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# Effects of dietary supplementation of kefir on body measurements, weight of visceral organs, and gut morphology in geese

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**ABSTRACT** - This study was designed to evaluate the effects of adding kefir on body measurements, giblet weight, and gut morphological patterns of geese through drinking water. A total of 54 birds were divided into three groups, each comprising 18 birds, and each group was further divided into three subgroups containing six birds each. One group served as the control group, while the other two groups were given kefirtreated drinking water at the ratios of 2.5 and 7.5%, respectively. No significant effects of kefir supplementation on body measurements, spleen weight, bursa weight, and gut morphology were observed. It is concluded that kefir may be used in poultry feed with an inclusion rate of 7.5% without imposing any adverse effect on the measurements of body structures, weights of visceral organs, and gut morphology.

**Keywords:** crypt depth, geese, natural alternatives, probiotic, villus length

# Introduction

The recent developments in the poultry sector have led to several innovations in profitable farming and production of safer foods for consumers. At the start of the 21st century, it was earnestly observed that administration of low-level doses of antibiotics in poultry feed for growth promotion resulted in chronic drug resistance leading to serious human health concerns. Ultimately in 2003, the use of antibiotics was completely banned in Europe, which urged the need to explore alternative feed additives that could fill the gap created by the ban of antibiotics and replace them in poultry feeds, while maintaining the same production levels.

A variety of feed additives are being used in the poultry sector, including phytogenic additives, enzymes, prebiotics, probiotics, and organic acids as alternatives to antibiotics (Karademir and Karademir, 2003). Probiotics are complex mixtures of microbes and are claimed to promote growth rate and health of animals by preventing the attachment of coliform bacteria, minimizing or inhibiting the growth of pathogenic bacteria, changing the absorption of nutrients from the intestine and modifying the balance of the microbes in the gut. They also seem effective in preventing or treating digestive disorders caused by stress (Reinhardt, 2015).

Kefir is one of the few products among probiotics that are being used in the poultry industry. It contains lactic acid bacteria and yeast (Marshall and Cole, 1985), 900 g kg<sup>-1</sup> moisture, about 100 g kg<sup>-1</sup> dry

matter, 30 g kg<sup>-1</sup> fat, 70 g kg<sup>-1</sup> ash, 300 g kg<sup>-1</sup> protein content, and 550 g kg<sup>-1</sup> nitrogen free extracts (Sekkal-Taleb, 2016). Kefir is made from the fermentation of milk or kefir grains by using a starter culture (Liu and Moon, 1983). It contains alcohol with a slightly acidic pH and is milky in appearance. It is well known that probiotics have potential effects on the growth performance of poultry including body weight gain and feed conversion ratio (Huang et al., 2004) and improve some other parameters such as meat and carcass quality (Pelicano et al., 2003; Kalavathy et al., 2006).

Kefir has been studied in many other poultry species (Kandir and Yardimci, 2015), but there is still a lack of sufficient literature to explore the effects of kefir as a probiotic in geese. Considering the aforementioned, this study was designed to investigate the effects of supplementation of kefir in drinking water on the body measurements, giblet weight, and intestinal morphology of geese.

# **Material and Methods**

This study was conducted on a commercial farm in the province of Afyonkarahisar in Turkey (38°45'24.7896" N latitude, 30°32'19.3344" E longitude, and 826 m altitude).

A total of 54 goslings, 21 days old each, were obtained from a hatchery and reared on floor pens with wood dust as litter. All managerial practices were performed as recommended for geese production. The temperature was adjusted at 25 and 20 °C for the 3rd and 4th weeks, respectively, and 20 °C for the remaining period. A 24-h lighting schedule was followed during the experiment.

Kefir was prepared from kefir grains that were procured from the Department of Food Hygiene and Technology, Faculty of Veterinary Medicine at Afyon Kocatepe University. It was manufactured by mixing 5% of active kefir grains with 3% of UHT cow milk and kept at 22 °C for 20 h for incubation (Marshall and Cole, 1985).

The birds were divided into three groups containing 18 birds in each, and each group was further subdivided into three subgroups comprising six birds each. One group was offered normal drinking water without any supplementation, as the control group, while the other two groups received 2.5 and 7.5% kefir in drinking water, respectively, by using individual drinker types for each replicate.

