Effect of dietary raw and fermented sour cherry kernel (*Prunus cerasus* L.) on digestibility, intestinal morphology and caecal microflora in broiler chickens

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ABSTRACT This study was conducted to investigate the effect of dietary raw sour cherry kernel (RC) or fermented sour cherry kernel (FC) on apparent digestibility, ileal morphology, and caecal microflora in broiler chickens. Raw sour cherry kernel was fermented by *Aspergillus niger* for 7 D. A total of 343 one-day-old Ross 308 male chicks were assigned to 7 dietary treatments consisting of 7 replicates of 7 broilers each. All birds were fed with a commercial diet or diets supplemented with 1%, 2%, or 4% RC or FC. The experimental period was 42 D. Apparent dry matter (DM), nitrogen and ash digestibilities were diminished ($P < 0.05$) by dietary RC inclusion, although dietary FC did not negatively affect ($P > 0.05$) nutrient digestibility. Dietary 1% FC increased ($P < 0.01$) the villus height to crypt depth ratio (VH:CD) compared with the other treatment groups, although RC4 reduced the villus height (VH, $P < 0.001$) and VH:CD ($P < 0.01$), compared with the control group. Dietary treatments had no effect ($P > 0.05$) on the crypt depth (CD). Birds fed 1% FC had the highest ($P < 0.05$) caecal *Lactobacillus spp.* counts among the treatment groups. *Enterococcus spp.* and *Escherichia coli* counts in cecum were not affected ($P > 0.05$) by dietary treatments. The results showed that the dietary inclusion of 1% FC improved ileal morphology and caecal microflora without any adverse effect on the apparent digestibility. These results indicate that FC has the potential to be a feed additive which improves intestinal health for broiler diets.

Key words: sour cherry kernel, digestibility, intestinal morphology, caecal microflora, broiler chicken

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INTRODUCTION

Probiotics and herbal products are largely used for improving intestinal health, as well as controlling the growth of harmful bacteria in broiler chickens (Altop et al., 2018; Steiner, 2006). *Aspergillus niger* is one of the major probiotics supplemented to broiler diets (Harimurti and Hadisaputro, 2015). Moreover, *A. niger* has the capacity to produce enzymes, such as amylase, protease, cellulase, xylanase, lipase, and tannase (Couri et al., 2000; Wu et al., 2015).

Sour cherry (*Prunus cerasus* L.) is a stone fruit from the *Rosaceae* family. Total annual production of sour cherry reached 1.2 million tonnes worldwide in 2017 (FAOSTAT, 2017). Cherry kernels are discarded after being separated from the fleshy parts in fruit juice factories (Yilmaz and Gokmen, 2013). Kernel wastes might cause environmental pollution if not disposed properly. Hauling the waste will add to the transportation cost for the factory. Raw sour cherry kernel (**RC**) has a potential use in poultry as an intestinal health enhancer due to its phenolic content (Kim et al., 2005). However, RC involves cellulose in high quantities and contains antinutritional components, such as cyanogenic glycosides (Senica et al., 2016), which have harmful effects on poultry (Rodríguez et al., 2001). These properties can limit the use of RC in broiler diets.

Solid-state fermentation refers to microbial growth in moistened solid substrates without free water (Gungor et al., 2017). In recent years, solid-state fermentation has largely been used in the utilization of agri-industrial byproducts produced at a rate of billions of tonnes every year. Solid-state fermentation can improve nutritional composition, decrease cellulosic components, eliminate antinutritional factors, and increase the biological activity of phenolic compounds in organic materials, as well as enrich substrates with enzymes and unknown growth promoters (Altop, 2019; Cao et al., 2012; Gungor et al., 2017; Mathivanan et al., 2006). Fermented products were found to enhance intestinal health by acting in a similar way to a probiotic and raise nutrient digestibility in broiler chickens (Zhang et al., 2013; Zhao et al., 2013). It is suggested that dietary RC and fermented sour cherry kernel (**FC**) can enhance intestinal morphology and microflora of broilers due to phenolic contents and effects of fermentation. Additionally, nutrient digestibility can be increased by diminishing cellulose and antinutritional factors of RC through fermentation. Altop (2019) showed that dietary

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1% raw and fermented kernel of sweet cherry (*Prunus avium* L.) sharing the similar subgenus of cerasus with RC enhanced intestinal morphology and microflora in broiler chickens. The effect of dietary RC or FC at 1%, 2%, and 4% on apparent digestibility, ileal morphology, and caecal microflora in broiler chickens was investigated in this study.

