
Lactobacillus spp.	6.50±0.50
Candida spp.	4.17±0.31

* *±SE*

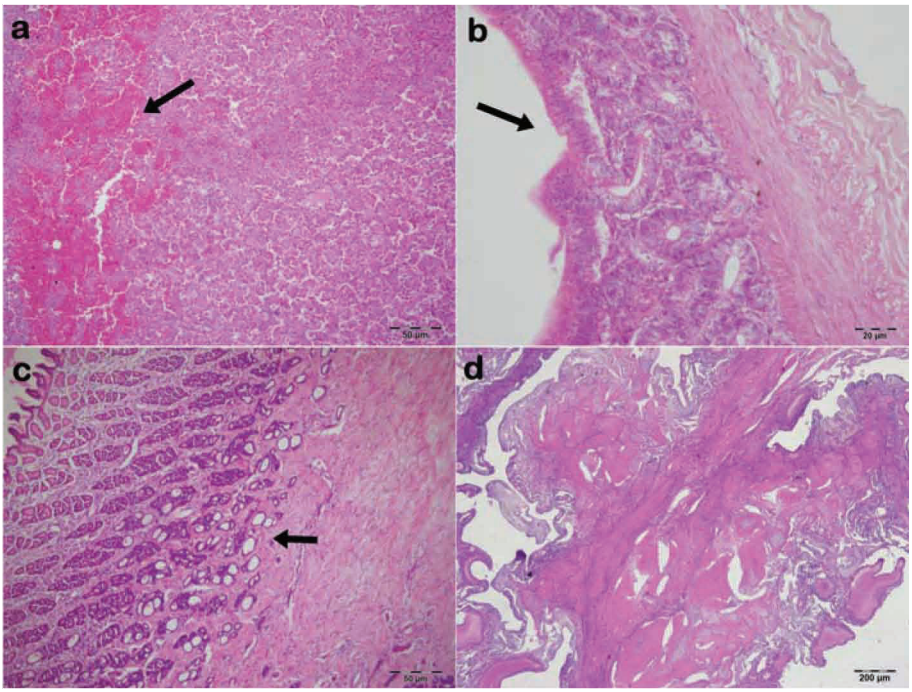


Fig 1. Histopathological findings of high concentration (10 cc/L) of kefir-treated group (Group A) in laying hens

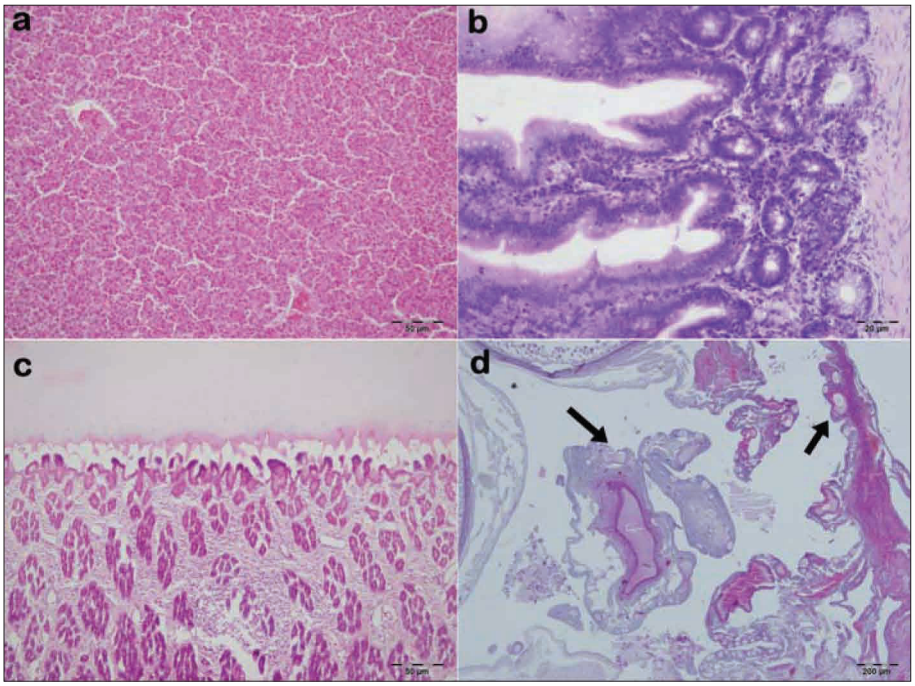
Group A; a) haemorrhagic foci in the liver tissue *(arrow),* x200, H-E, **b)** disappearances of villous architecture *(arrow)*, x400, H-E, **c)** dilatations in the glandules of gizzard *(arrow)*, x200, H-E and **d)** disappearances of normal follicular architecture in ovarium, x40, H-E