During 3-6 weeks of age, the goslings were fed a starter diet containing 220 g kg<sup>-1</sup> crude protein (CP), 2,900 kcal kg<sup>-1</sup> metabolizable energy (ME), and then, grower diet was provided during 7-12 weeks of age with 150 g kg<sup>-1</sup> CP and 2,900 kcal kg<sup>-1</sup> ME (Table 1). The experiment lasted for 63 days (nine weeks). At the end of the experiment, six birds from each group (from amongst 18) were slaughtered. Body length, neck length, wing length, and chest circumference values of the birds were recorded. After slaughtering, spleens and bursae were collected and weighed. For sample collection from the intestines, the whole intestinal tract was taken out. The tissues were prepared from the intestines for histopathological examination by the method described by Awad et al. (2011). The samples were collected from different parts of the intestines to measure the villus height, crypt depth, and tunica muscularis thickness. The gut samples were then preserved in a 10% buffered formalin solution for 48 h.

A 1.5-2-cm segment sample from the duodenum was taken 10 cm beyond the gizzard-duodenal junction, 5 cm proximal to the Meckel's diverticulum for jejunum and 5 cm proximal to the ileocecal junction for ileum. From the preserved samples, a proper section was cut and embedded in paraffin for routine histological examinations. After embedding, 4-µm sections were cut from each block by a microtome, mounted on a slide, and stained by hematoxylin & eosin (H&E) (Sakamoto et al., 2000; Solis de los Santos et al., 2005). The slides that were prepared were then examined in a light microscope equipped with a digital camera (Olympus CX41 attached Kameram R Digital Image Analyze System) from 10 different places for each parameter in each slide. The villus height was measured from the top of the villus up to the lamina propria (Sakamoto et al., 2000). The crypt depth was analyzed and noted between the crypt and villus, while the mucosa (*Muscularis* thickness) was measured from the top of the villus to the base of the *muscularis mucosa* (Aptekmann et al., 2001).

Table 1 - Ingredient and chemical composition of diet given to different groups of geese at 3-12 weeks of age

Item	Starter diet (g kg <sup>-1</sup> )	Grower diet (g kg <sup>-1</sup> )		
Ingredient				
Corn	480.0	480.0		
Wheat	100.0	100.0		
Wheat bran	0	100.1		
Maize bran	0	70.0		
Sorghum	0	37.5		
Sunflower oil	0	10.0		
Full fat soybean	100.0	0		
Sunflower meal	200.0	150.0		
Soybean meal	70.0	0		
Molasses	20.7	20.7		
Limestone	15.3	15.3		
Dicalcium phosphate (DCP)	7.6	10.0		
Salt	2.5	2.5		
Vitamin-mineral premix <sup>1</sup>	3.5	3.5		
Methionine	0.4	0.4		
Nutrient composition				
Dry matter (g kg <sup>-1</sup> )	890	890		
Crude protein (g kg <sup>-1</sup> )	220	150		
Metabolizable energy (kcal kg <sup>-1</sup> )	2900	2900		

<sup>&</sup>lt;sup>1</sup> Provided per kg of diet: vitamin A, 12,000,000 IU; Vitamin D3, 3,000,000 IU; vitamin E, 35,000 IU; vitamin K3, 3,500 IU; vitamin B1, 2,750 IU; vitamin B2, 5,500 IU; vitamin B3, 30,000 IU; Ca-D panthotenate, 10,000 IU; vitamin B6, 4,000 IU; vitamin B12, 15 IU; folic acid, 1,000 IU; D-biotin, 50 IU; choline chloride, 150,000 IU; manganese, 80,000 mg; iron, 60,000 mg; zinc, 60,000 mg; copper, 5,000 mg; iodine, 2,000 mg; cobalt, 500 mg; selenium, 150 mg; antioxidant, 15,000 mg.

The model assumptions of normality and homogeneity of variance were examined by Shapiro-Wilk and Levene's tests, respectively. The statistical analysis was performed by SPSS-10. An ANOVA was used for group comparisons, followed by Duncan's multiple range test for *post-hoc* analysis. The statistical model used to test the effects of treatment on the variables was:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

in which  $Y_{ij}$  = the response variable,  $\mu$  = general mean,  $\alpha_i$  = the effect of dietary treatments (DF = 2), and  $e_{ii}$  = the random error. The significance level was considered as P<0.05.