MATERIALS AND METHODS

Preparation of FC

The RC used in the present study was obtained from a juice factory in Tokat, Turkey, and ground in order to pass through a 2-mm sieve. *Aspergillus niger* (ATCC 200345) was cultured in potato dextrose agar at 24°C for 7 D. RC was enriched with nutritive salt (glucose: urea:(NH4)2SO4:peptone:KH2PO4:MgSO4.7H2O = 4: 2:6:1:4:1, 1 l for 1 kg of kernels) after autoclave sterilization. *Aspergillus niger* seeds (10^4 spores/mL) were inoculated in RC at 1 mL for each 100 g sample. The mixture was incubated at 30°C for 7 D. After the fermentation period, the sample was spread on a polyethylene sheet in a room at 30 to 40°C to achieve 90% dry matter (**DM**). Finally, the FC was milled to a size of 2 mm after drying.

Experimental Birds and Diets

A total of 343 one-day-old male Ross 308 broiler chicks with an average initial BW (38.91 ± 0.61 g) were randomly divided into 7 treatment groups. Each treatment was allotted to 7 replicates with 7 birds per replicate. The temperature was maintained at 32°C during the first 3 D. Then, the temperature was gradually lowered by 1°C every 2 D until it reached 20°C; this temperature was maintained until the end of the study. Lights were kept on continuously for the first 3 D after hatching, after which a 23L:1D (23 h of light and 1 h of dark) lighting schedule was maintained for the duration of the experiment. All birds received feed and fresh water ad libitum throughout the experiment. All birds were vaccinated against Newcastle disease on days 0 and 9, against Gumboro on day 15 and against infectious bursal disease on days 0, 9, and 19.

Diets were formulated according to the recommendations for Ross 308 male birds from the Ross breeders. Chicks were fed either a maize−soybean meal-based basal diet (control) or a basal diet supplemented with RC at 1% (**RC1**), 2% (**RC2**), and 4% (**RC4**) or FC at 1% (**FC1**), 2% (**FC2**), and 4% (**FC4**). The composition of the diets and the calculated contents are shown in Tables 1 and 2. The nutritional composition of RC and FC was 29.6 and 34.9% CP, 3220.6 and 3280.6 kcal/kg ME, 16.6 and 24.6% ether extract, 3.1 and 5.1% ash, 27.5 and 20.3% crude fiber, respectively. The experimental design and procedures were approved by the Ethical Committee of Ondokuz Mayis University (protocol number: 2017/09).

Digestibility

A digestibility trial was conducted using the acid insoluble ash method, according to Emami et al. (2012). On the 37th day of age, fecal samples were collected from each replicate to measure the apparent DM, nitrogen and ash digestibility. For the determination of apparent digestibility, 48 h before the collection of fecal samples, 5 g of celite (a source of acid insoluble ash; celite, Merck KGaA, Darmstadt, Germany) was added per kg of diet, with the acid insoluble ash content of diets and feces measured according to McCarthy et al. (1974). Analyses of DM (method 930.15), nitrogen (method 981.10), and ash (method 942.05) were performed according to the methods of the Association of Official Analytical Chemists (AOAC, 2000) while apparent digestibility values were calculated by the following equation:

$$\text{Apparent digestibility (\%)} = 100 - \left(\frac{\text{marker in diet (\%)} \times \text{nutrient in feces (\%)}}{\text{marker in feces (\%)} \times \text{nutrient in diet (\%)}}\right) \times 100$$

Gut Morphology

Ileum samples were taken from between Meckel's diverticulum and the ilea−caecal junction and cut into 1.5-cm pieces and placed into 10% formalin for further analyses. Tissue sections were placed into tissue cassettes for the dehydration process and embedded in paraffin blocks. They were subsequently cut with a 5-µ thickness and placed on a slide. Each ileal histomorphologic tissue sample was prepared and stained with hematoxylin and eosin solution by using standard paraffin-embedding methods (Xu et al., 2003). After the embedding process, villus height (**VH**) and crypt depth (**CD**) were evaluated by using a light microscope (Primo Star, Zeiss, Jena, Germany) and image processing and analysis software (ZEN 2012 SP2, Zeiss).

