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Performance, Immunity, Meat Composition and Fatty Acid Pattern in Broilers after Dietary Supplementation of Fermented *Ginkgo biloba* and *Citrus junos*

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Abstract

Dietary Fermented *Ginkgo biloba* (FGLP) and *Citrus junos* Probiotics (FCJP) was evaluated on growth performance, immunity, cecal microbiology, meat composition and fatty acid profile in broilers. A total of 150 one day old Ross 308 broilers were randomly allocated to the 5 treatments (5 replicated pen of 6 birds/pen): 1) Control (corn-soybean meal based basal diet), 2) FGLP1: Corn-soybean meal based basal diet+5% FGLP, 3) FGLP2: Corn-soybean meal based basal diet+10% FGLP, 4) FCJP1: Corn-soybean meal based basal diet+5% FCJP, and 5) FCJP2: Corn-soybean meal based basal diet+10% FCJP. A significant increase in weight gain during starter (0-21 days), finisher (22-35 days) and overall period (0-35 days) was exhibited in FGLP2 supplemented group compared to control and other treatment groups ($P<0.05$); where better feed conversion efficiency was found after FCJP2 supplementation during finisher and overall period compared to control and other treatment groups ($P<0.05$). Serum IgG was elevated in FGLP and FCJP supplemented group relative to control birds ($P<0.05$). Meat composition data elucidated that, thigh meat crude protein and crude fat content was increased in FCJP1 supplemented group compared to control ($P<0.05$). There was found no significant differences in meat cholesterol content between control and treatment groups ($P>0.05$); however, among meat fatty acids, sum of SFA in thigh meat was diminished in FGLP group and sum of MUFA in breast meat was diminuted in FGLP and FCJP group compared to control ($P<0.05$). Sum of n-3 PUFA of breast and thigh meat was elevated after supplementation of FGLP and FCJP compared to control ($P<0.05$). To sum up, dietary FGLP and FCJP supplementation significantly improved performance and immunity, decreased SFA and elevated n-3 PUFA of broiler meat. Therefore, FGLP and FCJP probiotics could be supplemented as functional feed additives in broilers diet.

Keywords: *Ginkgo biloba* probiotics; Fermented *Citrus junos* probiotics; Growth performance; Immunity; Meat quality

Introduction

Since 5000 BC aromatic plants or medicinal plants (herbs and spices) have been used in the Middle East for their medicinal properties, preservative capabilities, aroma and flavor enhancing in the food and food products [1]. Due to their beneficial properties, according to World Health Organization (WHO) around 80% of the total global populations (especially in the developing countries) depends on the medicinal plants based medicines for their health care [2,3] On the other hand, antibiotic growth promoters were utilized since 1950s for long time in animal production for allowing adequate productivity [4]; however due to their antimicrobial resistance in humans it was banned in the European Union on January 1st 2006, and then all over the world it becomes an important issue of banning in the animal production [5,6]. Therefore, the aromatic plants attracted by the researchers as alternative source for the animal production and health, as they are being utilized for long in the human medicines and food products. Different the aromatic plants, their extracts, essential oils tested as natural feed additives found to be advantageous as an alternative to antibiotic growth promoters, and they are considered as the generally recognized as safe and are residue free [7]. The feed additives derived from the natural plants are known as the botanicals or phytogenics or phytobiotics which can be utilized in the animal's diet to promote the performance and quality of the resulted feed and animal products [5,6,8].

Aromatic plants are found all over the world as wild or in some case as cultivated, all of them composed of chemical substances which are called primary and secondary metabolites such as flavonoids, polyphenols, polypeptides, alkaloids [9,10]. These bioactive compounds can act as therapeutics with having antioxidant and antiseptic properties [11]; antimicrobials and can contribute in the microbial growth retardation activities on the food and food products [12,13]; helps is inhibiting the oxidative rancidity and delaying the off-flavour development in food products [14,15]. The term probiotics become very common both for human and animal study; where the word "Probiotics" actually derived from the Greek word and meaning is 'for life' ("Antibiotic" means 'against life') and denoted the beneficial microorganisms and tested as single or combined state for promoting the growth performance in animal and poultry nutrition [5,16,17], immune enhancer through enhancing T-cell function and antibodies, antigenic stimulation by the secretion of immunoglobulin [18,19]. Fermentation of feed or agricultural byproducts can enhance nutritional quality of the product and able to improve the performance of animals and reduce cost in broiler [8,20]. Fermentation of plant materials along with probiotic can improve performance in broilers and cattle [21,22] and no adverse impact on broiler performance [23]. The benefit of utilizing natural plants or their products along with

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fermentation is cost-effective, environmental friendly and performance enhancer [8,24]. Fermentation of plant materials (*Punica granatum*, *Ginkgo biloba, Camellia sinensis, Alisma canaliculatum*) and multi-microbe probiotics reported that it can enhance the performance as well as the meat quality in broilers [9,21-24].

Several types of researches are going on all over the world to get more efficacies through utilization of probiotics and natural plant materials in poultry nutrition as well as in animal nutrition. The single or multi-microbial addition in basal diet; single or multi-plant material addition in basal diet; or combination of probiotic organism along with natural plant material, in single or multi-state dimension could be the research interest to test the efficacy in animal and poultry nutrition. Kim et al. [25] reported that a combinations of multi-plant materials along with multi-microbe fermented product exhibited better productive performance in case of broiler; where Bostami et al., [26] conducted a combination of two plant material along with multi-microbe fermented product in case of broiler, where both the performance and meat quality was improved in case of broiler. Since there is a huge amount of natural plants on the planet, the estimated amount is 70,000 species of folk medicine reported by Farnsworth and Soejarto [27], and around 21,000 plant taxa utilized [28] for medicinal purpose reported by WHO [29]. Different plant materials are composed of different secondary metabolites while the probiotic microorganisms also possess different organic substances which can exhibit synergistic actions in the poultry and animal nutrition [25,29,30]. Among different plant materials utilized for the human and animal medicinal purpose in Korea, China and Japan, *Alisma canaliculatum*, *Houyttunia cordata*, *Camelia sinensis*, *Citrus junos*, *Ginkgo biloba*, *Laminaria cordata*, *Salicornia herbacea, Glycyrrhiza glabra* and so on are available and under research investigation. Where *Ginkgo biloa* is under the family of *Ginkgoaceae*; and *Citrus junos* is under the family of *Rutaceae*; *Punica granatum* is under the family of *Lythraceae* are composed of different bioactive compounds which are tested as single state in different separate studies with positive or neutral impact in poultry [24,25,31]. To the best of our knowledge, there is no study conducted yet on combination of these plant materials along with fermentation of multi-microbe probiotics.

