

## The Effects of Cereal Based Fermented Functional Food Supplementation on Growth, Cecal Microflora and Duodenum Histology of Quails (*Coturnix coturnix Japonica*)

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### ABSTRACT

The aim of this study was to determine the effect of dietary cereal based fermented functional food (CBFFF) supplementation on growth performance, duodenum histomorphology, internal organ development and cecum microflora of quails. In this study, a total of 100 mixed sex birds at 21 age of days were used as animal material. The birds were divided into 2 dietary groups as control (C) and 5 g/kg CBFFF supplementation (F) with 5 replicates (5 male average 64.33±0.32g and 5 female average 66.42±0.38g for each replicate). The experiment lasted 21 days. Live weight gain (LWG), feed intake (FI), and feed conversion ratio (FCR) determined weekly. At the end of the experiment, one female and one male bird were selected from each replicate (a total number of 20 quails) and their characteristics of cecum microbiota and duodenum histomorphology were determined. LWG, FI, FCR, inner organs, did not change among the groups. Lactic acid bacteria (LAB) and yeast count was tend to increase, *Enterobacteriaceae* was tend to decrease. *E. coli* and Coliforms count in cecum decreased statistically. Villi length increased in F group than in the C group. To conclude, CBFFF decreased pathogenic bacteria in cecum and developed intestinal microflora and increased the digestion surface area in duodenum. Therefore, it can be said that 5 g/kg dietary CBFFF can use to improve cecum microflora and duodenum histomorphology in quails. But further studies should be conducted to determine probiotics effects of CBFFF on other animal species, doses or stress factors such as feed restriction, high fiber or low protein in diet etc.

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## Tahıl Bazlı Fermente Fonksiyonel Gıda Katkısı İlavasının Bildiricilerin Büyüme, Sekal Mikroflora ve Duodenum Histolojisine Etkileri (*Coturnix coturnix Japonica*)

### MAKALE BİLGİSİ

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### ÖZ

Bu çalışmanın amacı, bildiricinin yemlerine tahıl bazlı fermente fonksiyonel gıda (TBFFG) ilavesinin bildiricilerin büyüme, duodenum histomorfolojisi, iç organ gelişimi ve sekum mikroflorası üzerindeki etkisini belirlemektir. Bu çalışmada 21 günlük yaşta 100 adet karışık cinsiyette bildiricinin kullanılmıştır. Kontrol (C) ve 5 g / kg CBFFF ilaveli (F) grupları 5'er tekerrürlü olarak (5 erkek ortalama 64,33 ± 0,32g ve 5 dişi ortalama 66,42±0,38) 2 muamele grubuna ayrılmıştır. Çalışma 21 gün sürmüştür. Canlı ağırlık artışı (CAA), yem tüketimi (YT) ve yemden yararlanma oranı (YYO) haftalık olarak belirlenmiştir. Çalışma sonunda her bir tekerrürden bir dişi ve bir erkek bildiricinin seçilerek (toplam 20 bildiricinin sayısı) sekum mikrobiyotası ve duodenum histomorfolojisi özellikleri belirlenmiştir. CAA, YT, YYO, iç organ ağırlıkları değişmemiştir. Laktik asit bakterileri ve maya sayısı artış eğilimi, *Enterobacteriaceae* sayısı ise düşüş eğilimi göstermiştir. Sekumda *E. coli* ve Coliform sayısı istatistiksel olarak azalmıştır. Villi uzunluğu F grubunda C grubuna göre artış göstermiştir. Sonuç olarak, TBFFG sekumdaki patojenik bakterileri baskılamış ve bağırsak mikroflorası gelişimini ve duodenumda sindirim yüzey alanını arttırmıştır. Bu nedenle 5g / kg diyet CBFFF'nin bildiricilerde sekum mikroflorasını ve duodenum histomorfolojisini iyileştirmek için kullanabileceği söylenebilir. Yine de TBFFG 'nın diğer hayvan türleri üzerindeki probiyotik etkilerini, farklı dozların veya yem kısıtlamasının, diyetle yüksek lif veya düşük protein gibi stres faktörlerinin etkilerini belirlemek için daha fazla çalışma yapılmalıdır.