Gut Microflora

At the end of the experiment, 1 bird per replicate (i.e., 7 birds per treatment) was randomly selected and euthanized by severing the jugular vein. The carcasses were subsequently opened and caecal samples were aseptically transferred to sterile bags and stored at −21°C until analysis. Each caecal digesta was serially diluted with Ringer solution from 10^{-1} to 10^{-9}. Dilutions were subsequently plated on selective agar media for the enumeration of target bacterial groups. *Lactobacillus spp.*, *Enterococcus spp.*, and *Escherichia coli* were enumerated on MRS (de Man, Rogosa and Sharpe) agar (Merck 110660), Slanetz and Bartley agar

Table 1. Ingredients and nutrient composition of experimental diets in starter (Days 1 to 11) and grower 1 (Days 12 to 21) periods.

	Starter (Days 1 to 11)[1]							Grower 1 (Days 12 to 21)[1]						
Ingredients (g/kg)	CON	RC1	RC2	RC4	FC1	FC2	FC4	CON	RC1	RC2	RC4	FC1	FC2	FC4
Corn	434.8	430.4	425.9	417.1	430.4	425.9	417.1	438.5	434.1	429.6	420.7	434.1	429.6	420.7
Soybean meal (46%)	255.2	252.6	250.0	244.8	252.6	250.0	244.8	155.0	153.4	151.9	148.7	153.4	151.9	148.7
Full-fat soybean (35%)	140.0	138.6	137.1	134.3	138.6	137.1	134.3	200.0	198.0	195.9	191.9	198.0	195.9	191.9
Corn gluten	100.0	99.0	98.0	95.9	99.0	98.0	95.9	100.0	99.0	98.0	95.9	99.0	98.0	95.9
Sunflower meal (36%)	–	–	–	–	–	–	–	35.0	34.6	34.3	33.6	34.6	34.3	33.6
Meat and bone meal (35%)	50.0	49.5	49.0	48.0	49.5	49.0	48.0	58.0	57.4	56.8	55.6	57.4	56.8	55.6
Raw sour cherry kernel	–	10.0	20.0	40.0	–	–	–	–	10.0	20.0	40.0	–	–	–
Fermented sour cherry kernel	–	–	–	–	10.0	20.0	40.0	–	–	–	–	10.0	20.0	40.0
Dicalcium phosphate (18%)	3.8	3.8	3.8	3.8	3.8	3.8	3.8	–	–	–	–	–	–	–
Marble dust (38%)	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Salt	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
DL Methionine	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.1	2.1	2.1	2.1	2.1	2.1	2.1
L Lysine HCl	1.1	1.1	1.1	1.1	1.1	1.1	1.1	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Threonine	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin and mineral premix[2]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin D3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sodium sulphate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Anticoccidial	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Toxin binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chemical analysis														
Crude protein	230	231	231	233	231	232	235	220	221	222	223	221	223	225
Ether extract	55.8	56.9	58.0	60.2	57.7	59.6	63.4	68.1	69.1	70.1	72.0	69.9	71.7	75.2
Crude fibre	33.9	36.3	38.7	43.6	35.6	37.3	40.6	40.3	42.7	45.0	49.7	41.9	43.5	46.8
Ash	53.0	52.5	52.0	51.0	52.5	52.0	51.1	48.5	48.0	47.6	46.7	48.1	47.6	46.8
Calculated analysis														
Metabolizable energy (kcal/kg)	3,000	3,002	3,004	3,009	3,003	3,006	3,011	3,050	3,052	3,053	3,057	3,052	3,055	3,059
Lysine	14.0	14.0	14.0	14.0	14.0	14.0	14.0	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Methionine	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.3	5.3	5.3	5.3	5.3	5.3	5.3
Methionine and cystine	10.5	10.5	10.5	10.5	10.5	10.5	10.5	9.9	9.9	9.9	9.9	9.9	9.9	9.9
Threonine	9.8	9.8	9.8	9.8	9.8	9.8	9.8	8.9	8.9	8.9	8.9	8.9	8.9	8.9
Tryptophan	2.9	2.9	2.9	2.9	2.9	2.9	2.9	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Calcium	10.0	10.0	10.0	10.0	10.0	10.0	10.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Total P	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.1	7.1	7.1	7.1	7.1	7.1	7.1
Available P	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.6	4.6	4.6	4.6	4.6	4.6	4.6
Na	1.8	1.8	1.8	1.8	1.8	1.8	1.8	2.1	2.1	2.1	2.1	2.1	2.1	2.1

[1]CON = basal diet, RC1, RC2, and RC4 = basal diet containing 1, 2, and 4% raw sour cherry kernel, respectively, FC1, FC2, and FC4 = basal diet containing 1, 2, and 4% fermented sour cherry kernel, respectively.