Şekil 1. Yüksek düzeyde (10 cc/L) kefir uygulanan gruptaki (Grup A) yumurtacı tavuklarda histopatolojik bulgular

Fig 2. Histopathological findings of low concentration (7.5 cc/L) of kefir-treated group (Group B) in laying hens

Group B; a) normal histological appearance of liver, x200, H-E, **b)** normal villous architecture and limited cellular infiltration of small intestine, x400, H-E, **c)** normal histological appearance of gizzard, x200, H-E and **d)** normal follicular appearance *(arrows)* of ovarium, x40, H-E

Şekil 2. Düşük düzeyde (7.5 cc/L) kefir uygulanan gruptaki (Grup B) yumurtacı tavuklarda histopatolojik bulgular



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

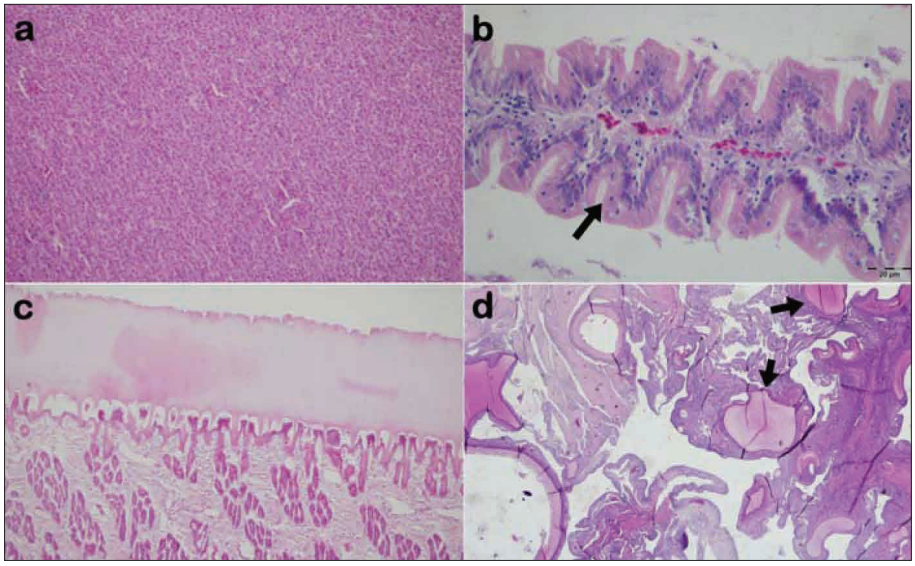


Fig 3. Histopathological findings of the control group (Group C) in laying hens

Group C; a) normal histological appearance of liver, x200, H-E, **b)** normal villous architecture *(arrow)* of small intestine, x400, H-E, **c)** normal histological appearance of gizzard, x200, H-E and **d)** normal follicular appearance *(arrows)* of ovarium, x40, H-E

Şekil 3. Kontrol grubundaki (Grup C) yumurtacı tavuklarda histopatolojik bulgular