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## Introduction

After the ban of antibiotic use in animal production, researchers have focused on new probiotic feed additives such as probiotics and symbiotics instead of antibiotic growth promoters in animals. It has been reported that probiotics increases poultry performance (Samli et al., 2007; Coskun et al., 2017; Sözcü and Ipek, 2017; Asadi et al., 2018). There is a trend on the using of dairy and non-dairy fermented foods as a probiotic source on animals in last decade. Cenesiz et al., (2008) reported that fermented dairy products (kefir, yogurt) works like commercial probiotics, dietary kefir supplementation increased growth of broilers and also, Boostani et al., (2013) reported that dietary yogurt supplementation increased growth performance. General effects of these dairy fermented food additives is that they increases growth of chicks (Boostani et al., 2013; Cenesiz et al., 2008; Karademir and Unal, 2009; Yaman et al., 2006) by increasing gut health (Yaman et al., 2006; Yenice et al., 2014). Fermented dairy products are functional foods for human nutrition, determined that they work like probiotic in the body. There are a lot of functional foods that is not dairy product and they are also used as functional foods for human nutrition. They are fermented product and they contain live probiotic bacteria. Fermented foods with yeast or lactic acid bacteria have been using in human nutrition since ancient times. These foods have been also known as functional foods and they are alternative probiotic source for human consumption. Cereal based fermented functional food can be a candidate of these functional foods as known tarhana traditional Turkish fermented cereal food (Özdemir et al., 2007). LAB and yeast fermentations occur simultaneously during CBFFF production (Bilgiçli et al., 2006). It is produced by mixing wheat flour, yoghurt, yeast, tomatoes, onions, green and red pepper, salt, herbs, mint and thyme. Fermentation occurs until the 5 days (Ekinci, 2005). Different ingredients such as milk, soybean, lentil, chickpea, corn flour, and egg can also be added (Ekinci and Kadakal, 2005) to increase nutritional value. Organic acids are produced in fermentation, low moisture content (6–10%) and low pH (3.3–5.0) provide bacteriostatic effects on pathogenic microorganisms and so increase shelf life. At the end of fermentation, it was determined that CBFFF includes average  $8 \times 10^8$  *L. acidophilus* and  $6 \times 10^9$  *S. thermophilus* (Daglioglu, 2000). In earlier studies, it was well documented that CBFFF has symbiotic effect as a functional food in humans (Gabrial et al., 2010). Although CBFFF has been determined to be used as a functional food in humans, there has been no study about the using of CBFFF as a probiotics source in animals.

Therefore, the aim of this study was to investigate the effect of CBFFF on quail growth performance, inner organ development, caecal LAB, yeast, *Enterobacteriaceae*, *E. coli*, total coliform count and duodenum histomorphological parameters.

## Material and Methods

### Cereal Based Fermented Functional Food

One kilo of tomatoes, red peppers, onions were boiled and crushed and mixed with 500 grams of soured yoghurt, two kilograms of flour and 100 grams of salt, and left to

fermentation for 3 days. The product obtained at the end of fermentation has 4.0 pH and left to dry for 2 days. 22% crude protein (CP) and  $7 \times 10^7$  cfu/g live LAB is determined in the dried fermented product.

### Animals and Housing

In this study, 21 d old 100 chicks (60 males and 60 females) equalized live weights selected for study. The quails were divided into two experimental groups with five replicates (10 chicks, 5 males  $64.33 \text{ g} \pm 0.32 \text{ g}$  and 5 females  $66.42 \text{ g} \pm 0.38 \text{ g}$ ) at equal body weights for each replicate. Experimental groups were as follows: control (C) and 5 g CBFFF supplemented group per kg diet (F). CBFFF contains 22% Crude protein, 1.2% Crude ash, 3.5% Crude fibre. All quail chicks in this study were housed in floor pens with wood shavings. They were fed ad libitum and offered fresh water daily with plastic waterer seized 1 litre. The experimental feed was purchased in local feed supplier in Kırşehir province and nutritional composition diet is given in Table 1. Illumination was provided during the experiment. Room temperature was 28°C at the beginning of the study and, then, gradually decreased to 26°C on d 21 of the trial. Live weight gain and feed intake were measured at 7, 14, and 21 d of study. At the end of study, two birds were slaughtered (one female and one male per replicate), to determine the weights of internal organs (heart, liver, gizzard, proventriculus, gastrointestinal tract length (GITL) and duodenum histomorphology. Mortality was not observed during experimental period.

Table 1. Composition of the experimental diet (%)

Feed Ingredients	g/kg
Maize	44.00
Soybean meal (44)	41.15
Meat and bone meal (35)	4.00
Soybean oil	6.50
Dicalciumphosphate	2.50
L-lysine HCl	0.70
DL-methionine	0.35
Salt	0.30
Vitamin Premix*	0.25
Mineral Piremix**	0.25
Calculated nutrient composition	
ME (kcal/kg)	3080
Crude protein	22.39
Crude fibre	2.80
Ether extract	8.50
Calcium	7.60
Utilizable phosphorus	3.80

\*Premix provided per kg of diet: \* Vitamin A, 12,000 IU; Vitamin D3, 2,400 IU; Vitamin E, 30 mg; Vitamin K3, 4 mg; Vitamin B1, 3 mg; Vitamin B2, 7 mg; Vitamin B6, 5 mg; Vitamin B12, 15 µg; niacin, 25 mg; \*\* Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; Choline, 125000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.