[2]Premix provided per kilogram of diet: 12,000 IU retinol; 2,400 IU cholecalciferol; 40 mg α-tocopherol; 4 mg menadione; 3 mg thiamine; 6 mg riboflavin; 25 mg nicotinic acid; 10 mg pantothenic acid; 5 mg pyridoxine; 0.03 mg cyanocobalamin; 0.05 mg biotin; 1 mg folic acid; 80 mg Mn; 60 mg Zn; 60 mg Fe; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se, 200 mg choline chloride.

(Merck 105262), and EMB (eosin methylene blue) agar (Merck 101347), respectively. The plates were incubated according to the manufacturer's recommendations for each microorganism. Subsequently, colonies were counted, while the average number of live bacteria was calculated based on the weight of the wet caecum digesta sample. Bacteria counts were converted to $\log_{10}$ cfu per gram sample.

Statistical Analysis

All data were analyzed by one-way ANOVA with post hoc Duncan multiple comparison tests using the SPSS statistical software (Version 21.0 for Windows, SPSS, Inc., Chicago, IL, IBM, 2012). The orthogonal polynomial contrast test was performed to determine the linear and quadratic effects of increasing the inclusion level of RC or FC in the diet. Values in the tables were means and pooled SEM. The statistical significance level for the difference was set at $P < 0.05$.

RESULTS

Digestibility

There was a linear decrease in apparent DM ($P < 0.001$) and nitrogen ($P = 0.004$) digestibility with an inclusion level of RC in the diet (Table 3). Apparent ash digestibility showed a linear ($P < 0.001$) and quadratic ($P = 0.028$) decrease with incremental RC inclusion. Chickens fed the RC2 and RC4 diets had lower ($P < 0.001$, $P < 0.05$, and $P < 0.001$, respectively) apparent DM, nitrogen and ash digestibility, compared with the control group. Chickens fed the FC4 diets had higher apparent DM ($P < 0.001$) and ash ($P < 0.001$) digestibility, compared with the other FC groups. The apparent nitrogen digestibility of birds in FC4 was increased ($P < 0.05$), compared with that in the FC1 group. There was a quadratic ($P = 0.005$ and $P = 0.004$, respectively) influence on apparent DM and nitrogen digestibility with increasing levels of FC in the diet. Apparent ash digestibility showed a linear ($P = 0.018$)

Table 2. Ingredients and nutrient composition of experimental diets in grower 2 (Days 22 to 35) and finisher (Days 36 to 42) periods.

