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Influence of Dietary Vegetable Wasps with Fly Pupa on Gastrointestinal Tract Microflora, Carcass Characteristics and Growth Performance in Broiler Chickens

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Abstract: This study evaluated the effects of the addition of different levels of VWFP (Vegetable Wasps with Fly Pupa) on the growth performance, carcass characteristics and gastrointestinal microorganism populations of broiler chickens. About 400 animals (Ross 308, 1 day old) with an average body weight of 45 g were sorted randomly into 4 treatment groups and 4 repetition groups of 25 animals each. The treatment groups were divided into a control group not fed with VWFP and treatment groups fed with VWFP 2.0, 3.5 and 5.0%. The feeding test lasted 5 weeks separated by the grower period (0-21 days) and the finisher period (22-35 days). Although, the broilers' weight gain and feed efficiency were significantly higher in the VWFP 3.5% ($p<0.05$) group throughout the entirety of the test period, no statistically significant differences were noted between the control group and other treatment groups. Triglyceride in the blood, total cholesterol and LDL-C were significantly lower in the VWFP treatment groups than in the control group ($p<0.05$). The blood lipid reduction rate ranged from 5.32-10.63% for triglycerides from 9.23-2.62% for total cholesterol and from 44.67-53.81% for LDL-C in the VWFP treatment groups relative to the control group. The abdominal fat weight ratio was reduced significantly in the VWFP treatment groups ($p<0.05$) compared with the control group with a reduction rate range of 17.67-21.68%. Broiler carcass weight, carcass rate and breast muscle, skin and thigh muscle weights against carcass weight were significantly higher in the VWFP 3.5% treatment group and a statistically significant difference was noted between the control group and other treatment groups ($p<0.05$). Enteropathogenic *E. coli* and Salmonella were lower in the VWFP treatment groups than in the control group whereas the beneficial bacteria Bifidobacteria were significantly higher in the VWFP treatment groups than in the control group ($p<0.05$).

Key words: Vegetable wasps, fly pupa, growth performance, carcass, microflora, skin

INTRODUCTION

Vegetable wasps also known as Plant worms or Cordyceps are small mushrooms of the family Clavicipitaceae and the order Clavicipitales. They are parasites of insect hosts in the Winter and grow either by generating fruiting bodies on the insect carcass or by forming spores on the bodies of insects in the hot and humid Summer. There are 300 kinds of Cordyceps and their main host insects are cicadas, butterflies, ants, bees, dragonflies and beetles. *Cordyceps militaris* is parasitic against the pupae of various insects belonging to the butterfly order (Sung *et al*., 1998). The hyphae of Cordycepts are Ascomycetes, Clavicipitales and Clavicipitaceae and generally, the Cordyceps, Podonectria and Torrubiella genera are the most widely known (Koo and Lee, 2004). Cordyceps are found on arthropods including insects as well as fungi or any parasitic fungi on seeds of higher plants (Sung *et al*., 1998). Cordyceps contain carbohydrate, protein, essential fatty acids, iron, vitamins A, C, B_{12}, Cordyceptin and Ergosterol, a vitamin D precursor (Choi *et al*., 2004; Koo and Lee, 2004; Oh *et al*., 2003; Ohmori *et al*., 1999; Shimizu, 1997). Cordyceps have been reported to effect a broad range of functions including nutritional and tonic functions (Yong-Lu *et al*., 1977), endurance enhancement and immune regulation (Kang *et al*., 2003; Kuo *et al*., 1996), cancer resistance (Kuo *et al*., 1994; Furuya *et al*., 1983), blood glucose and cholesterol reduction (Kiho *et al*., 1993, 1996), antibiotic, antispasmodic and calming effects and have also been associated with improvements in kidney function and sexual function (Kang *et al*., 2003; Lin, 1999; Ying *et al*., 1987).

It has been previously reported that when broilers are fed fermented feed inoculated with snow flower Cordyceps strains, breast and thigh muscle weight can increase significantly while making no difference in growth performance (Kang *et al*., 2003). However, very few studies have evaluated the growth performance and carcass characteristics of broilers fed with VWFP.