# **Results**

The results of the study indicated that there was no significant effect (P>0.05) of kefir supplementation on body, neck, and wing length or chest circumference (Table 2). Similarly, no effect was observed in the cases of spleen and bursa weights in all supplemented groups in comparison with the control group (Table 3). The differences in muscular thickness, crypt depth, villus height, and villus height:crypt depth ratio were recorded as non-significant in all groups in comparison with the control group (Tables 4 and 5).

Table 2 - Body measurements of geese at 3-12 weeks (63 days) of age offered different kefir treatments

Body measurement (cm)	0% kefir		2.5% kefir		7.5% kefir		- P-value
	Mean	SEM	Mean	SEM	Mean	SEM	r-value
Body length	33.00	0.37	32.20	1.20	33.00	0.68	0.69
Neck length	20.50	0.50	20.20	1.11	22.17	0.31	0.13
Wing length	45.67	0.95	45.20	1.46	46.00	1.06	0.88
Chest circumference	36.00	0.68	36.40	0.81	35.83	0.79	0.87

SEM - standard error of the mean.

Table 3 - Weight of visceral organs of geese at 3-12 weeks (63 days) of age offered different kefir treatments

Visceral measurement (g)	0% kefir		2.5% kefir		7.5% kefir		D .1 .
	Mean	SEM	Mean	SEM	Mean	SEM	P-value
Spleen	1.83	0.07	1.98	0.14	1.92	0.29	0.86
Bursa	1.93	0.20	1.93	0.13	2.45	0.34	0.31

SEM - standard error of the mean.

**Table 4 -** Villus height, crypt depth, and tunica muscularis thickness of geese intestine at 3-12 weeks (63 days) of age offered different kefir treatments

Muscular layer thickness (μm)	0% kefir		2.5% kefir		7.5% kefir		D .1 .
	Mean	SEM	Mean	SEM	Mean	SEM	- P-value
Duodenum	184.13	8.95	172.80	20.75	187.57	11.60	0.63
Ileum	187.77	29.85	204.39	8.37	212.09	20.21	0.52
Cecum	181.68	8.33	198.43	6.40	198.98	10.05	0.29
Colon	373.11	28.45	388.83	28.84	310.21	26.87	0.15
Crypt depth (µm)							
Duodenum	86.88	3.75	98.93	10.49	93.89	2.85	0.43
Ileum	81.56	2.39	89.96	5.85	81.24	4.24	0.37
Cecum	66.79	6.11	63.69	7.16	59.17	4.10	0.65
Colon	75.26	4.33	80.96	1.93	80.50	2.59	0.34
Villus height (μm)							
Duodenum	394.83	46.11	491.00	18.28	420.64	6.71	0.16
Ileum	460.35	39.32	434.42	10.10	364.65	40.17	0.16
Cecum	263.49	47.39	192.71	14.37	186.36	9.06	0.19
Colon	417.91	34.91	424.02	16.02	416.69	16.38	0.94

SEM - standard error of the mean.

**Table 5** - Relative villus height and crypt depth of the intestine of geese at 3-12 weeks (63 days) of age offered different kefir treatments

Villus height:crypt depth ratio	0% kefir		2.5% kefir		7.5% kefir		Danalasa
	Mean	SEM	Mean	SEM	Mean	SEM	- P-value
Duodenum	4.64	0.62	5.20	0.60	4.50	0.16	0.60
Ileum	5.73	0.67	4.90	0.32	4.50	0.52	0.28
Cecum	3.80	0.42	3.10	0.42	3.20	0.31	0.39
Colon	5.60	0.57	5.20	0.27	5.20	0.30	0.71

SEM - standard error of the mean.

### **Discussion**

Kefir belongs to a probiotic group that may exert positive and healthy effects on the host when ingested, by changing the microbial environment of the gut (Fuller, 1989). Probiotics may produce beneficial effects as they contain microorganisms such as lactic acid-producing bacteria lactobacilli, streptococcus, and bifidobacterial and may also induce some adverse effects because of the enterobacteria, clostridium, and enterococcus species. It is also known that probiotics inflict predominantly good effects on intestinal microbiota in enhancing its population and making it able enough to protect the intestines from pathogens, while maintaining the microbial population balance in the gut (Urdaneta et al., 2007; Yaman et al., 2006).