	Grower 2 (Days 22 to 35)[1]							Finisher (Days 36 to 42)[1]						
Ingredients (g/kg)	CON	RC1	RC2	RC4	FC1	FC2	FC4	CON	RC1	RC2	RC4	FC1	FC2	FC4
Corn	493.5	488.5	483.5	473.5	488.5	483.5	473.5	533.6	528.2	522.8	512.1	528.2	522.8	512.1
Soybean meal (46%)	101.1	100.1	99.1	97.0	100.1	99.1	97.0	53.0	52.5	51.9	50.9	52.5	51.9	50.9
Full-fat soybean (35%)	200.0	198.0	195.9	191.9	198.0	195.9	191.9	210.0	207.9	205.8	201.5	207.9	205.8	201.5
Corn gluten	100.0	99.0	98.0	95.9	99.0	98.0	95.9	100.0	99.0	98.0	96.0	99.0	98.0	96.0
Sunflower meal (36%)	35.0	34.6	34.3	33.6	34.6	34.3	33.6	35.0	34.6	34.3	33.6	34.6	34.3	33.6
Meat and bone meal (35%)	58.0	57.4	56.8	55.7	57.4	56.8	55.7	60.0	59.4	58.8	57.6	59.4	58.8	57.6
Raw sour cherry kernel	–	10.0	20.0	40.0	–	–	–	–	10.0	20.0	40.0	–	–	–
Fermented sour cherry kernel	–	–	–	–	10.0	20.0	40.0	–	–	–	–	10.0	20.0	40.0
Marble dust (38%)	2.7	2.7	2.7	2.7	2.7	2.7	2.7	–	–	–	–	–	–	–
Salt	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DL Methionine	2.1	2.1	2.1	2.1	2.1	2.1	2.1	1.9	1.9	1.9	1.9	1.9	1.9	1.9
L Lysine HCl	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Threonine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin and mineral premix[2]	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Sodium sulphate	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Anticoccidial	0.6	0.6	0.6	0.6	0.6	0.6	0.6	–	–	–	–	–	–	–
Chemical analysis														
Crude protein	200	201	202	204	201	203	206	185	186	187	189	187	188	192
Ether extract	69.2	70.2	71.1	73.1	71.0	72.7	76.3	72.0	72.9	73.9	75.8	73.7	75.5	79.0
Crude fibre	39.4	41.8	44.1	48.8	41.0	42.7	45.9	39.1	41.5	43.8	48.5	40.7	42.4	45.6
Ash	44.5	44.1	43.7	42.8	44.1	43.7	42.9	39.7	39.3	39.0	38.2	39.4	39.0	38.3
Calculated analysis														
Metabolizable energy (kcal/kg)	3,100	3,101	3,102	3,105	3,102	3,104	3,107	3,150	3,151	3,151	3,153	3,151	3,153	3,155
Lysine	11.6	11.6	11.6	11.6	11.6	11.6	11.6	10.4	10.4	10.4	10.4	10.4	10.4	10.4
Methionine	5.0	5.0	5.0	5.0	5.0	5.0	5.0	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Methionine and cystine	9.2	9.2	9.2	9.2	9.2	9.2	9.2	8.6	8.6	8.6	8.6	8.6	8.6	8.6
Threonine	8.1	8.1	8.1	8.1	8.1	8.1	8.1	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Tryptophan	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Calcium	8.5	8.5	8.5	8.5	8.5	8.5	8.5	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Total P	6.9	6.9	6.9	6.9	6.9	6.9	6.9	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Available P	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.7	4.7	4.7	4.7	4.7	4.7	4.7
Na	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.7	1.7	1.7	1.7	1.7	1.7	1.7

[1]CON = basal diet, RC1, RC2, and RC4 = basal diet containing 1, 2, and 4% raw sour cherry kernel, respectively, FC1, FC2, and FC4 = basal diet containing 1, 2, and 4% fermented sour cherry kernel, respectively.

[2]Premix provided per kilogram of diet: 12,000 IU retinol; 2,400 IU cholecalciferol; 40 mg α-tocopherol; 4 mg menadione; 3 mg thiamine; 6 mg riboflavin; 25 mg nicotinic acid; 10 mg pantothenic acid; 5 mg pyridoxine; 0.03 mg cyanocobalamin; 0.05 mg biotin; 1 mg folic acid; 80 mg Mn; 60 mg Zn; 60 mg Fe; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se, 200 mg choline chloride.

Table 3. Effects of dietary treatments on apparent nutrient digestibility.

	Dietary treatment[1]									Effects of RC[2]		Effects of FC[3]	
Items	CON	RC1	RC2	RC4	FC1	FC2	FC4	SEM	P	L	Q	L	Q
DM, %	76.7[a,b]	76.3[a,b]	67.1[c]	66.4[c]	71.6[b,c]	71.3[b,c]	78.3[a]	1.06	***	***	NS	NS	**
Nitrogen, %	69.3[a,b]	68.8[a,b]	60.3[c]	60.1[c]	64.4[b,c]	64.9[a,b,c]	72.7[a]	1.18	*	**	NS	NS	**
Ash, %	37.2[b]	39.8[a,b]	17.6[c]	13.4[c]	35.6[b]	37.0[b]	42.8[a]	2.15	***	***	*	*	*

[a–c]Means in the same row that do not share a common superscript are significantly different ($P < 0.05$). DM = dry matter, RC = raw sour cherry kernel, FC = fermented sour cherry kernel, L = Linear, Q = Quadratic, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

[1]CON = basal diet, RC1, RC2, and RC4 = basal diet containing 1, 2, and 4% raw sour cherry kernel, respectively, FC1, FC2, and FC4 = basal diet containing 1, 2, and 4% fermented sour cherry kernel, respectively.

[2,3]Orthogonal polynomials were used to determine linear and quadratic effect of raw and fermented sour cherry kernel.

and quadratic ($P = 0.024$) increase with the inclusion level of FC in the diet.