Therefore, in this study, the purpose is to prepare two fermented products using the plant materials and probiotics and then compare that two fermented products with the control diet. The fermented products were as follows: 1) FGLP: Combination of *Ginkgo biloba+Punica granatum* and fermentation with *Lactobacillus plantarum, Lactobacillus acidophilus* and *Saccharomyces cerevisiae;* 2) FCJP: Combination of *Citrus Junos+Punica granatum* and fermentation with *Lactobacillus plantarum, Lactobacillus acidophilus* and *Saccharomyces cerevisiae.* Where fermented probiotic product was prepared using two level (5% and 10%) of *Ginkgo biloba* and *Citrus Junos* and then added at the rate of 0.4% of the total basal diet in case of broiler. As a continuation of our target of development of functional feed additives by the proper utilization of natural plant materials with the beneficial microorganisms through testing the efficacy in broiler, pigs and cattle, and to suggest the farmers; the objective of the present study is testing the efficacy of fermented *Ginkgo biloba* and *Citrus Junos* probiotic in broilers on their performance, immunity, cecal microbiology, meat composition and meat fatty acid profiles.

Materials and Methods

Preparation of fermented *Gingko biloba* and *Citrus junos* probiotics

Gingko biloba leaf and *Citrus junos* rind was obtained from Boseong, Republic of Korea. *Punica granatum* byproduct, which is a Goheung-gun cultivar, was collected from a juice manufacturing company. The byproduct was composed of about 80% peels and rinds and 20% seed. *Gingko biloba* leaf, *Citrus junos* rind and *Punica granatum* were then dried in a forced air oven (Doori TEC, Doori TEC, FA, Co., Ltd.) at 80°C for 3 days and subsequently ground into powder that could pass through a 0.15 mm sieve using a milling machine [32]. Samples were then tightly packed in polythene plastic bags, after which they were sealed and kept at room temperature until needed. *Gingko biloba* leaf, *Citrus junos* rind and *Punica granatum* skin were analyzed in triplicate for Crude Protein (CP), Ether Extract (EE), moisture and ash as described by the Association of Official Analytical Chemists [33]. The fatty acid composition was determined by a direct method for Fatty Acid Methyl Ester (FAME) synthesis using a Gas Chromatograph (GC). The pH was measured using a digital pH meter (Docu-pH+meter, Sartorius, USA).

Fermented *Ginkgo biloba* Probiotics (FGLP1) contains 65% defatted rice bran, 30% pomegranate peel extract, and 5% *Ginkgo bloba* leaf powder; Fermented *Ginkgo biloba* Probiotics (FGLP2) contains 60% defatted rice bran, 30% pomegranate peel extract, and 10% *Ginkgo bloba* leaf powder. Whereas Fermented *Citrus junos* Probiotics (FCJP1) contains 65% defatted rice bran, 30% pomegranate peel extract, and 5% *Citrus junos* rind powder; Fermented *Citrus junos* Probiotics (FCJP2) contains 60% defatted rice bran, 30% pomegranate peel extract, and 10% *Citrus junos* rind powder. After mixing the ingredients to prepare FGLP and FCJP, samples were inoculated with 30% (v/w) *Lactobacillus plantarum* KCTC 3099 and *Lactobacillus acidophilus* KCTC 3111 and fermented for 2 days at 37°C and 40% moisture in a commercial fermenter (W-1000; Wonbalhyo Industry Co., Incheon, South Korea). After fermentation the medium was again inoculated with 30% (w/v) *Saccharomyces cerevisiae* KCTC 7904 and fermented for 3 days at 37°C. Fermentation with microbial inoculum was conducted using a cycle of 5 h standing and 3 h shaking to ensure proper mixing and fermentation. Subsequently, the fermented sample was dried in a forced air oven (Doori TEC, Doori TEC, FA, Co., Ltd.) at 32°C for 2 days to reduce the moisture levels. During fermentation with microbial inoculum, there was a cycle of 5 h standing and 3 h shaking to obtain the proper mixing and fermentation. Finally, FGLP and FCJP were stored in an air-tight plastic bag until being mixed with basal diet. The microbial concentration, proximate composition, trace minerals, fatty acids and pH of FGLP and FCJP were analyzed in triplicate. To determine the number of cells, 1 g of FGLP and FCJP was diluted with sterilized distilled water (10 ml) at room temperature. After approximately 10 min, 1 ml of the dilution was serially diluted 10-fold in NaCl (8.5 g/kg) solution and cultured in agar media. Then the culture plates were incubated at 37°C for 24-48 h and the number of colonies was counted carefully. Chemical compositions of FGLP and FCJP were determined by following the method of AOAC [32]. After preparation of FGLP and FCJP, 0.4% was added with the corn-soybean meal based basal diet. The ingredients and chemical composition of the starter and finisher diet was presented in the Tables 1 and 2. The experimental FGLP and FCJP composition was presented in Table 3. The pH of the FGLP was 3.22–3.29, whereas in FCJP it was 3.35–3.39.

Experimental design, dietary treatments and bird's management

Experimental birds were reared in the Sunchon National University experimental farm, Suncheon, Republic of Korea. A total of 150 one day-old Ross 308 broiler chicks were assigned to five treatment groups having five replications with six birds based on the

Item	Starter diet (0 to 21 days)			Finisher diet (22 to 35 days)		
	Control	FGLP[1]	FGLP[2]	Control	FGLP[1]	FGLP[2]
Ingredients (%, as fed basis)						
Corn grain	57.58	57.18	57.18	60.64	60.24	60.24
Soybean meal	26.80	26.80	26.80	24.90	24.90	24.90
Corn gluten	5.00	5.00	5.00	3.50	3.50	3.50
Soybean oil	2.20	2.20	2.20	2.20	2.20	2.20
Animal fats	4.50	4.50	4.50	5.00	5.00	5.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	2.14	2.14	2.14	2.00	2.00	2.00
Limestone	0.92	0.92	0.92	0.88	0.88	0.88
Vitamin-mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30
Choline	0.08	0.08	0.08	0.07	0.07	0.07
L-lysine HCL (78%)	0.24	0.24	0.24	0.16	0.16	0.16
DL-Methionine	0.20	0.20	0.20	0.10	0.10	0.10
Ginkgo probiotics 5%	-	0.40	-	-	0.40	-
Ginkgo probiotics 10%	-	-	0.40	-	-	0.40
Composition (% DM)						
ME (MJ/kg)	13.03	13.01	13.01	13.27	13.25	13.23
Moisture	12.07	11.25	11.02	13.08	13.28	13.25
Crude protein	20.89	20.67	20.98	19.12	18.53	18.11
Ether extract	4.65	4.57	5.93	2.43	3.68	3.33
Crude fiber	4.42	4.45	4.47	3.71	3.00	3.07
Crude ash	5.63	5.14	5.44	5.61	4.99	5.04
Calcium	1.05	1.05	1.05	0.81	0.81	0.81
Available phosphorus	0.55	0.55	0.55	0.45	0.45	0.45
Lysine	1.42	1.42	1.42	1.10	1.10	1.10
Methionine	0.49	0.49	0.49	0.45	0.45	0.45

[1]Vitamin-mineral mixture provided the following nutrients per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 1,500 IU; vitamin E, 20.0 mg; vitamin K3, 0.70 mg; vitamin B12, 0.02 mg; niacin, 22.5 mg; thiamine, 5.0 mg; folic acid, 0.70 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10.0 mg; Fe, 72 mg; Co, 2.5 mg (Bayer Korea Ltd., Dongjak-Ku, Seoul, Korea).
Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 1: Feed ingredients and chemical composition of experimental diet with fermented *Ginkgo biloba* probiotics.