This study revealed that supplementation of kefir at the ratio of 2.5 and 7.5% in drinking water did not show any significance effect on body measurements in comparison with the control group. Kandir and

Yardimci (2015) also observed no significant effect of kefir supplementation with respect to head and foot size at the same dose levels in ducks. Likewise, it is evident that there is no well-known relationship between supplementation of probiotics and mineral absorption or bone measurements (Stavric and Kornegay, 1995; Jin et al., 1997; Simmering and Blaut, 2001; Patterson and Burkholder, 2003).

Although probiotics have been investigated extensively in poultry to explore their effects on various parameters such as performance and immune parameters and proven to be effective in many cases by improving performance, maintaining digestive health, and reducing dependence on antibiotics (Reinhardt, 2015), kefir has not been investigated much to record its effect on body measurements. Another study observed that probiotic (yeast) supplementation reduced tibial dyschondroplasia and improved bone strength (Plavnik and Scott, 1980), which demonstrated that probiotics may have beneficial impacts on bone parameters that might be due to the positive correlation between usage of probiotics and Ca:P retention (Nahashon et al., 1994).

The spleen and bursa weights were also not affected significantly by kefir supplementation on any level. Some other researchers also reported no significant increase in giblet weight in geese fed a kefir-supplemented diet (Sahin and Yardimci, 2009). Likewise, Karademir and Unal (2009) observed no significant difference in giblet organ weight in broilers given a diet with kefir. These results indicate that kefir may improve carcass hygiene by influencing the microbial balance in intestine (Yaman et al., 2006) but has no effect on body measurements. Similarly, Yenice et al. (2014) reported no significant increase in heart weight, but contrary to our results, they showed an increase in live and gizzard weight in kefir-supplemented groups. In our study, there was no significant increase or decrease in spleen and bursa weights, which might be related to feed intake, which remained unchanged in the kefir-treated groups.

Limited data are available regarding the effects of kefir supplementation on intestinal morphology. A study conducted by Urdaneta et al. (2007) demonstrated that kefir supplementation had a pronounced effect on intestinal enzyme activity and increased absorption of nutrients with no change in the morphologic structure in jejunum. These findings were similar to our results, indicating that supplementing kefir in drinking water has no effects on villus height, crypt depth, muscularis thickness, or on villus height:crypt depth ratio. Although kefir ingestion resulted in improvement of the population of beneficial lactobacillus microflora and decreasing of the population of aerobic microflora to maintain good health of the gut (Yaman et al., 2006), no effect was found in the gut in terms of morphology.

Based on the results of this study, it may be stated that kefir has no adverse effects if supplemented for geese via drinking water. Likewise, some studies reported that the use of kefir is beneficial in enhancing performance, immunity, suggested gut microbiota, and blood parameters (Cavazzoni et al., 1998; Abdulrahim et al., 1999; Santoso et al., 2001; Kalavathy et al., 2006; Arslan and Saatci, 2004; Karademir and Unal, 2009; Cenesiz et al., 2008; Salarmoini and Fooladi, 2011; Cho et al., 2013; Thoreux and Schmucker, 2001; Marquina et al., 2002; Vinderola et al., 2006; Urdaneta et al., 2007).

# **Conclusions**

The use of kefir in drinking water at the ratios of 2.5 and 7.5% show no significant effect on the weights of visceral organs, body measurements, and gut morphology. It may be recommended to use kefir in geese up to 7.5% without causing adverse effects on body structure and gut morphology of geese.

## **Conflict of Interest**

The authors declare no conflict of interest.

### **Author Contributions**

Conceptualization: I.S. Cetingul. Data curation: E.E. Gultepe. Formal analysis: A. Ulucan. Investigation: A.B. Akkaya. Methodology: E.E. Gultepe and A. Ulucan. Project administration: I. Bayram. Resources:

C. Uyarlar and A.B. Akkaya. Supervision: I. Bayram. Visualization: I. Bayram. Writing-original draft: A. Rahman. Writing-review & editing: A. Rahman and C. Uyarlar.

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