Therefore, the principal objective of this study was to evaluate the effects of the addition of different levels of vegetable wasps with fly pupae to broiler diets on growth performance, carcass characteristics and the gastrointestinal microorganisms of broiler chickens.

MATERIALS AND METHODS

This experiment was approved by the Institutional Animal Care and Use Committees (IACUC) of Kangwon National University, South Korea. After 400 1 day old male chicks (45 g±0.3 BW) from a gender-examined Ross 308 strain were purchased from the Han-Yang hatchery (located in Ichon, Gyeonggido), they were allocated randomly to 4 treatment groups and 4 repetition groups. About 100 broilers were allocated to each treatment group and 25 to each repetition group. Treatment groups were divided into a control group (T1) fed with no added VWFP and treatment groups fed with added VWFP 2.0% (T2), VWFP 3.5% (T3) and VWFP 5.0% (T4). The VWFP was provided by Korea Beneficial Insects Lab. Co., Ltd. and its chemical analysis showed a composition of 4.50% moisture, 56.27% crude protein, 8.69% crude fat, 5.44% crude ash, 8.79% crude fiber, 16.31% NFE (nitrogen-free extracts) and 3,650 kcal kg^{-1} of gross energy. The composition of fatty acids shows oleic acid to be highest in Table 1. The levels of VWFP addition were determined as above in a preliminary experiment in which VWFP was added at levels of 5.0, 7.5 and 10.0% to broiler feed; the results revealed that the broilers' growth rate was substantially reduced when >5.0% VWFP was added to the broiler feed. Kang *et al.* (2003) reported the same results as were reported in the preliminary experiment, demonstrating how broilers' growth rate and chicken meat production were reduced when the broilers were fed with fermented feed inoculated with >5.0% of snow flower Cordyceps strains.

Table 1: Fatty acid composition of vegetable worm with house fly pupa (percentage of total fatty acid)

Formula	Common name	Percentage
8:0	Octanoic acid	-
10:0	Decanoic acid	3.77
12:0	Lauric acid	1.18
14:0	Myristic acid	2.23
16:0	Palmitic acid	20.96
16:1n-7	Palmitoleic acid	11.69
18:0	Stearic acid	7.71
18:1n-9	Oleic acid	42.15
18:2n-6	Linoleic acid	8.55
18:3n-3	Linolenic acid	-
20:0	Arachdic acid	1.77
SFA[1]	-	37.62
UFA[2]	-	62.38
UFA/SFA	-	1.66
Total	-	100.00

[1]SFA: Saturated Fatty Acid; [2]UFA: Unsaturated Fatty Acid

For experimental feed, mainly corn and soybean meals were mixed to either meet or exceed the nutrient requirements of broilers laid out in the United States NRC feeding standards (NRC, 1994) and the crude protein and metabolic energy contents of experimental feed were also adjusted to match the standards (Table 2 and 3). The mixed feed, stored in a cool place was provided freely to broilers with water for 35 days under standard environmental conditions in which temperature, humidity, ventilation, illumination and noise are automatically controlled from the day of hatching until the broilers reach market weight. Broilers were bred under normal conditions (density 10 animals m^{-2}) with each pen padded with a bedding of rice husks reaching up to 10 cm from the floor. The feeding period was separated into the grower period (0-21st day) and the finisher period (22-35th day) with the breeding room temperature maintained at 33°C from the 1st day for 3 days and lowered by 2-3°C $week^{-1}$ until the 21st day then maintained at 25°C from the 22nd day. Relative humidity was maintained at 70% with 24 h continuous illumination and ventilation 3-5 times a day provided by an auto ventilation system.