Item	Starter diet (0 to 21 days)			Finisher diet (22 to 35 days		
	Control	FCJP[1]	FCJP[2]	Control	FCJP[1]	FCJP[2]
Ingredients (%, as fed basis)						
Corn grain	57.58	57.18	57.18	60.64	60.24	60.24
Soybean meal	26.80	26.80	26.80	24.90	24.90	24.90
Corn gluten	5.00	5.00	5.00	3.50	3.50	3.50
Soybean oil	2.20	2.20	2.20	2.20	2.20	2.20
Animal fats	4.50	4.50	4.50	5.00	5.00	5.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	2.14	2.14	2.14	2.00	2.00	2.00
Limestone	0.92	0.92	0.92	0.88	0.88	0.88
Vitamin-mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30
Choline	0.08	0.08	0.08	0.07	0.07	0.07
L-lysine HCL (78%)	0.24	0.24	0.24	0.16	0.16	0.16
DL-Methionine	0.20	0.20	0.20	0.10	0.10	0.10
Citron probiotics 5%	-	0.40	-	-	0.40	-
Citron probiotics 10%	-	-	0.40	-	-	0.40
Chemical composition (%DM)						
ME (MJ/kg)	13.03	13.01	13.01	13.27	13.25	13.23
Moisture	12.07	11.84	11.94	13.08	12.71	12.75
Crude protein	20.89	20.67	20.98	19.12	19.22	19.59
Ether extract	4.65	4.05	4.40	2.43	2.50	2.40
Crude fiber	4.42	4.58	4.46	3.71	3.45	3.00
Crude ash	5.63	5.14	5.44	5.61	5.08	5.02
Calcium	1.05	1.05	1.05	0.81	0.81	0.81
Available phosphorus	0.55	0.55	0.55	0.45	0.45	0.45
Lysine	1.42	1.42	1.42	1.10	1.10	1.10
Methionine	0.49	0.49	0.49	0.45	0.45	0.45

[1]Vitamin-mineral mixture provided the following nutrients per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 1,500 IU; vitamin E, 20.0 mg; vitamin K3, 0.70 mg; vitamin B12, 0.02 mg; niacin, 22.5 mg; thiamine, 5.0 mg; folic acid, 0.70 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10.0 mg; Fe, 72 mg; Co, 2.5 mg (Bayer Korea Ltd., Dongjak-Ku, Seoul, Korea).
Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 2: Feed ingredients and chemical composition of experimental diet with *Citrus junos* probiotics.

completely randomized design. Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: Corn-soybean meal based basal diet+5% FGLP, 3) FGLP2: Corn-soybean meal based basal diet+10% FGLP, 4) FCJP1: Corn-soybean meal based basal diet+5% FCJP, 5) FCJP2: Corn-soybean meal based basal diet+10% FCJP. The basal diet was formulated to meet the Nutrient Requirements of Poultry (National Research Council, NRC, 1994, Washington DC, USA) and applied for a total of 5 weeks in two stages: Starter (0-3 weeks) and finisher (4-5 weeks). All diets were in mashed form.

To conduct the present experiment, all guidelines for the care and use of animals in research were followed based on the Korean Ministry for Food, Agriculture, Forestry and Fisheries (2008). Broilers were reared in a closed, ventilated, wire-floor caged broiler house (100 cm long × 90 cm wide × 40 cm high/cage) with a floor space of 1,125 cm^2/bird. The cages had a linear feeder in the front and a nipple drinker in the back to provide *ad libitum* feed intake and free access to water. The internal temperature of the broiler house was set and maintained at 34°C for the first week, after which it was gradually reduced to 23°C at 3°C per week, and then maintained at this temperature until the end of the total experimental period. The internal relative humidity was maintained at around 50% throughout the experimental period.

Measurement of growth performance

Continuous lighting was provided for the entire experimental period, and there was no vaccination or medication program. Chicks were inspected daily and dead birds were removed following recoding of the mortality (pen, date and body weight). Feed intake and Body Weight (BW) were recorded weekly by replicate, and the Average Daily Feed Intake (ADFI), Average Daily Gain (ADG), and FCR (feed

Item	Dietary treatments			
	FGLP[1]	FGLP[2]	FCJP[2]	FCJP
Chemical composition				
Moisture (%)	23.42	26.12	30.62	29.80
Crude protein (%)	10.98	15.13	14.84	15.72
Crude fat (%)	2.41	2.07	1.72	1.51
Crude fiber (%)	9.83	9.33	8.14	7.38
Crude Ash (%)	6.66	6.49	5.60	5.52
Nitrogen free extract (%)	53.30	40.86	39.08	40.07
Microbial strains				
Lactobacillus plantarum KCTC 3099	2.0×10^9	2.0×10^9	2.0×10^9	2.0×10^9
Lactobacillus acidophilus KCTC 3111	2.2×10^9	2.2×10^9	2.1×10^9	2.1×10^9
Saccharomyces cerevisiae KCTC 7904	2.4×10^8	2.4×10^8	2.5×10^8	2.5×10^8

Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 3: Chemical composition of fermented *Ginkgo biloba* probiotics and *Citrus junos* probiotics.

to gain ratio) per cage were then calculated by period and for the total experimental period.

Collection and analyses of blood and caecal samples

At the termination of the feeding trial, 2 birds close to the mean body weight were randomly selected from each pen for blood sample collection. Blood samples were collected (10 mL) from the wing veins of the selected birds into a 10-mL anticoagulant-free vacutainer tube (Greiner Bio-One GmbH, Kremsmunster, Austria). The samples were subsequently stored on ice during the period of collection and then immediately centrifuged to separate the serum (centrifugation for 15 min at 1,610 × *g* at 4°C). Then, the serum samples were carefully transferred to plastic vials and stored at −20°C until immunoglobulin analysis was performed. The concentrations of serum IgG, IgA, and IgM were assayed using appropriately diluted samples by a sandwich ELISA with chicken-specific IgG (Cat. No. E30–104), IgA (Cat. No. E30–103), and IgM (Cat. No. E10–101) ELISA quantitation kits (Bethyl Laboratories Inc., Montgomery, TX) according to the manufacturer's instructions. Each experiment was run in duplicate and the results represent the means of triplicate experiments. The absorbance of each well at 450 nm was measured within 30 min using a microplate autoreader (Thermo Lab Systems, Helsinki, Finland). The concentrations of IgG, IgA, and IgM were determined using standard curves constructed from the respective immunoglobulin standards and the results were expressed as mg/ml of serum.