Table 4. *The effects of kefir upon the microbial loads (Log cfu ml^{-1}) and pH of small and large intestines in laying hens*
Tablo 4. *Yumurtacı tavuklarda ince ve kalın bağırsakların mikrobiyel yükü (Log cfu ml^{-1}) ve pH'sı üzerine kefirin etkileri*

Parameters Studied		Groups			Statistics
		A (10 ml/L) (n=6)	B (7.5 ml/L) (n=6)	Control (n=6)	Significance
Small Intestine	*E. coli*	1.95±0.00^{b}	2.13±0.17^{ab}	2.67±0.33^{a}	*
	Enterobacter spp.	2.13±0.17	2.64±0.67	2.49±0.23	NS
	Lactobacillus spp.	2.46±0.51^{b}	3.15±0.41^{b}	5.00±0.52^{a}	**
	Peptostreptococcus spp.	1.95±0.00^{b}	1.95±0.00^{b}	3.99±0.52^{a}	***
	pH	5.81±0.32	6.47±0.13	6.19±0.37	NS
Large Intestine	*E.coli*	3.30±1.00	3.30±1.00	4.00±0.26	NS
	Enterobacter spp.	1.95±0.00	2.63±0.67	2.82±0.32	NS
	Lactobacillus spp.	1.95±0.00^{b}	3.64±1.09^{ab}	5.49±0.81^{a}	*
	Peptostreptococcus spp.	2.30±0.34^{b}	2.48±0.23^{b}	7.50±0.85^{a}	***
	pH	6.67±0.59^{b}	5.98±0.41^{b}	8.82±0.11^{a}	**

^{abc} Means (±SEM) within the same row having different superscripts are significantly different from each other, ***NS:*** *Non significant (P>0.05), * P<0.05, ** P<0.01, *** P<0.001*

Table 5. *The effects of kefir on production and egg quality parameters of hens*
Tablo 5. *Kefirin yumurtacı tavuklarda yumurta verimi ve kalitesi üzerine etkileri*

Parameters Studied	Groups			Statistics
	A (10 ml/L)	B (7.5 ml/L)	Control	Significance
Egg yield (%)	89.40±0.91^{b}	92.86±0.87^{a}	92.50±0.84^{a}	**
Egg weight (g)	63.14±0.27^{a}	61.93±0.29^{b}	63.04±0.28^{a}	**
Eggshell thickness (mm)	0.385±0.005^{a}	0.372±0.004^{b}	0.380±0.005^{ab}	*

^{ab} Means (±SEM) within the same row having different superscripts are significantly different from each other, ***NS:*** *Non significant (P>0.05), * P<0.05, ** P<0.01*

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.

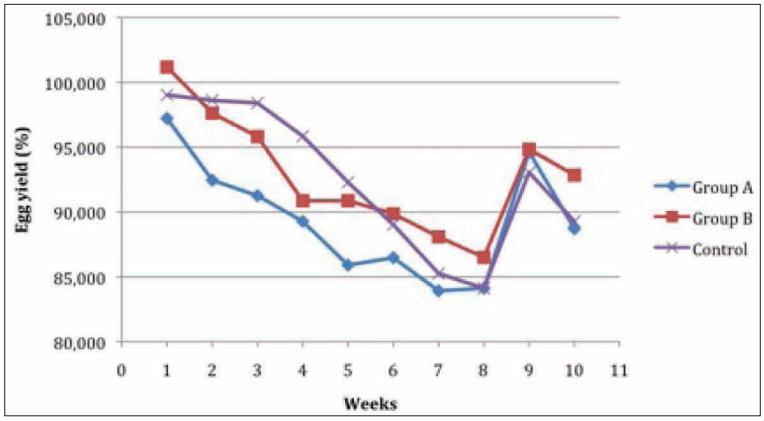


Fig 4. Weekly egg yields of kefir-treated groups* in laying hens

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

Şekil 4. Kefir uygulanan gruplardaki* yumurtacı tavuklarda haftalık yumurta verimleri