Table 2: Formula and chemical composition of the experimental diets for broiler chickens (0-3 weeks) (percentage as-fed)

	Groups[1]			
Ingredients	T1	T2	T3	T4
Yellow corn	55.60	56.80	56.40	55.70
Full fat soy	2.70	0.30	-	-
Soybean meal	29.80	28.90	27.90	27.70
Fish meal (CP 50%)	3.00	3.00	2.90	2.20
Corn gluten meal	2.00	2.00	2.00	2.00
Soy oil	3.00	3.00	3.00	3.00
Vegetable worm	-	2.00	3.50	5.00
Limestone	1.73	1.70	1.95	1.92
Dicalcium phosphate	0.86	0.93	0.98	1.12
Salt	0.22	0.22	0.22	0.22
DL-methionine (99%)	0.19	0.19	0.18	0.17
L-lysine (78%)	0.36	0.40	0.40	0.40
L-threonine (50%)	0.23	0.27	0.27	0.27
Phyzyme 1,000 FTU	0.06	0.05	0.05	0.05
Choline (50%)	0.05	0.05	0.05	0.05
Vit.+min. mix[2]	0.20	0.20	0.20	0.20
Calculated values[3]				
ME (mcal kg^{-1})	3.05	3.05	3.05	3.05
Crude protein (%)	22.00	22.00	22.00	22.24
Calcium (%)	1.00	1.00	1.00	1.06
Available P (%)	0.50	0.50	0.50	0.50
Lysine (%)	1.40	1.40	1.40	1.40
Methionine (%)	0.51	0.51	0.51	0.51
Met.+cystine (%)	0.90	0.90	0.90	0.90

[1]T1: Control, T2: Vegetable worms 2.0%, T3: Vegetable worms 3.5%, T4: Vegetable worms; 5.0%. [2]Supplied per kilogram of diet: Fe, 80 mg; Zn, 80 mg; Mn, 70 mg; Cu, 7 mg; I, 1.20 mg; Se, 0.30 mg; Co, 0.70 mg. vitamin A (retinyl acetate), 10,500 IU; vitamin D_3, 4,100 IU; vitamin E (DL-α-tocopheryl acetate), 45 mg; vitamin K_3, 3.0 mg; thiamin, 2.5 mg; riboflavin, 5 mg; vitamin, B_6, 5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.18 mg; niacin, 44 mg; pantothenicacid, 17 mg; folic acid, 1.5 mg. [3]Calculated from NRC (1994)

Table 3: Formula and chemical composition of the experimental diets for broiler chickens (4-5 weeks) (percentage as-fed)

	Groups[1]			
Ingredients	T1	T2	T3	T4
Yellow corn	61.80	61.90	62.20	62.60
Full fat soy	0.50	-	-	-
Soybean meal	26.40	25.30	23.40	21.40
Fish meal (CP 50%)	3.00	2.00	2.00	2.00
Corn gluten meal	2.00	2.00	2.00	2.00
Soy oil	3.00	3.00	3.00	3.00
Vegetable worm	-	2.00	3.50	5.00
Limestone	1.65	1.88	1.89	1.90
Dicalcium phosphate	0.46	0.66	0.72	0.78
Salt	0.22	0.22	0.22	0.22
DL-methionine (99%)	0.17	0.17	0.16	0.15
L-lysine (78%)	0.27	0.31	0.33	0.35
L-threonine (50%)	0.23	0.26	0.28	0.30
Phyzyme 1,000 FTU	0.06	0.05	0.05	0.05
Choline (50%)	0.05	0.05	0.05	0.05
Vit.+min.mix[2]	0.20	0.20	0.20	0.20
Calculated values[3]				
ME (mcal kg^{-1})	3.10	3.10	3.10	3.12
Crude protein (%)	20.00	20.00	20.00	20.00
Calcium (%)	0.90	0.93	1.00	1.00
Available P (%)	0.50	0.50	0.50	0.50
Lysine (%)	1.20	1.20	1.20	1.20
Methionine (%)	0.46	0.46	0.46	0.46
Met.+cystine (%)	0.82	0.82	0.82	0.82