Selected chickens were slaughtered at the end of 5th week of experimental period to measure the micro flora concentration of caeca, where caecal contents were collected carefully from each bird. Feed withdrawal period of 12 h were maintained. The collected caecal contents were serially diluted in sterile saline in the 1:10 dilution and then cultured on agar media (duplicate for each). The culture media for *E. coli*, *Salmonella*, *Lactobacillus* and yeast were MacConkey Sorbitol Agar; Salmonella Shigella Agar; Lactobacilli MRS (Mann, Rogosa and Sharpe) Agar; and Potato Dextrose Agar, respectively. Incubation in the anaerobic condition at 37°C for 24 h (*E. coli* and *Salmonella)* and 48 h (*Lactobacillus* and yeast) were done followed by the smearing of supernatant of 100 µl onto the agar plate. Following enumeration of microbial colonies in the duplicate incubated agar plates, microbial counts were expressed as log10 CFU/ml.

Slaughtering and meat sampling procedure

At the end of the experimental period, two broilers from each replicate cage were randomly selected and slaughtered following the halal slaughtering method. The breast and thigh meat were then excised from the carcass by removing the skin, bones and connective tissue. After weighing the breast and thigh meat samples from each bird, they were ground separately using a meat grinder. The samples were subsequently divided into two parts, one for the oxidative stability analysis and another for the fatty acid composition analysis. Finally, the samples were poured into plastic sample bottles, after which those for oxidative rancidity analysis were refrigerated at 4°C and samples for other analyses were stored at -20°C.

Determination of breast and thigh meat fatty acids

The fatty acids compositions of breast and thigh meat were determined by a direct method for Fatty Acid Methyl Ester (FAME) synthesis using a slight modification of the method described by O'Fallon et al. 1 g of minced meat sample was placed into a 15 ml Falcon tube, after which 0.7 ml of 10 N KOH in water and 6.3 ml of methanol were added. The tube was then incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking for 10 s every 30 min to properly permeate, dissolve and hydrolyze the sample. After cooling to below room temperature in a cold tap water bath, 0.58 ml of 24 N H_2SO_4 in water was added. The tube was then mixed by inversion, after which K_2SO_4 precipitated. The sample with the precipitate was incubated again in a 55°C water bath for 1.5 h with vigorous hand-shaking for 10 s every 30 min. After FAME synthesis, the tube was cooled in a cold water bath. Next, 3 ml of hexane were added and the tube was vortexed for 5 min on a multitude vortexed. The tube was subsequently centrifuged for 5 min at 3000 × g (HANIL, Combi-514R, and Korea), after which the top (hexane) layer containing the FAME was dehydrated through the anhydrous Na_2SO_4. The extracted and dehydrated hexane was then concentrated to 1.5 ml and placed into a GC vial for analysis.

The fatty acid composition of the FAME was determined using a Gas Chromatograph (Agilent, 7890A series, USA) equipped with a flame ionization detector and a Hewlett Packard HP-88 capillary column (J&W Scientific, USA) with a length of 60 m, a 0.52 mm internal diameter and a 0.20 µm polyethylene glycol-film thickness. Samples were injected using an auto-sampler (Agilent Technologies 7693, USA). The initial oven temperature was 125°C, which was held for 1 min, then increased to 145°C at 10°C/min, where it was held for 26 min, then further increased to 220°C at 2°C/min, where it was held for 2 min. Purified air and hydrogen were applied at a flow rate

of 400 ml/min and 40 ml/min as the carrier gas, whereas helium was applied at 40 ml/min as the makeup gas. Both the injector and detector temperature were set at 260°C, and the split ratio was 30:1. Fatty acids were identified by comparison of their retention times with those of a standard FAME mixture (Supelco™ 37 Component FAME Mix, 10 mg/ml in CH_2Cl_2. Catalog Number 47885-U. Supelco, Bellefonte, PA 16823-0048, USA). Sums and ratios useful for evaluating the nutritional value and healthiness of the fatty acid profile were also determined; specifically, the sum of saturated fatty acids (ΣSFA), monounsaturated fatty acids (ΣMUFA), polyunsaturated fatty acids (ΣPUFA), n-3 fatty acids (Σn-3), n-6 fatty acids (Σn-6) and the ratios of MUFA to SFA (MUFA/SFA), PUFA to SFA (PUFA/SFA), n-6 to n-3 (n-3/n-6) and hypocholesterolemic to hypocholesterolemic (H/H) fatty acid ratio. The H/H ratio was determined as follows:

H/H=[(sum of C18:1 cis-9, C18:2 n-6, C20:4n-6, C18:3 n-3, C20:3n-6, C20:5 n-3, and C22:6 n-3)/(sum of C14:0 and C16:0)].

Statistical analyses

All data were subjected to ANOVA using the General Linear Models (GLM) function of the Statistical Analysis System (SAS, 2003, Version 9.1, SAS Institute, Cary, NC, USA). Each cage was considered as the experimental unit for growth performance parameters (BW, BWG, FI and FCR), whereas an individual bird served as the experimental unit for immunity and caecal microbiology. A probability level of $P<0.05$ was considered as statistically significant and a level of $P<0.10$ was considered as statistical tendency.

Results

Performance of broilers

The result of the growth performance of the broilers supplemented with fermented ginkgo leaf probiotics and fermented citron rind probiotics was presented in Table 4. The result depicted that, the body weight gain and feed intake of broilers were significantly improved after supplementation of FGLP2 during starter, finisher and overall period while compared with the control diet and FCJP group ($P<0.05$). Feed conversion efficiency (FCR) did not differ during starter and finisher period, however, it was found better in FCJP2 in comparison to the control group during overall period of experiment ($P<0.05$). During finisher period, FCJP2 differed with FGLP1 and FCJP1 ($P<0.05$), while during overall period, FCJP differed with FGLP1, FGLP2 and FCJP1 ($P<0.05$).

Mortality and immunity of birds

The mortality rate of the birds as 5.56% in control group and 4.5% FGLP; while FCJP2 showed the zero mortality (data not shown). The serum immunoglobulin status is shown in Table 4. The serum immunoglobulin G (IgG) levels were significantly elevated in the FGLP and FCJP supplemented groups relative to control group ($P<0.05$). Where, serum IgM level differed in FCJP2 with FGLP1, FGLP2 and FCJP1 group ($P<0.05$).