Table 6. *The effects of kefir on some organs weights of hens*
Tablo 6. *Yumurtacı tavuklarda kefirin bazı organ ağırlıkları üzerine etkileri*

Organs Studied (g/100 g CA)	Groups			Statistics
	A (10 ml/L) (n=6)	B (7.5 ml/L) (n=6)	Control (n=6)	Significance
Liver	2.80±0.10^{a}	2.82±0.19^{a}	2.22±0.10^{b}	*
Heart	0.48±0.03	0.48±0.02	0.45±0.01	NS
Spleen	0.12±0.01	0.13±0.01	0.13±0.00	NS
Gizzard	2.88±0.15^{a}	2.54±0.97^{ab}	2.24±0.10^{b}	*

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

Probiotics affect the host animal beneficially by both improving its intestinal balance and creating gut micro-ecological conditions that suppress harmful micro-organisms like *Campylobacters, Clostridium, Salmonella* and *Coliforms* [5,15,19,26], 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 [18,19]. As with the previous findings, the increasing levels markedly lowered *Coliform* counts in small intestines. *Lactobacillus* can be classified as 'colonising' species [1], 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 [8,17,26,27]. Further, an increase of beneficial micro-organisms in the intestine affected the performance favourably [28]. There was an obvious reduction in total bacterial count of ileum, while the number of *Lactobacilli* was increased with various yeast levels [29]. *Coliform* counts in the caecum of broilers receiving 0.05-0.10% *Lactobacillus* cultures were markedly lower than those of the controls [27]. Kefir, as *Lactobacilli*-yeast supplement (0.20% and 0.50%) markedly increased the numbers of *Lactobacilli,* while decreasing the numbers of total aerobic bacteria, *Coliforms*, *Enterobacteriaceae,* and *Enterococci* in faeces of goslings [30]. In our study, although the amount of intestinal microorganisms was decreased greatly, there was no increase in the population of beneficial ones. These observations may imply the apparent necessity of the optimisation of kefir concentration to be used.

The pH value is one of the main factors for flora competition and suppression of pathogenic bacteria [31]. Probiotics tend the cause favourable impact on nutrient absorption by reducing the pH [28]. 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 (5.98±0.41) 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 [8]. Also, the live yeast supplementation reduced the ileal pH in layers [29]. However, the probiotic had no effect on the caecal pH in quails [31]. 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 *(Fig. 4)*, there was some increase (from the 6th week onwards) in 7.5 ml/L 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 [32]. In another study, although the probiotic had no effect on the yield, but a marked decline was noted in egg weight [33]. 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 [34]. Likewise, there were proportional

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 [35]. It can bee 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 lipidosis occurred. By contrast, kefir was reported to have no marked effect on the organ weights in geese [36] and broilers [3].

In broilers, the probiotic led to a marked increase in the serum levels of LH, FSH and T_3 hormones [35]. 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 T_3 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 [37]. 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 *Bioplus 2B* caused a marked proliferation of lymphatic system in the lamina propria layer, along with hyperplasia in intestine of layers [38]. On the other hand, the probiotic had no deleterious effect on the morphology of gastrointestinal tract, liver and pancreas [39]. 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 [26]. Moreover, the yeast derivatives improved the numbers of intestinal goblet cells, while reducing those of enterocytes undergoing apoptosis in broilers [40].

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

1. Huyghebaert G, Ducatelle R, Immerseel FV: An update on alternatives to antimicrobial growth promoters for broilers. *Vet J*, 187, 182-188, 2011.

2. FAO/WHO: Evaluation of health and nutritional properties of powder milk with live lactic acid bacteria. Report from FAO/WHO Expert Consultation, 1-4 Oct, Cordoba, Argentina, 2001.