[1]T1: Control, T2: Vegetable worms 2.0%, T3: Vegetable worms 3.5%, T4: Vegetable; worms 5.0%. [2]Supplied per kilogram of diet: Fe, 80 mg; Zn, 80 mg; Mn, 70 mg; Cu, 7 mg; I, 1.20 mg; Se, 0.30 mg; Co, 0.70 mg. vitamin A (retinyl acetate), 10,500 IU; vitamin D_3, 4,100 IU; vitamin E (DL-α-tocopheryl acetate), 45 mg; vitamin K_3, 3.0 mg; thiamin, 2.5 mg; riboflavin, 5 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.02 mg; biotin, 0.18 mg; niacin, 44 mg; pantothenicacid, 17 mg; folic acid, 1.5 mg. [3]Calculated from NRC (1994)

The broilers' growth performance (feed intake, weight gain and feed demand ratio) by growth stage was measured at the 3rd and 5th weeks where feed efficiency was calculated by dividing feed intake over a certain period of time by weight gain. After 35 days of breeding, 24 animals (6 per repetition pen) having reached average weight were selected from each treatment group and sacrificed via cervical dislocation without any stress procedures according to the test animal euthanasia recommendations (Close *et al*., 1997). The carcasses were submerged in hot water (58-60°C) for 4 min prior to evisceration and depilated by passing them through a dehairing machine for 2 min. Evisceration was carried out 15 min after the broilers were sacrificed and the carcasses were maintained for 1 h at approximately 18°C where they were sacrificed and then in a chilling room in 4°C until the 24th h after being sacrificed.

The broilers' carcass rate was calculated by the ratio of carcass weight (weight excluding feather, blood, head, leg and digestive tract contents) to live weight. The head was cut off at the first neck bone and the leg was cut at the knee and shin and all the inedible gut contents were removed. Abdominal fat was measured by extracting all the fat around the abdominal cavity and gizzard. The weight ratio of breast, skin and leg was calculated by the ratio of each of these weights to the carcass weight and the weights of the liver, gizzard, abdominal fat and immune organs (thymus, spleen, bursa of Fabricius) were expressed as a ratio to live weight. On the day of sacrifice, pH was measured for 12 carcasses from treatment groups, using experimental materials drawn from one-half of each carcass. The breast muscle was sampled for the analysis of water holding capacity and meat color and the thigh muscle samples for TBARS measurement were extracted from 24 of the 24 h old carcasses per treatment group.

On the day of experiment completion, 1 mL blood samples were taken from the wing veins of 24 randomly selected animals (6 per repetition pen) per treatment group via Heparin injection. After the blood plasma was separated from the blood via 15 min of centrifugation at 3,000 rpm, it was quickly frozen with liquid nitrogen gas (-196°C) and stored in a freezer at -20°C until the time of biochemical analysis. TAG (Triglyceride), TC (Total Cholesterol) and HDL-C (High-Density Lipoprotein Cholesterol content) were analyzed using a commercial enzyme kit (Bioclinical system auto kits, BCS, Korea) and LDL-C (Low Density Lipoprotein Cholesterol content) was calculated by the total cholesterol-(Triglyceride/5+HDL.C) formula (Friedewald *et al*., 1972). In order to assess gastrointestinal microorganisms, 24 animals (6 per repetition pen) per treatment group were selected from broilers sacrificed on the day of experiment completion. The caecal content was removed using sterile techniques, immediately put on ice and then anaerobically moved to an experimental room in order to determine the total numbers of microflora of Bifidobactera, Lactobacillus, *E. coli* and Samonella. After homogenizing, the gut contents removed using sterile techniques, 1 g of sample was dissolved in 9 mL of sterilized anaerobic saline (Phosphorus Buffered Saline (PBS)) and mixed and then diluted 10 times (wt/vol). In a preliminary experiment, the adequate dilution factor was selected after serial 10x dilutions to a final 10^{-8}. At 10^{-7} which was selected as the adequate dilution factor in the preliminary experiment, 0.1 mL each was injected into 4 sterilized selective plate media (Lactobacillus, BL medium; Bifidobactera, BS medium; Salmonella, SS medium; *E. coli*, MacConkey medium). After stationary culture for 40 h in the 37°C CO_2 incubator under anaerobic conditions regulated by the Gas-Pak® system (BBL), the number of colonies was investigated as a counter of microorganisms on each plate medium. The measurements were presented in common logarithms for the numbers of fungi per caecal content in g (CFU, Colony Forming Unit/g wet caecal content).