Gut Morphology

The VH and the villus height to crypt depth ratio (**VH:CD**) of chickens fed 4% RC were decreased ($P < 0.001$ and $P < 0.01$, respectively), compared with the control group (Table 4). Chickens fed the FC1 diet had the highest ($P < 0.01$) VH:CD among the treatment groups. Dietary treatments had no effect ($P > 0.05$) on the CD.

There was a linear ($P = 0.033$) and quadratic ($P = 0.001$) influence on VH and also a linear ($P = 0.032$) effect on the VH:CD of birds when increasing the level of RC in the diet. There was a quadratic ($P = 0.016$) influence on VH and a linear ($P = 0.021$) and quadratic ($P = 0.015$) effect on the VH:CD of broilers when increasing the level of FC in the diet.

Table 4. Effects of dietary treatments on ileum morphology.

Items	Dietary treatment[1]							SEM	P	Effects of RC[2]		Effects of FC[3]	
	CON	RC1	RC2	RC4	FC1	FC2	FC4			L	Q	L	Q
VH, μm	2,211[a,b]	2,462[a]	2,375[a]	1,836[c]	2,276[a]	2,461[a]	1,867[b,c]	51.8	***	*	**	NS	*
CD, μm	143.9	166.9	166.9	163.5	115.8	173.3	163.0	4.96	NS	NS	NS	NS	NS
VH:CD, μm:μm	16.0[b]	15.5[b,c]	15.0[b,c]	11.5[c]	20.9[a]	15.5[b,c]	12.6[b,c]	0.57	**	*	NS	*	*

[a–c]Means in the same row that do not share a common superscript are significantly different ($P < 0.05$). VH = villus height, CD = crypt depth, VH:CD = villus height to crypt depth ratio, RC = raw sour cherry kernel, FC = fermented sour cherry kernel, L = Linear, Q = Quadratic, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.
[1]CON = basal diet, RC1, RC2, and RC4 = basal diet containing 1, 2, and 4% raw sour cherry kernel, respectively, FC1, FC2, and FC4 = basal diet containing 1, 2, and 4% fermented sour cherry kernel, respectively.
[2,3]Orthogonal polynomials were used to determine linear and quadratic effect of raw and fermented sour cherry kernel.

Table 5. Effects of dietary treatments on intestinal microflora.

Items (log10 cfu/g)	Dietary treatment[1]							SEM	P	Effects of RC[2]		Effects of FC[3]	
	CON	RC1	RC2	RC4	FC1	FC2	FC4			L	Q	L	Q
Lactobacillus spp.	8.3[b]	8.3[b]	8.7[b]	8.5[b]	9.3[a]	8.4[b]	8.1[b]	0.11	*	NS	NS	NS	**
Enterococcus spp.	7.3	7.7	7.4	7.7	7.4	7.7	7.3	0.11	NS	NS	NS	NS	NS
Escherichia coli	7.3	7.8	7.2	7.2	7.5	7.9	7.7	0.15	NS	NS	NS	NS	NS

[a,b]Means in the same row that do not share a common superscript are significantly different ($P < 0.05$). RC = raw sour cherry kernel, FC = fermented sour cherry kernel, L = Linear, Q = Quadratic, * = $P < 0.05$, ** = $P < 0.01$.
[1]CON = basal diet, RC1, RC2, and RC4 = basal diet containing 1, 2, and 4% raw sour cherry kernel, respectively, FC1, FC2, and FC4 = basal diet containing 1, 2, and 4% fermented sour cherry kernel, respectively.
[2,3]Orthogonal polynomials were used to determine linear and quadratic effect of raw and fermented sour cherry kernel.

Gut Microflora

Increased levels of RC did not affect ($P > 0.05$) *Lactobacillus* spp. while increasing levels of dietary FC increased ($P < 0.05$) the *Lactobacillus* spp. in a quadratic ($P = 0.008$) manner in the intestines (Table 5). Dietary treatments had no effect ($P > 0.05$) on caecal *Enterococcus spp.* and *E. coli* counts.