### Cecum Microbiota

Samples of the caecal contents were collected into sterile glass tubes in which they were kept on ice until subsequent inoculation into agars. MRS agar (MERCK, Darmstadt, Germany, 1.10660) was used for enumeration of LAB at 37°C for a 3-d incubation period and malt extract agar (MERCK, Darmstadt, Germany, 1.05398) was used

for enumeration of yeast at 30°C for a 3-d incubation period. VRBD (Violet Red Bile Dextrose) (MERCK, Darmstadt, Germany, 1.01406) agar was used for enumeration of *Enterobacteriaceae* at 37°C for an 18-20 h incubation period. 3 M Petrifilm TM (3 M Microbiology Products St. Paul MN 55114 USA) was used to determine *Escherichia coli* and Coliforms count in caecal samples. The following manufacturer's instructions for incubation conditions were used to determine the microbial counts of samples: *Escherichia coli*: at 32°C for 24 h; Coliforms: at 35°C for 24 h. Bacterial colonies were counted by determining the average number of live bacteria per g caecal content. LAB, Yeast, *Enterobacteriaceae*, *E. coli* and Coliforms counts of the samples were converted into logarithmic colony forming units (CFU/g).

### Duodenum Histomorphology

Duodenum samples were cut into 1.0 cm pieces and placed into 10% formalin for histomorphological processing. Tissues sections were inserted into tissue cassettes. After dehydration process, tissue sections were embedded in paraffin blocks, cut to 10-µ thickness, and placed on a slide. Each sample of ileum histomorphological tissue was prepared and stained with PAS staining procedure by using standard paraffin-embedding methods. The following the manufacturer's instructions for PAS staining were used to illustrate of duodenum mucosa. After embedding process, villi length and width were evaluated by using an image processing and analysis system (ZEN 2012 SP2) for Zeiss Primo Star HD Light Microscope.

### Statistical Analyses

The data were analysed using the independent samples T test procedure of SPSS software (SPSS 15).

### Results

LWG, FI, FCR did not change (Table 2). But live weight gain was tended to increase with dietary CBFFF supplementation. Internal organs (Table 3), heart, liver, gizzard, proventriculus and gastro intestinal length (GITL) did not affect with dietary CBFFF supplementation (P>0.05).

Dietary CBFFF supplementation decreased the colonization of *E. coli* and coliforms (P<0.05) without affecting LAB, yeast and *Enterobacteriaceae* colonization in cecum (Table 4). But LAB and yeast colonization were tended to increase and also, *Enterobacteriaceae* colonization was tend to decrease in cecum with CBFFF supplementation. Dietary CBFFF supplementation increased villi length (P<0.05) without effecting crypt depth and villi crypt ratio (VCR) (Table 5).

Table 2. The effect of CBFFF on quail growth

Traits	C	F	Pe
LWG (g)	175.88±4.26	180.89±2.62	0.37
FI (g)	474.79±3.85	482.28±9.12	0.25
FCR	2.70±0.66	2.67±0.18	0.15

LWG= Live weight gain, FI= Feed intake, FCR= Feed conversion ratio. CBFFF= Cereal base fermented functional food.

Table 3. The effect of CBFFF on quail inner organ development

Traits	C	F	P
Heart	1.06±0.03	1.03±0.06	0.16
Liver	2.34±0.12	2.43±0.11	0.39
Gizzard	2.95±0.12	2.96±0.16	0.57
Proventriculus	0.44±0.028	0.46±0.015	0.07
GITL	27.47±0.91	27.95±1.74	0.22

GITL= Gastro Intestinal Tract length. CBFFF= Cereal base fermented functional food

Table 4. The effect of CBFFF on cecal microflora

Traits	C	F	P
LAB	6.21±0.29	7.09±0.30	0.58
Yeast	6.67±0.22	7.22±0.15	0.55
E coli	6.82±0.28 <sup>a</sup>	6.53±0.10 <sup>b</sup>	0.05
Coliforms	7.17±0.45 <sup>a</sup>	6.67±0.14 <sup>b</sup>	0.01
<i>Enterobacteriaceae</i>	7.72±0.25	7.03±0.24	0.73

a-b-Means in a row with no common superscript letters differ significantly (P<0.05). LAB=Lactic acid bacteria. CBFFF=Cereal base fermented functional food.

Table 5. The effect of CBFFF on duodenum histomorphology

Traits	C	F	P
Villi Length	547.68±29.40 <sup>b</sup>	665.60±17.30 <sup>a</sup>	0.01
Crypt depth	26.83±1.24	27.19±0.87	0.81
VCR	20.97±1.40	24.86±0.95	0.17

a-b-Means in a row with no common superscript letters differ significantly (P<0.01). VCR= Villi crypt ratio. CBFFF=Cereal base fermented functional food.