Statistical analysis: Analysis of variance was carried out for statistical treatment of data with the GLM procedure of SAS (2000). According to Duncan's multiple range test, the statistical significance between treatment means was evaluated at the 95% level ($p<0.05$).

RESULTS AND DISCUSSION

The effects of the addition of different levels of VWFP on growth performance are shown in Table 4. Throughout the entire period, the broilers' weight and feed intake were significantly higher in T3 ($p<0.05$) but no statistically significant difference was observed among T1, T2 and T4. Although, feed intake in the grower period was higher in T3, no statistically significant differences were observed among T1, T2 and T4. In the finisher period, feed intake was higher in T3 and lowest in T4 and no statistically significant differences were observed between T1 and T2. These results affected feed intake changes throughout the entire period to a significant degree ($p<0.05$). Feed efficiency did not differ between treatment groups during the grower period, however in the finisher period, feed intake was highest in the T3 group and lowest in the T4 group ($p<0.05$) and no statistically significant differences were observed between T1 and T2. Feed efficiency during the entire period was significantly higher in T3 ($p<0.05$) and no statistically significant differences were observed among T1, T2 and T4.

Since, body weight was significantly higher in the VWFP 3.5% treatment group without any significant differences between the control group and other treatment groups and was significantly lower in the VWFP 2.0 and 5.0% treatment groups than in the VWFP 3.5% treatment at the time of experimental completion, it is estimated that simultaneously improves immunocompetence, anti-group a plateau will occur at which the broilers' weight gain does not increase further with the gradual addition of VWFP.

An important fact discovered in this experiment was that broiler mixed feed with added VWFP at a level of 3.5% might improve the growth performance of the broilers. Since, the addition of VWFP stimulates the growth of beneficial microorganisms in the broiler caecum and oxidant activity and antibiotic activity (Kang *et al*., 2003; Lin, 1999; Kuo *et al*., 1996; Ying *et al*., 1987), it is further assumed to prevent diarrhea in chicks, increase feed efficiency and ultimately improve the broilers' productivity.

The effects of the addition of different levels of VWFP on broilers' blood lipids are shown in Table 5. Blood triglycerides, total cholesterol and LDL-C were significantly lower in the VWFP treatment groups as compared to the control group whereas HDL-C was increased significantly in the VWFP treatment groups relative to the control group ($p<0.05$). The blood lipid reduction ranges were 5.32-10.63% for triglycerides, 9.23-12.62% for total cholesterol and 44.67-53.81% for LDL-C in the VWFP treatment groups relative to the control group; these levels of reduction may indeed cause a reduction in abdominal fat (Table 6). Kiho *et al*. (1993, 1996) reported that Cordyceps may reduce blood

Table 4: Body weight gain, feed intake and feed efficiency of broilers fed experimental diets for 35 days

	Groups[1]				
Days	T1	T2	T3	T4	PSE[2]
	---------Body weight gain (g)--------				
0-21	846[b]	850[b]	876[a]	848[b]	20.3715
22-35	952[b]	952[b]	1,008[a]	962[b]	32.0728
0-35	1,798[b]	1,802[b]	1,884[a]	1,810[b]	30.0218
	----------Feed intake (g)----------				
0-21	980[b]	984[b]	1,027[a]	982[b]	10.3730
22-35	1,255[b]	1,262[b]	1,412[a]	1,215[c]	12.3154
0-35	2,235[b]	2,246[b]	2,439[a]	2,197[c]	21.4620
	---------Feed conversion ratio--------				
0-21	1.15	1.15	1.17	1.15	0.0312
22-35	1.31[b]	1.32[b]	1.40[a]	1.26[c]	0.0262
0-35	1.24[b]	1.24[b]	1.29[a]	1.21[b]	0.0324