Cecal microbiology and pH

The cecal microbiology data elucidated that, there was no significant differences among the dietary treatments of FGLP and FCJP (Table 5). The pH of the cecal content did not differ after dietary inoculation of FGLP and FCJP in the broiler diet ($P>0.05$).

Chemical composition and cholesterol content of breast and thigh meat

Table 6 shows the meat composition of breast and thigh portion, where it was depicted that, breast and thigh meat crude protein and crude fat content was affected after dietary inclusion of FGLP and FCJP

Items	Dietary treatments					SEM	P value
	Control	FGLP[1]	FGLP[2]	FCJP[1]	FCJP[2]		
0-3 weeks							
Initial weight(g)	39	39	39	39	39	0.02	0.21
Final weight(g)	764^{c}	759^{c}	855^{a}	798^{b}	763^{c}	7.41	<0.0001
Weight gain(g)	724^{c}	720^{c}	816^{a}	759^{b}	724^{c}	7.41	<0.0001
Feed intake(g)	1,013^{b}	1,027^{b}	1,167^{a}	1,025^{b}	998^{b}	19.18	0.001
FCR (Feed/Gain)	1.40	1.43	1.43	1.35	1.38	0.02	0.27
3-5 weeks							
Initial weight(g)	764^{c}	759^{c}	855^{a}	798^{b}	763^{c}	7.41	<0.0001
Final weight(g)	1,870^{c}	1,890^{c}	2,097^{a}	1,895^{c}	1,964^{b}	15.40	0.001
Weight gain(g)	1,106^{bc}	1,130^{bc}	1,242^{a}	1,097^{c}	1,201^{ab}	20.07	0.02
Feed intake(g)	1,786^{c}	1,896^{ab}	1,977^{a}	1,818^{bc}	1,860^{bc}	26.75	0.01
FCR (Feed/Gain)	1.61^{ab}	1.68^{a}	1.60^{ab}	1.66^{a}	1.55^{b}	0.03	0.08
0-5 weeks							
Initial weight(g)	39	39	39	39	39	0.02	0.21
Final weight(g)	1,870^{c}	1,890^{c}	2,097^{a}	1,895^{c}	1,964^{b}	10.89	<0.0001
Weight gain(g)	1,830^{c}	1,850^{c}	2,058^{a}	1,855^{c}	1,925^{b}	10.88	<0.0001
Feed intake(g)	2,800^{d}	2,924^{b}	3,144^{a}	2,844^{cd}	2,858^{c}	12.15	<0.0001
FCR (Feed/Gain)	1.53^{b}	1.58^{a}	1.53^{b}	1.53^{b}	1.48^{c}	0.01	0.001

$^{a-d}$Mean with different superscripts within the same row are significantly different ($P<0.05$).
SEM: Standard Error of Mean.
Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 4: Effects of dietary fermented *Ginkgo biloba* probiotics and *Citrus junos* probiotics on growth performance of broilers.

Item	Dietary treatments					SEM	P value
	Control	FGLP[1]	FGLP[2]	FCJP[1]	FCJP[2]		
Immunoglobulins (mg/mL)							
Immunoglobulin M (IgM)	0.21[a]	0.16[b]	0.14[b]	0.14[b]	0.25[a]	0.01	0.01
Immunoglobulin A (IgA)	0.51	0.52	0.58	0.61	0.54	0.03	0.17
Immunoglobulin G (IgG)	0.23[b]	0.29[ab]	0.32[a]	0.32[a]	0.33[a]	0.01	0.04
Cecum microbiota (log10 cfu/g)							
Lactic acid bacteria	7.33	6.79	7.60	7.39	8.01	0.35	0.27
Yeast & mold	7.36	6.90	7.41	7.22	7.62	0.29	0.61
Escherichia coli	6.49	4.73	7.64	6.59	8.55	0.53	0.20
pH	5.85	6.12	6.20	5.72	5.59	0.30	0.62

[a,b]Mean with different superscripts within the same row are significantly different (P<0.05).
SEM: Standard Error of Mean.
Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 5: Effects of dietary fermented *Ginkgo biloba* probiotics and *Citrus junos* probiotics on serum immunoglobulin concentration and cecal microbiology in broilers.

Meat composition	Dietary treatments					SEM	P value
	Control	FGLP[1]	FGLP[2]	FCJP[1]	FCJP[2]		
Breast meat							
Crude protein (%)	24.95[a]	23.52[ab]	22.80[b]	23.71[ab]	22.22[b]	0.46	0.01
Crude fat (%)	0.95[d]	1.05[cd]	2.04[a]	1.22[bc]	1.43[b]	0.08	<0.0001
Moisture (%)	74.35[ab]	74.62[a]	74.17[ab]	73.85[b]	74.60[a]	0.21	0.10
Crude ash (%)	1.36[a]	1.29[ab]	1.21[b]	1.40[a]	1.29[ab]	0.03	0.01
Thigh meat							
Crude protein (%)	19.03[c]	19.34[bc]	19.70[ab]	20.70[a]	20.45[ab]	0.39	0.03
Crude fat (%)	2.00[b]	2.90[a]	3.37a	3.53[a]	3.01[a]	0.28	0.01
Moisture (%)	75.35	74.34	74.51	74.43	75.12	0.37	0.27
Crude ash (%)	1.08	1.18	1.09	1.21	1.12	0.03	0.16
Cholesterol (mg/100 g)							
Breast meat	140.89[ab]	156.99[a]	147.66[ab]	120.99[b]	122.16[b]	5.53	0.01
Thigh meat	165.39[ab]	183.27[a]	146.14[ab]	162.30[ab]	133.41[b]	8.85	0.03

[a,b]Mean with different superscripts within the same row are significantly different (P<0.05).
SEM: Standard Error of Mean.
Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 6: Effects of dietary fermented *Ginkgo biloba* probiotics and *Citrus junos* probiotics on breast and thigh meat composition in broilers.

in the broiler diet. In case of breast meat the crude protein content was decreased whereas the crude fat content was increased in FGLP2 and FCJP2 relative to control group (P<0.05). Moisture content did not differ with control group; but there was found higher moisture content in the FGLP1 and FCJP2 group compared to FCJP1 group (P<0.05). The crude ash content was lower in FGLP2 relative to the control group. Where in case of thigh meat both the crude protein and crude fat content was augmented in FGLP and FCJP supplemented group compared to control (P<0.05). The meat cholesterol content did not differ in FGLP and FCJP while compared with the control group; however, it did differ between the FGLP and FCJP group (P<0.05).