3. Karademir G, Ünal Y: The use kefir as probiotic in broiler. *Lalahan Hay Araşt Enst Derg*, 49 (1): 47-54, 2008.

4. Otles S, Cagindi O: Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. *Pak J Nutr*, 2 (2): 54-59, 2003.

5. Santini C, Baffoni L, Gaggia F, Granata M, Gasbarri R, Di Gioia D, Biavati B: Characterization of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*. *Int J Food Microbiol*, 141, 98-108, 2010.

6. Placha I, Simonova MP, Cobanova K, Laukova A, Faix S: Effect of *Enterococcus faecium* AL41 and *Thymus vulgaris* essential oil on small intestine integrity and antioxidative status of laying hens. *Res Vet Sci*, 89 (2): 257-261, 2010.

7. Alp M, Kahraman R: The use of probiotics in animal nutrition. *İstanbul Üniv Vet Fak Derg*, 22 (1): 1-8, 1996.

8. Jin LZ, Ho YW, Abdullah N, Ali, MA, Jalaludin S: Effects of adherent *Lactobacillus* cultures on growth, weight of organs and intestinal microflora and volatile fatty acids in broilers. *Anim Feed Sci Technol*, 70 (3): 197-209, 1998.

9. Cenesiz S, Yaman H, Ozcan A, Kart A, Karademir G: Effects of kefir as a probiotic on serum cholesterol, total lipid, aspartate amino transferase and alanine amino transferase activities in broiler chicks. *Medycyna Wet*, 64 (2): 168-170, 2008.

10. Panda AK, Rao SSR, Raju MVLN, Sharma SS: Effect of probiotic *(Lactobacillus sporogenes)* feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. *J Sci Food Agric*, 88 (1): 43-47, 2008.

11. Kurtoglu V, Kurtoglu F, Seker E, Coskun B, Balevi T, Polat ES: Effect of probiotic supplementation on laying hen diets on yield performance

and serum and egg yolk cholesterol. *Food Addit Contam*, 21 (9): 817-823, 2004.

12. Xu CL, Ji C, Ma Q, Hao K, Jin ZY, Li K: Effects of a dried *Bacillus subtilis* culture on egg quality. *Poult Sci*, 85, 364-368, 2006.

13. Alkhalf A, Alhaj M, Al-Homidan I: Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. *Saudi J Biol Sci*, 17, 219-225, 2010.

14. Ross RP, Desmond C, Fitzgerald GF, Stanton C: Overcoming the technological hurdles in the development of probiotic foods. *J Appl Microbiol*, 98 (6): 1410-1417, 2005.

15. Santos A, San Mauro M, Sanchez A, Torres JM, Marquina D: The antimicrobial properties of different strains of *Lactobacillus spp.* isolated from kefir. *Syst Appl Microbiol*, 26 (3): 434-437, 2003.

16. Abraham AG, De Antoni GL: Characterization of kefir grains grown in cows' milk and in soya milk. *J Dairy Res*, 66, 327-333, 1999.

17. Marquina D, Santos A, Corpas I, Muñoz J, Zazo J, Peinado JM: Dietary influence of kefir on microbial activities in the mouse bowel. *Lett Appl Microbiol*, 35, 136-140, 2002.

18. Rodrigues KL, Caputo LRG, Carvalho JCT, Evangelista J, Schneedorf JM: Antimicrobial and healing activity of kefir and kefiran extract. *Int J Antimicrob Agents*, 25 (5): 404-408, 2005.

19. Golowczyc MA, Mobili P, Garrote GL, Abraham AG, De Antoni GL: Protective action of *Lactobacillus* kefir carrying S-layer protein against *Salmonella enterica serovar enteritidis*. *Int J Food Microbiol*, 118, 264-273, 2007.

20. NRC: Nutrient Requirements of Poultry. 9th Revised ed., Natl Acad Press, Washington, DC, 1994.

21. AOAC: Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists, Arlington, VA, 1990.

22. Bryant MP, Burkey LA: Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. *J Dairy Sci*, 36, 205-217, 1953.

23. Jin LZ, Abdullah N, Ho YW, Jalaludin S: Comparison of different media for isolation of anaerobic bacteria from the duodenum, jejunum and cecum of chicken. **In,** *Proc of the 17th Malaysian Microbiology Symposiom,* 1994, Penang, Malaysia, 87-90.