[1]T1: Control, T2: Vegetable worms 2.0%, T3: Vegetable worms 3.5%, T4: Vegetable worms 5.0%; [2]PSE: Pooled Standard Error; [3]Feed conversion ratios: Feed intake devided to weight gain; [a-c]Mean values with different superscripts are significantly different at $p<0.05$

Table 5: Levels of TAG, TC, HDL.C and LDL.C in plasma from broilers fed the experimental diets for 35 days[1] (mg dL^{-1})

	Groups[1]				
Levels	T1	T2	T3	T4	PSE[2]
TAG	125.56[a]	114.98[b]	112.21[b]	118.87[b]	0.9420
TC	124.95[a]	110.06[b]	113.41[b]	109.18[b]	0.8740
HDL.C	51.48[c]	64.73[a]	64.21[a]	62.72[b]	0.3595
LDL.C	48.35[a]	22.33[c]	26.75[b]	22.68[c]	0.3034

[1]T1: Control, T2: Vegetable worms 2.0%, T3: Vegetable worms 3.5%, T4: Vegetable worms 5.0%; [2]PSE: Pooled Standard Error; [a-d]Mean values with different superscripts differ significantly ($p<0.05$)

Table 6: Characteristics of carcass of broilers fed the experimental diets for 35 days

	Groups[1]				
Items[2]	T1	T2	T3	T4	PSE[3]
Carcass weight (g)	1,297.00[c]	1,317.00[b]	1,442.00[a]	1,335.00[b]	28.2022
Carcass yield (%)	72.14[c]	73.11[b]	76.54[a]	73.74[b]	19.4584
Breast muscle	23.58[c]	24.55[b]	26.88[a]	24.77[b]	0.0541
Thigh muscle	16.45[c]	17.05[b]	18.22[a]	17.18[b]	0.0700
Liver	2.36	2.43	2.35	2.31	0.0312
Thymus	0.10	0.11	0.13	0.12	0.0239
Spleen	0.09	0.11	0.08	0.10	0.0180
Bursa of fabricius	0.15	0.12	0.14	0.16	0.0281
Abdominal fat	2.49[a]	2.05[b]	1.95[b]	1.96[b]	0.0107

[1]T1: Control, T2: Vegetable worms 2.0%, T3: Vegetable worms 3.5%, T4: Vegetable worms 5.0%; [2]Percentage of breast and thigh muscle weight to carcass weight, percentage of abdominal fat, bursa, spleen and thymus weights to live weight; [3]PSE: Pooled Standard Error; [a-c]Mean values with different superscripts are significantly different at $p<0.05$

glucose and blood cholesterol and that they exert a lipid peroxidation inhibition effect when 3% of Cordyceps fruiting body and hypha powder was fed to Sprague-Dawley rats with hypercholesterolemia (Shen and Chen, 1968), a finding which supports the results of this experiment. Broilers' carcass characteristics according to the addition of different levels of VWFP are shown in Table 6. Broilers' carcass weight, carcass rate and breast, skin and thigh muscle weights against carcass weight were significantly higher in T3 followed by T4 and T2 and a statistically significant difference was observed between T1 and the rest of T2-T4 ($p<0.05$). With regard to the weight ratios of immune organs such as the bursa of Fabricius, thymus and spleen to live weight, no statistically significant differences were observed between treatment groups. The abdominal fat weight ratio was significantly reduced in T2-T4 compared with T1 ($p<0.05$) and the range of abdominal fat reduction rates was 17.67-21.68%. The reduction of broilers' abdominal fat along with the addition of VWFP can be attributed to the reduction of blood lipids (Table 5). It is generally recognized that in lipid metabolism, blood lipids migrate to living tissues where they are used to generate energy with the remainder being stored in abdominal cavity tissues. Therefore, the reduction of abdominal fat can likely be explained by a reduction in the amounts of lipids that moved to abdominal cavity tissues due to the lowering of blood lipids by VWFP (Park and Park, 2009).