Fatty acid composition of breast and thigh meat

The fatty acid composition of breast and thigh portion of broiler carcass was presented in the Tables 7 and 8. In Table 7, for the breast meat, it was observed that, sum of Saturated Fatty Acid (SFA) and sum of Polyunsaturated Fatty Acid (PUFA) did not differ in FGLP and FCJP in comparison to the control; however, the sum of Monounsaturated Fatty Acid (MUFA) was lower in FGLP and FCJP group relative to the control group (P<0.05). The sum of n-3 fatty acid was observed higher in FGLP1 compared to control whereas the sum of n-6 fatty acid was found lower in FGLP1 in comparison to the control group (P<0.05). There was found no significant differences in the ratio of MUFA to SFA and PUFA to SFA; however, the ratio of n-6 PUFA to n-3 PUFA was differed in FGLP1, FCJP1 and FCJP2 compared to the control group (P<0.05).

As shown in Table 8, in case of thigh meat, the sum of SFA was downtrended in the FGLP1 and FGLP2 group relative to the control group (P<0.05). The sum of MUFA and PUFA did not show any difference due to supplementation of FGLP and FCJP. The sum of n-3 PUFA did show difference among the control, FGLP and FCJP group. While the sum of n-6 PUFA significantly differed in the FGLP1 in relation to the control group (P<0.05).

Discussion

Medicinal plants or their materials contain several types of bioactive compounds. The chemical constituents of the *Ginkgo biloba* leaf are polysaccharides, flavonoids, flavone glycosides, quercetin, terpenoids, bilobalides and ginkolides [33,34]. The *Citrus junos* composed of flavonoids, naringin, hesperidin, carotinoids, polyphenols, and

Fatty acid (g/100 g FA)	Dietary treatments					SEM	P value
	Control	FGLP[1]	FGLP[2]	FCJP[1]	FCJP[2]		
Myristic acid (C14:0)	0.93[b]	1.65[ab]	1.60[ab]	2.00[a]	2.14[a]	0.17	0.04
Pentadecanoic acid (C15:0)	2.24[a]	2.28[a]	1.09[b]	0.96[b]	0.97[b]	0.13	<0.0001
Palmitic acid (C16:0)	21.11	20.45	19.92	20.23	19.96	0.34	0.27
Palmitoleic acid (C16:1n7)	4.38	4.15	4.39	4.03	3.76	0.23	0.39
Stearic acid (C18:0)	0.21[b]	5.50[a]	9.71[a]	7.36[a]	7.01[a]	1.35	0.01
Oleic acid (C18:1 n9)	35.08[a]	32.95[b]	36.16[a]	34.73[a]	34.73[a]	0.38	0.001
Linoleic acid (C18:2n6)	17.85[a]	15.25[c]	17.37[a]	16.08[b]	17.33[a]	0.26	0.002
α-linolenic acid (C18:3n3)	0.43[a]	0.34[b]	0.45[a]	0.47[a]	0.44[a]	0.02	0.06
Arachidic acid (C20:0)	0.74[a]	0.48[b]	0.77[a]	0.64[a]	0.71[a]	0.04	0
Eicosaenoic acid (C20:1n9)	0.5	0.45	0.45	0.55	0.59	0.05	0.29
Eicosapentanoic acid (C20:5n3)	4.19[b]	5.70[a]	3.23[b]	4.28[b]	4.27[b]	0.34	0.01
DGLA (C20:3n6)	1.99	1.72	1.75	2.11	2.01	0.14	0.43
Docosahexaenoic acid (C22:6n3)	1.12[b]	2.78[a]	1.31[b]	1.94[ab]	1.61[b]	0.25	0.02
Tetracosaenoic acid (C24:1n9)	1.40[ab]	1.54[ab]	1.10[b]	1.66[ab]	1.91[a]	0.16	0.05
∑SFA	34.01	35.35	33.56	34.52	34.11	0.68	0.59
∑MUFA	41.35[a]	44.08[b]	42.09[b]	40.96[b]	40.99[b]	0.53	0.04
∑PUFA	25.57	25.78	24.1	24.88	25.65	0.51	0.21
∑n-3	5.74[bc]	8.81[a]	4.98[c]	6.69[b]	6.31[b]	0.37	<0.0001
∑n-6	19.84[ab]	16.96[c]	19.11[a]	18.20[b]	19.34[ab]	0.4	0.001
MUFA/SFA	1.22	1.12	1.25	1.19	1.21	0.03	0.17
PUFA/SFA	0.75	0.74	0.72	0.72	0.75	0.02	0.77
n-6/n-3	3.53[ab]	1.97[d]	3.87[a]	2.77[c]	3.12[bc]	0.18	<0.0001

[a,b]Mean with different superscripts within the same row are significantly different (P<0.05).
SEM: Standard Error of Mean.
∑SFA: Saturated fatty acid; ∑MUFA: Mono-unsaturated fatty acid; ∑PUFA: Poly-unsaturated fatty acid, ∑n-3: Total omega 3 fatty acid; ∑n-6: Total omega 6 fatty acid.
Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 7: Effects of dietary fermented *Ginkgo biloba* probiotics and *Citrus junos* probiotics on the fatty acid composition of broiler thigh meat.

Fatty acid (g/100g FA)	Dietary treatments					SEM	P value
	Control	FGLP[1]	FGLP[2]	FCJP[1]	FCJP[2]		
Myristic acid (C14:0)	1.1	1.03	1.07	1.02	1.07	0.02	0.16
Myristoleic acid (C14:1 n5)	0.38[a]	0.37[ab]	0.34[b]	0.35[b]	0.34[b]	0.01	0.03
Pentadecanoic (C15:0)	0.79	0.83	0.71	0.81	0.85	0.07	0.72
Palmitic acid (C16:0)	21.54[a]	20.84[b]	20.06[c]	21.18[ab]	20.86[b]	0.17	0.001
Palmitoleic acid (C16:1n7)	6.14[ab]	6.75[a]	5.52[b]	5.84[b]	5.57[b]	0.23	0.01
Stearic acid (C18:0)	7	6.66	7.42	7.12	7.31	0.21	0.13
Oleic acid (C18:1 n9)	39.72	40.35	39.94	40.18	40.06	0.45	0.92
Linoleic acid (C18:2n6)	17.85[ab]	16.70[c]	18.22[a]	17.21[bc]	18.22[a]	0.26	0.001
α-linolenic acid (C18:3n3)	0.57	0.58	0.61	0.61	0.58	0.02	0.43
Arachidic acid (C20:0)	1.03[ab]	0.94[c]	0.99[bc]	0.99[bc]	1.06[a]	0.02	0.01
Eicosaenoic acid (C20:1n9)	0.21[b]	0.21[b]	0.25[a]	0.23[ab]	0.24[b]	0.01	0.01
Eicosadienoic acid (C20:2 n6)	0.14	0.15	0.15	0.16	0.14	0.01	0.33
DGLA(C20:3n6)	0.37	0.4	0.37	0.46	0.41	0.04	0.59
Eicosapentanoic acid (C20:5n3)	2.15[b]	3.07[a]	2.19[b]	2.70[ab]	2.23[b]	0.36	0.04
Docosahexaenoic acid (C22:6n3)	0.88	0.52	0.36	0.99	0.83	0.19	0.16
∑SFA	31.46[a]	30.30[b]	30.25[b]	31.10[a]	31.15a	0.24	0.001
∑MUFA	46.44	47.68	46.05	46.6	46.21	0.61	0.43
∑PUFA	21.95	21.43	21.89	22.11	22.41	0.6	0.86
∑n-3	3.61[b]	4.17[a]	3.16[b]	4.28[a]	3.64[b]	0.48	0.05
∑n-6	18.35[ab]	17.25[c]	18.73[a]	17.83[bc]	18.76[a]	0.25	0.001
USFA/SFA	1.48	1.57	1.52	1.5	1.49	0.03	0.15
PUFA/SFA	0.7	0.71	0.73	0.71	0.72	0.02	0.86
n-6/n-3	5.44	4.5	2.77	4.44	5.18	1.46	0.11