24. Sofos JN, Fagerberg DJ, Quarles CL: Effects of sorbic acid feed fungistat on the intestinal microflora of floor-reared broiler chickens. *Poult Sci*, 64 (5): 832-840, 1985.

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

26. Sen S, Ingale SL, Kim YW, Kim JS, Kim KH, Lohakare JD, Kim EK, Kim HS, Ryu MH, Kwon IK, Chae BJ: Effect of supplementation of *Bacillus subtilis LS 1-2* to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Res Vet Sci*, 93 (1): 264-268, 2012.

27. Jin LZ, Ho YW, Abdullah N, Jalaludin S: Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing *Lactobacillus* cultures. *Poult Sci*, 77 (9): 1259-1265, 1998.

28. Chambers JR, Gong J: The intestinal microbiota and its modulation for *Salmonella* control in chickens. *Food Res Int*, 44, 3149-3159, 2011.

29. Saadia MH, Nagla KS: Effect of probiotic *(Saccharomyces cerevisiae)* adding to diets on intestinal microflora and performance of Hy-Line layers hens. *J Am Sci*, 6 (11): 159-169, 2010.

30. Yaman H, Ulukanli Z, Elmali M, Unal Y: The effect of a fermented probiotic, the kefir, on intestinal flora of poultry domesticated geese *(Anser anser). Revue Méd Vét*, 157 (7): 379-386, 2006.

31. Siriken B, Bayram I, Onol AG: Effects of probiotics: alone and in a mixture of Biosacc® plus Zinc Bacitracin on the caecal microflora of Japanese quail. *Res Vet Sci*, 75 (1): 9-14, 2003.

32. Horniakova E, Busta L, Flatnitzer F: Application of probiotic preparation IMB 52 in laying hens´ nutrition. *Slovak J Anim Sci, 39 (4): 191-196, 2006.*

33. Dizaji SB, Pirmohammadi R: Effect of *Saccharomyces cerevisiae* and Bioplus 2B on performance of laying hens. *Int J Agric Biol*, 11 (4): 495-497, 2009.

34. Panda AK, Reddy MR, Ramarao SV, Praharaj NK: Effect of dietary supplementation of probiotic on performance and immune response of layers in the decline phase of production. *Indian J Poult Sci*, 35 (1): 102-104, 2000.

35. Khalid HS, Saeb YAR: Effect of probiotic on some physiological parameters in broiler breeders. *Int J Poult Sci*, 10 (8): 626-628, 2011.

36. Sahin HE, Yardimci M: Effects of kefir as a probiotic on growth performance and carcass characteristics in geese *(Anser anser). J Anim Vet Adv*, 8 (3): 562-567, 2009.

37. Cebi C, Akcay E: Reproductive hormonal regulation in hens. *Lalahan Hay Araşt Enst Derg*, 50 (2): 103-115, 2010.

38. Mahdavi AH, Rahmani HR, Pourreza J: Effect of probiotic inclusion in different levels of barley substitution for corn diets on laying hen's histological changes of duodenum. *Proc 15th European Symp Poult Nutr,* Balatonfüred, Hungary, 25-29 Sept, 321-323, 2005.

39. Babińska I, Rotkiewicz T, Otrocka-Domagała I: The effect of *Lactobacillus acidophilus* and *Bifidobacterium spp.* administration on the morphology of the gastrointestinal tract, liver and pancreas in piglets. *Pol J Vet Sci*, 8 (1): 29-35, 2005.

40. Reisinger N, Ganner A, Masching S, Schatzmayr G, Applegate TJ: Efficacy of a yeast derivative on broiler performance, intestinal morphology and blood profile. *Livestock Sci*, 142 (2-3): 195-200, 2012.