DISCUSSION

Digestibility

Senica et al. (2016) reported that cyanogenic glycosides of RC, such as amygdalin and prunasin, have toxic effect on broiler chickens. Cyanogenic glycosides are converted to hydrogen cyanide in intestinal epithelium and deteriorate growth performance and nutrient digestibility in broiler chickens (Rodríguez et al., 2001; Senica et al., 2016). In this study, the dietary inclusion of RC linearly decreased the apparent nutrient digestibility in broiler chickens. Cellulose is also one of the major factors affecting nutrient digestibility in broilers. The decrease in apparent nutrient digestibility could be due to cyanogenic glycosides and the high crude fiber content of RC. Altop (2019) indicated that dietary 1% sweet cherry kernel impairs the FCR in broilers. Similarly, Arbouche et al. (2012) demonstrated that apricot kernel from the *Rosaceae* family, containing cyanogenic glycosides similar to RC, impairs the broiler FCR. Increasing levels of palm kernel meal in the diet decreased apparent DM, nitrogen and ash digestibilities in broiler chickens (Alshelmani et al., 2016; Navidshad et al., 2016). Nesseim et al. (2015) also reported a decline in apparent nitrogen digestibility when increasing levels of *Jatropha curcas* kernel in broiler diets.

Solid-state fermentation can eliminate antinutritional components in agri-industrial byproducts. Zhang et al. (2013) showed that ginkgolic acid, an antinutritional factor of *Ginkgo biloba* leaves, is eliminated by fermentation. Similarly, solid-state fermentation decreased the tannic acid in olive leaves (Xie et al., 2016). Jazi et al. (2017) also reported that the free gossypol level of cottonseed meal is decreased by fermentation. Therefore, cyanogenic glycosides or other possible antinutritional factors in RC could be eliminated or reduced by fermentation in the present study. This may be the most important reason for the increase in the apparent nutrient digestibility of RC by solid-state fermentation. Similarly, Zhang et al. (2013) reported an improvement in intestinal absorption with the elimination of ginkgolic acid in *G. biloba* leaves through fermentation.

Solid-state fermentation could enrich substrate with enzymes, such as cellulase, hemicellulase, xylanase, protease, amylase, lipase, and tannase, which help nutrient digestibility (Couri et al., 2000; Wu et al., 2015). The enzymes produced by the fermentation process could have played a role in the improvement of apparent nutrient digestibility. Lawal et al. (2010) reported that chickens fed on diets supplemented with fermented palm kernel cake had higher apparent DM, nitrogen and ash digestibility than in the case of the birds in the control group. Likewise, Apata (2011) noted that the dietary inclusion of fermented almond meal increased apparent nitrogen digestibility, compared with the control group.

The higher apparent nutrient digestibility in chickens fed FC4, compared to the birds fed RC4, may be mostly due to the inactivation of anti-nutritional factors and enrichment with digestive enzymes and the decreasing crude fiber content of RC by solid-state fermentation.

Gut Morphology

Jia et al. (2010) reported that VH, CD, and VH:CD are important parameters of healthy intestines in birds and directly related to the absorptive capacity of the mucous membrane. Altop (2019) found that dietary 1% raw sweet cherry kernel increased VH and VH:CD in the ileum of broiler chickens. Although RC has more phenolic compounds than sweet cherry kernel (Kim et al., 2005), 1% and 2% RC had no effect on intestinal morphology and 4% RC lowered VH and VH:CD, compared with the control group. The decrease in VH and VH:CD should correlate with a lowered absorptive capability of the small intestine (Yamauchi et al., 2006), as in this study, and also indicate the presence of toxins (Xu et al., 2003). The adverse effect on ileal morphology with dietary RC inclusion could be due to its cyanogenic glycosides of RC.