### Discussion

The results of the current study showed that cereal based fermented functional food improved gut health and digestion surface area in Japanese quails. It was expected that dietary CBFFF supplementation can increase weight gain, but quails live weight gain at 42 d of life but not important statistically. In this study, quail chicks reared 21 day for separate sexuality to determine accurately breast feather colour. After 21 d male and female chick's weight determined and separated with equal weight belonging treatments and the study conducted in fully controlled poultry house. Thus, there were no any stress factors during experimental period. Live weight gain may not be affected because of this reason. It was reported that performance parameters may affect from multiple factors such as genetic, sanitation of poultry house or other environmental stress factors (Ozturk and Yildirim, 2004).

The current study is the first experiment on the effect of CBFFF on quail's growth. The result of this study is consisted with the earlier studies about different fermented feed supplementation to poultry diet. Namely, Yasar and Gok, (2014) reported that dry fermented wheat in diet increased quail growth and also Coskun (2018), reported that dietary dried sourdough supplementation increased body weight gain, yeast colonization in cecum and villi length in ileum of quails. Ashayerizadeh et al., (2018), reported that dietary fermented rapeseed supplementation increased growth performance of broiler chicks. Wang et al. (2018), reported that 5% dietary fermented corn gluten meal supplementation increased broiler growth performance. Yasar and Yegen, (2017) reported that 0.5 and 1% *Saccharomyces cerevisiae* fermented food

additive supplementation increased growth performance and developed FCR of broilers. Without any detrimental effect of CBFFF on growth and internal organ development of quails, our results consistent with the earlier studies that determined non-dairy fermented feed supplementation increases growth of chickens.

It was determined that *E. coli* and Coliform count in cecum content decreased by statistically ( $P < 0.05$ ). Although there was no difference among the groups in terms of *Enterobacteriaceae*, yeast and LAB count, *Enterobacteriaceae* population was tended to decrease, yeast and LAB count were tended to increase in quails consumed CBFFF. So, it can be said that dietary CBFFF supplementation improved quail gut health as functioned as commercial probiotics in quail's gut. Samli et al. (2007), reported that dietary probiotics prevented growth of pathogen bacteria in digestive system of broiler chickens.

Probiotics facilitates a wall between the intestinal wall and the lumen of gut for the pathogenic bacteria. Probiotics in a bird's digestive tract increases the production of volatile fatty acids which in turn decrease the pH level in the digestive tract. Lowering the pH level and decreasing high volatile fatty acid create an unfavourable environment for pathogens. The result of our study showed that CBFFF has a promising probiotic for livestock. Because, dietary CBFFF supplementation decreased pathogenic bacteria population and increased beneficial bacteria population in quail's cecum. Differentiation of cecum microbiota showed that LAB in CBFFF protected liveability and suppressed pathogenic bacteria. Ozdemir et al., (2007) reported that CBFFF can store with 6 month with any loss due to its low pH (3.5-5.0) and moisture content. Indeed, in our laboratory we determined that CBFFF included  $7 \times 10^7$  live LAB. According to the results, it can be said that fermented foods can be used for probiotics source in livestock.

The result of the fermented food supplementation on the intestine histomorphology is finite. In these finite studies, Altop, (2019) reported that fermented cherry kernels supplementation increased ileum villi length in broilers. Also, Gungor and Erener, (2020a-b) reported that fermented cherry kernels supplementation increased growth performance, ileum villi length and increased LAB count in caecum of broilers. Chu et al., (2017) reported that fermented wheat bran increased ileum histomorphological parameters in broilers. Dietary CBFFF supplementation increased villi length in duodenum and our findings about histomorphology is consistent with earlier studies. According to the earlier studies, differences in gut histomorphology such as small villi and deeper crypts have been related to toxins (Yason et al., 1987). Drop of villi length leads to reduced digestive surface area. Crypts include stem cells responsible for renewal (Buclaw, 2016) and epithel cells regeneration (Yasar and Forbes, 1999) of villi. The current histomorphological parameters showed that dietary CBFFF supplementation increased digestion surface in duodenum. There are a lot of functional foods peculiar to countries including live probiotic bacteria worldwide. These functional foods were well documented by Granato et al., (2010) and liquid functional foods were well documented by Martins et al., (2013). But there has been no study about these functional foods using as a probiotic source in animals.

## Conclusion

It was determined that CBFFF improves gut health and digestive surface of quails and it can be used as probiotic feed additive in chickens. CBFFF increased beneficial bacteria population and decreased pathogenic bacteria population in caecum. But further studies should be conducted to investigate the effects of CBFFF and other functional foods including live probiotic bacteria on different bacteria development or under different stress factors in other animal species.

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