[a-c]Mean with different superscripts within the same row are significantly different (P<0.05).
SEM: Standard Error of Mean.
∑SFA: Saturated fatty acid; ∑MUFA: Mono-unsaturated fatty acid; ∑PUFA: Poly-unsaturated fatty acid; ∑n-3: Total omega 3 fatty acid; ∑n-6: Total omega 6 fatty acid.
Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: 5% FGLP (corn-soybean meal based basal diet+5% FGLP), 3) FGLP2: 10% FGLP (corn-soybean meal based basal diet+10% FGLP), 4) FCJP1: 5% FCJP (corn-soybean meal based basal diet+5% FCJP), 5) FCJP2: 10% FCJP (corn-soybean meal based basal diet+10% FCJP).

Table 8: Effects of dietary fermented *Ginkgo biloba* probiotics and *Citrus junos* probiotics on the fatty acid composition of broiler thigh meat.

flavonols, pectins, acids, volatile oils, enzymes and vitamin C [35,36]. The active phytochemicals present in *Punica granatum* are punicalagin, flavonoids, peduculagin, ellagitannin, and punicalin, some other phenolic compounds and anthocyanins [36]. Fermentation of plant materials with the beneficial microbe's results improvement of the fermented product with the enrichment of the enzymes, vitamins and growth factors which can helps to promote the performance of animals [37,38]. The combination of probiotics and prebiotics that include both beneficial microorganisms and substrates exerts synergistic effects on the gastro-intestinal tract and consequently promotes the growth of animals [39]. Ginkgo biloba while fermented with *Aspergillus* spp. reported that there was no impact on weight gain in broilers [40]. However, in the present study, the weight gain was improved in the FGLP2 group compared to control and FCJP group. Supporting to the study, it was reported that, supplementation of fermented *Ginkgo biloba* along with multi-microbe probiotic was effective in the improvement of the growth performance of broilers [26].

Fermentation of *Citrus junos* with *Saccharomyces cerevisiae* or *Bacillsu subtilis* can improve the nutritive value and antioxidative value in the fermented product resulting in the improvement of the weight gain and feed efficiency [41,42]. No improvement in the feed efficiency after supplementation of citrus fruits by-products with fermentation of multi-microbe probiotics was reported in the study of Ahmed et al. [31]. However, in the present study, although the weight was not significantly improved, the feed conversation efficiency was improved in the FCJP group compared to control. The pectin content of *Citrus junos* might be influenced by the probiotic organisms and activate to generate pectinolytic enzymes resulting in higher production of lactic acid bacteria [36,43,44]; where lactic acid bacteria can complement with the other beneficial microbes can exhibit synergism to utilize the feed with higher efficiency in broiler [45]. Therefore, combination of flavonoids, naringin, hesperidin, carotinoids, polyphenols, and flavonols, pectins, punicalagin, flavonoids, peduculagin, ellagitannin, and punicalin, some other phenolic compounds with the microbial generated organic compounds [34-36], the feed efficiency was improved in the FCJP2 group. The feed efficiency data depicted that, lower level of both the FGLP and FCJP was more efficient in case of broiler. It was reported that lower percentage of fermented *Citrus junos* or *Alisma canaliculatum* along with multimicrobe probiotic might be able to improve the performance of broiler but higher percentage is not able to promote the growth performance of broilers [23,31]. Studies of the modes of action of combined phytobiotic substances and probiotics suggested the possibility of synergistic effects between these classes of compounds, which might help improve microbial balance, immune development and animal performance as well [23]. In addition, polysaccharides of *Ginkgo biloba extract* and *Citrus junos* with *Punica granatum* along with probiotics might play synnergysm for the improvement of performance and immunity in birds. The combination of benficial microbes are able to improve the performance of broilers [17,24].

The improved growth enhancement was concurrent with the lower mortality rate and elevation of Immunoglobulin G (IgG) in the FGLP and FCJP supplemented birds in the present study. Plant derived flavones and terpenes from the combination of natural plants along with probiotic ferementation might have immune promoting activites that help enhance the lymphocyte synthesis, phagocytosis activity and cytokin release [46]. Supporting these findings, improvement in the immune status and lower mortality was reported after inclusion of *Ginkgo biloba extract* and *Camelia sinensis* in broilers. The elevation serum immnonoglobulin level indicating the natural antibodies impact which might be attributable to enlargement of the splenic lymphocite due to the probiotic effect and combination of polyphenolic compounds (derived from *Punica granatum*, *Ginkgo biloba* and *Citrus junos*) [47]. Supplementation of probiotics elevate the capability of the splenic lymphocytes to the proliferation in tesponse to the B-cell and T-cell mitogenic stimulation [48,49]. Dietary inclusion of 10% fermented *Gingkgo biloba* reported to be beneficial to improve immune function in the case of weaned piglets [50], which in turn supports the results of our study regarding higher serum immunoglobulin levels in the case of FGLP supplementation of broilers. The presence of flavonoids (dominance of hesperidin) and vitamin C (L-ascorbic acid) in case of *Citrus junos* and fermentation with probiotics ascribed to be the reason of improvement in the humoral immunity of birds [47,51].