Altop (2019) indicated that 1% inclusion of fermented sweet cherry kernel to diets reduced VH and CD, although there was no change in the VH:CD in broiler chickens. In this study, VH:CD was enhanced in birds fed on 1% FC, compared with the other groups. Similarly, Zhang et al. (2012) reported that fermented *G. biloba* leaves increased VH:CD and reduced CD in jejunum, while also raising the VH in duodenum and jejunum. The improved VH:CD in FC1 might be associated with the increased numbers of beneficial bacteria (Xu et al., 2003). In this study, *Lactobacillus spp.* counts of the birds in the FC1 groups were highest among the treatment groups. Similarly, Altop (2019) showed that 1% dietary sweet cherry kernel enhanced intestinal morphology by increasing caecal *L. acidophilus* counts. This view is supported by Zhang et al. (2015) who reported that fermented *G. biloba* leaves raised ileum *Lactobacillus spp.* count and increased the ileum VH and VH:CD ratio, while reducing CD in broiler chickens. Fermented rapeseed meal elevated caecal and colonial *Lactobacillus spp.* counts while raising the VH and VH:CD ratio in jejunum and ileum, compared with unfermented rapeseed meal (Chiang et al., 2010). Similarly, Jazi et al. (2017) noted that fermented cottonseed meal reduced coliform bacteria in ileum, raised *Lactobacillus spp.* counts in crop and increased the VH and VH:CD in duodenum and jejunum, as well as reducing CD in jejunum. In this study, the VH of chickens receiving a diet supplemented with 4% FC was lower than that of the birds in FC1 group. This may be attributed to the fact that cyanogenic glycosides in FC were high enough to deteriorate the intestinal morphology of broiler chickens by 4% dietary FC inclusion, although it could have been decreased by fermentation. Mathivanan et al. (2006) indicated that fermented soybean elevated VH at 0.5 and 1%, although the VH of birds fed on diets supplemented with 1.5% fermented soybean was not changed, compared with the control group.

The VH, CD, and VH:CD are highly useful measurements to estimate the absorption capacity of the small intestine (Montagne et al., 2003). In this study, the highest VH:CD was observed in the chickens in the FC1 group, although the apparent nutrient digestibility of birds in the FC4 group was higher than that of chickens in the other FC groups. This result may be attributed to the enzymes produced by *A. niger* during solid-state fermentation. Similarly, Nie et al. (2015) indicated that an increase in the apparent nutrient digestibility of diets supplemented with cottonseed meal may be caused by exoenzymes produced through solid-state fermentation.

Gut Microflora

Fermented feeds have been reported to have a probiotic effect on broiler chickens (Chiang et al., 2010). *Aspergillus niger* raised *Lactobacillus spp.* counts in broilers when supplementing diets either as spores (Mountzouris et al., 2007) or fermented feed (Zhao et al., 2013). *Lactobacillus spp.* was also increased in the caecum of the chickens fed with 1% FC, compared with birds in the control and other treatment groups. In parallel with the results of the present study, fermented *G. biloba* leaves raised *Lactobacillus* counts in the ileum of broilers (Zhang et al., 2015) and in the ileum and caecum of laying hens (Zhao et al., 2013). Raised Lactobacillus count in the ileum of broilers (Zhang et al., 2015) and in ileum and caecum of laying hens (Zhao et al., 2013). Ashayerizadeh et al. (2018) noted that rapeseed meal, fermented with a mix of *L. acidophilus*, *Bacillus subtilis* bacteria and *A. niger*, elevated *Lactobacillus spp.* counts in the crop of broiler chickens. Similar findings were reported by Jazi et al. (2017) who investigated the effects of cottonseed meal fermented with *B. subtilis*, *A. niger*, and *A. oryzae* on broilers. Conversely, Altop (2019) noted that fermented sweet cherry kernel did not affect caecal *L. acidophilus* counts in broilers.

Fermented *G. biloba* leaves also reduced caecal *E. coli* counts in laying hens (Zhao et al., 2013). Similarly, fermented rapeseed meal and cottonseed meal reduced ileal coliform bacteria in broilers (Ashayerizadeh et al., 2018; Jazi et al., 2017). However, dietary FC had no effect on caecal *E. coli* and *Enterococcus spp.* counts in this study. Similar to the results of the present study, Altop (2019) reported no effect of dietary fermented sweet cherry kernel inclusion on *E. coli* and *Enterococcus spp.* in the caecum of broilers. Similarly, Zhang et al. (2015) reported no change in ileal and caecal *E. coli* counts with the addition of fermented *G. biloba* leaves in broiler diets.

Altop (2019) indicated that dietary sweet cherry kernel increased caecal *L. acidophilus* counts in broilers. Kim et al. (2005) reported that phenolic compounds of RC are greater than for sweet cherry kernel. However, dietary RC did not affect the caecal *Lactobacillus spp.* counts in this study. This can be explained by the differences between the cyanogenic glycoside content of RC and sweet cherry kernel, which is harmful to intestinal health.

In conclusion, the results of the present study indicated that FC is a potential feed additive which improves intestinal morphology and microflora in broiler chickens. Diets supplemented with 1% FC increased the VH:CD ratio in ileum and raised caecal *Lactobacillus spp.* counts without any adverse effect on apparent nutrient digestibility.

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