Broiler breeders hope to produce low-fat carcasses (leaner carcasses). Selection to improve feed efficiency and reduce triglyceride concentrations in the blood can reduce broilers' abdominal fat relative to body weight. Owing to the global trend toward healthy food, the broiler industry is currently showing increased interest in fat accumulation in carcasses. This growing awareness of animal fat in food, particularly its relation to the progression of arteriosclerosis has put pressure on the poultry industry to reduce the fat contents of chicken meat. Excessive fat accumulation in broilers reduces profits for the food processing industry and makes the treatment of waste fat more difficult. Several approaches have become available for the regulation of fat accumulation; genetic selection for low-fat meat production, nutritional treatment, drug therapy and the manipulation of environmental conditions. On average, abdominal fat takes up approximately 2~3% of broilers' live weight.

Part of the accumulated fat removed during processing reduces processing efficiency and increases the amount of inferior meat and fat that must be discarded into the wastewater of slaughterhouses. Remaining fat in ready to cook chicken products tends to give the impression that chicken meat contains a lot of fat thus weakening consumer preference for such products.

Table 7: Viable cell counts of microflora in cecal digesta of broilers fed the experimental diets for 35 days (log 10 cfu g^{-1} content)

	Groups[1]				
Microflora	T1	T2	T3	T4	PSE[2]
Total aerobic bacteria	6.44[a]	6.03[b]	6.27[b]	6.17[b]	0.0324
Bifidobacteria	6.13[b]	6.67[a]	6.77[a]	6.72[a]	0.0273
E. coli	5.06[a]	4.58[b]	4.56[b]	4.63[b]	0.0265
Salmonella	5.54[a]	4.46[b]	4.72[c]	4.83[c]	0.0289

[1]T1: Control, T2: Vegetable worms 2%, T3: Vegetable worms 3.5%, T4: Vegetable worms 5%; [2]Pooled standard error of the mean values; [a-c]Mean values with different superscripts differ significantly ($p<0.05$)

The changes in microorganism content according to the addition of different levels of VWFP were investigated in the caecum contents of broilers and are shown in Table 7. *E. coli*, Salmonella which are aerobic and pathogenic intestinal microorganisms were lower in the T2-T4 (groups fed with VWFP) than in the T1 group whereas the beneficial bacteria Bifidobacteria were higher in the T2-T4 groups than in the T1 group; a statistically significant difference between treatment groups was observed ($p<0.05$). The importance of microorganisms in the digestive tract is primarily attributable to their roles in the synthesis of fermentation products which supplies the energy necessary for intestinal epithelial cells as well as to their roles in the stimulation of the digestive tract immune system, vitamin K synthesis and resistance against the clustering of exogenous pathogenic organisms (Tako *et al*., 2008). While Lactobacillus and Bifidobacteria are well-known beneficial microorganisms for animal health, other microorganisms such as *E. coli* and *Clostridium perfringens*, may be pathogenic (Devaraj *et al*., 2002). As the intestinal microflora of Bifidobacteria and Lactobacillus compete with potential pathogens for nutrient and adhesion sites in the intestine, they reduce the number of intestinal pathogenic organism groups. Additionally, Bifidobacteria and Lactobacillus secrete the active substance of bacteriocin against *E. coli* and generate substrates for organic acid and other microorganisms. The majority of the organic acids generated by the fermentation of Lactobacillus are lactic acid and acetic acid. All of these substrates can inhibit the intestinal clustering of pathogenic organisms (Rolfe, 2000; Gibson and Wang, 1994). Part of this mechanism significantly reduced the number of microflora such as *E. coli* and Salmonella in the caecum in the VWFP treatment groups.

CONCLUSION

This study suggests that the addition of VWFP 3.5% to broiler feed not only improves weight gain and carcass characteristics but also has the effect of increasing beneficial intestinal microflora and inhibiting harmful microflora.

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