Hossain et al. [23] reported that, the composition of meat can be affected by *Alisma canaliculatum* with probiotics, where they reported that, crude protein was increased but crude fat is decreased. Similar trend was reported by Bostami et al. [26] while they tested the efficacy of Fermented *Salicornia herbacia* L. and *Houttuynia cordata* Thunb with multi-microbe probiotics in broilers. However, in the present study elevation of crude protein and diminishing of crude fat was exhibited in the FGLP2 and FCJP2 in case of breast meat; where for the thigh meat both crude protein and crude fat was increased in FGLP and FCJP compared to control. The reason of such types of result for the thigh meat is not clear to us. Currently, due to health aspects, peoples are paying attention to the new natural products having lipid reducing activity, so that cholesterol level can be controlled and checked the hyperlipidemic risk factor [52-54]. Plant derived secondary metabolites can play important role on the cholesterol content through the mode of action in the biological reactions. The plant bioactive compounds exhibited anti-oxidative action, anti-tumor and anti-lipidemic action (lowering the cholesterol level [40,55]. The biologically active substances can play in favor of diminishing the cholesterol level [48,56]. It was reported that, plant metabolites influences on abate mating the serum level of TC and LDL-C [56]. The plant bioactive compounds might be able to format an insoluble complex combining with the endogenous cholesterol which can further prevented to reabsorb by the action of bile in the physiological system [57,58]. Addition of probiotic in the diet of broiler reported to be effective on reducing the serum cholesterol, low density lipoprotein and triglycerides [59]. Therefore, in the present study, the physiological reaction of the combination of plant materials along with multi-microbes in the FCJP group might effectively exhibited on reducing the breast and thigh meat cholesterol content, which indicated the better quality of broiler meat. Cardiovascular disease is a common worldwide and major case of health which usually happened due to the cholesterol level [60]. Increment of the Low Density Lipoprotein (LDL) causes acceleration of atherosclerosis and finally coma and death, therefore limiting cholesterol intake might be the effective approach [40,55].

Fatty acids play major role in the human nutrition and also have different and important functions in plant, mammal, and animal metabolism. Fatty acid acts as precursor of the biosynthesis of eicosanoids which is considered as an important bio-regulator in the body physiology and cellular metabolic processes. Fatty acids profile in food has a direct impact on human health according to the epidemiological study [61,62]. It well known fact that, the unsaturated fats have a hypocholesterolemic effect, whereas the saturated fats tend to proliferate the level of the total cholesterol and Low-Density Lipoproteins (LDL) [63]. The diminishing of the unsaturated fatty acids will resulted on the compositional change in the membrane lipids which consequently will impact on the membrane lipid phase

transition [64]. Ahmed et al. [24] reported that, plant secondary metabolites of *Punica granatum* able to reduce the total SFA with different dose level in broiler breast and thigh meat. Bostami et al. [26] reported that supplementation of medicinal plants (*Salicornia herbacea* and *Houttuynia cordata*) and multi-microbe probiotics is potential to ameliorate the fatty acids due to the presence of plant phenolic compounds and organic acids of fermented product. In the present study, for breast meat there was found no impact on sum of SFA and sum of PUFA, but in case of thigh meat sum of SFA was diminuted in the FGLP group relative to control and FCJP group, which is indicated the positive manner on the aspect of meat quality and health risk.

Usually the variation in the SFA, MUFA and PUFA is happened due to the phenomena of conversion of one fatty acid to another, like stearic to oleic acid; and the action of enzyme (such as fatty acid synthase enzyme) in the formation and depletion of fatty acids [65]. The sum of MUFA was declined in the FGLP and FCJP group in the present study. The reduction of SFA and MUFA could be happed due to the action of plant derived flavonoids, tannins, mixture of polyphenolics in goat, sheep and broiler meat [66,67]. The key enzyme associated with the conversion and elevation-diminution process of fatty acid is the 9-desaturase enzyme [68]. It was postulated that, the flavonoids, polyphenols and organic acids from the FGLP and FCJP might influence on the function of 9-desaturase enzyme, which consequently resulted in the declining of the MUFA in the present study. The sum of n-3 PUFA was increased but n-6 PUFA was decreased in FGLP1 (both in breast and thigh meat) and in FCJP1 (in thigh meat) in the current study. The phenomena regarding the enlargement and abatement of the n-3 and n-6 PUFA in the present study might be attributable to the competition for the similar enzymes during elongation and desaturation metabolism of fatty acids in the physiological system [69]; the metabolites derived from the combination of plant materials along with multimicrobe probiotics in the FGLP and FCJP might affect the enzymatic system which influence in the composition of fatty acids in the present study. Consumers are now more interested in consuming the product having rich amount of n-3 PUFA to combat against the cardiovascular disease; therefore, Eicosapentaenoic Acid (EPA) and Docosahexaenoic (DHA) acids are two most important n-3 fatty acids for the human nutrition. EPA and DHA acts as the precursor of prostaglandin, thromoboxane, leukotriene eicosanoids and resolvins which play significant role against heat attack, stroke, and anti-inflammatory function [70,71]. DHA acts as the precursor of the protectins and resolvins which acts as anti-inflammatory and neuroprotective agent [70,72]; and also important for the functional development of the brain of young and for the maintenance of the brain of adult human being. In the present study, EPA and DHA (in breast meat) and EPA (in thigh meat) were proliferated in the FGLP1 supplemented group which is the excellent outcome of this research. The variation in the fatty acid and cholesterol content in the present study might be attributable to the variation in the composition of the chemical constituents and also the dose of bioflavonoids derived from the FGLP and FCJP [68].

Conclusion

Present study was conducted to observe the dietary effect of fermented *Ginkgo biloba* probiotics (FGLP) and Fermented *Citrus junos* Probiotics (FCJP) on growth performance, immunity, cecal microbiology, meat composition and fatty acid profile in broilers. Dietary treatments were: 1) Control (corn-soybean meal based basal diet), 2) FGLP1: Corn-soybean meal based basal diet+5% FGLP, 3) FGLP2: Corn-soybean meal based basal diet+10% FGLP, 4) FCJP1: Corn-soybean meal based basal diet+5% FCJP, and 5) FCJP2: Corn-soybean meal based basal diet+10% FCJP. A significant increase in weight gain during starter (0-21 days), finisher (22-35 days) and overall period (0-35 days); and feed intake during finisher period (22-35 days) and overall period (0-35 days) was exhibited in the FGLP2 supplemented group compared to control and other treatment groups. Better feed conversion efficiency was found after FCJP2 supplementation during finisher and overall period compared to control and other treatment groups. Serum IgG was elevated in FGLP and FCJP supplemented group relative to control birds ($P<0.05$). Meat composition data elucidated that, thigh meat crude protein and crude fat content was increased in FCJP1 supplemented group compared to control. There was found no significant differences in meat cholesterol content between control and treatment groups; however, among meat fatty acids, sum of SFA in thigh meat was diminished in FGLP group and sum of MUFA in breast meat was diminuted in the FGLP and FCJP group compared to control. Sum of n-3 PUFA of breast and thigh meat was elevated after supplementation of FGLP and FCJP compared to control. To sum up, dietary FGLP and FCJP supplementation significantly improved performance and immunity, decreased SFA and elevated n-3 PUFA of broiler meat. Therefore, FGLP and FCJP probiotics could be supplemented as functional feed additives in broilers diet. However, further detail research is required to confirm the dose level.

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