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Effect of feeding a diet containing housefly (*Musca domestica*) larvae extracts on growth performance in broiler chickens

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Abstract: Insects like housefly (*Musca domestica* L.) are future feed resources for economic animals because insects can improve growth performance by promoting the immune function and gut microbial balance. However, little is known about research reports on housefly larvae extracts containing an antibacterial peptide. This study investigated the effect of feeding diets supplemented with housefly larvae extract (HLE) containing antibacterial peptides on the growth performance of broiler chickens. Nine hundred broilers (Ross 308) were fed diets containing HLE for 35 days. Treatment groups consisted of CON (control without HLE), PC (positive control, 8 ppm of avilamycin), HLE50 (diet containing 50 ppm of HLE), HLE100 (diet containing 100 ppm of HLE), HLE150 (diet containing 150 ppm of HLE), and HLE200 (diet containing 200 ppm of HLE). Body weight gain and feed conversion ratio were significantly ($P < 0.05$) increased in HLE150 and HLE200 groups compared to those in the CON group. Spleen weights were higher in HLE150 and HLE200 groups than in the CON group. Caecal bifidobacteria and *Lactobacillus* counts were significantly ($P < 0.05$) higher in HLE150 and HLE200 groups whereas *E. coli* and coliform bacteria counts were higher in the CON group. Caecal acetic acid, propionic acid, and total short-chain fatty acid levels were significantly ($P < 0.05$) higher in HLE150 and HLE200 groups than in the CON group. These results show that feeding diets containing 150 ppm of HLE containing antimicrobial peptides could replace antibiotics to improve the growth performance of broiler chickens.

Keywords: acetic acid; antibacterial peptide; housefly larvae; immune organ; *Lactobacillus*

There are reviews on the use of insects as future animal feed resources that can replace feed antibiotics (Makkar et al. 2014; Jozefiak and Engberg 2017). In animal nutrition, the use of dietary antibiotics resulted in serious problems such as development of resistant bacteria, drug residues in the body of the birds, and imbalance of intestinal bacteria (Furtula et al. 2010; Mehdi et al. 2018). Antibiotics use may produce antibiotic resistance and leave residues in animal products (Gaskins et al. 2002; Nisha 2008). The world is tense with concerns over super bacteria that develop tolerance against antibiotics selectively in the body of the host as a result of misuse or abuse of antibiotics on humans or animals, such as methicillin, vancomycin and quinolone resistant *Streptococcus aureus* and strong antibiotic

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resistant bacteria such as *Salmonella typhimurium* DT 104 (An et al. 2004; Bexfield et al. 2004).

Research and development of new alternative substances to antibiotics that can replace the existing antibiotics is urgently required to alleviate possible damage and loss that may occur from not using antibiotics and to ensure continuous improvement of the productivity of stock farming (Mehdi et al. 2018). Analysis of housefly larvae extract (HLE) against multi-antibiotic resistant *Staphylococcus aureus* and *Bacillus subtilis* was performed and the existence of 5-kDa antibacterial peptide was revealed (Bexfield et al. 2004; Park et al. 2010). The exact structure and amino acid alignment of antibacterial protein derived from HLE and the mechanism of antibacterial action against various pathogenic bacteria in the intestine were not clearly known yet and especially, there have been no animal trials performed in standard environment or production field on the antibiotic substitution effect of HLE. The fact that HLE has an antibiotic substitution effect means that the productivity of stock farming can be achieved without using antibiotics and the production of antibiotics-free safe stock farm products will contribute to the quality improvement of domestic stock farm products in comparison with imported goods (Park et al. 2007; Park and Park 2015). However, no measurement has been taken for the active effect of bifidobacteria in the intestine of animals in livestock production sites and the quality of stock farm products that were produced using HLE as an appropriate substitute for antibiotics for the production of safe stock farm products. Park et al. (2007; 2010) performed structure analysis of HLE-derived antibiotic substance and developed a production and extraction system. Park and Park (2015) used antibacterial extracts from HLE, and added them to the diets of rats as animal biomodels to monogastric nutrition in order to establish their usefulness as bifidogenic effect for an antibiotic substitute. Obtained antibiotic candidates could be used for developing innovative antibiotics by using the structure-based drug discovery method. Housefly (*Musca domestica* L.) larvae are also known as a potential biomedical material that contains a nonspecific antibacterial peptide (Bexfield et al. 2004; Park et al. 2010). The objective of the present study was to investigate the effect of feeding a diet containing housefly larvae extracts to replace antibiotics to improve the growth performance of broiler chickens.

MATERIAL AND METHODS

Preparation of HLE

The housefly larvae samples dried on a warm floor at 40 °C were provided by HooinEcobio Co., Ltd (Hongseong, Republic of Korea). After housefly larvae were compressed at 1 000 psi, 150 °C for 30 min, 98% of the lipids were removed. The remaining lipids in the housefly larvae were completely removed using hexane, and the defatted housefly larvae residue and ethyl alcohol were mixed at a ratio of 1 : 10 and using a reflux condensing system ethanol extracts were obtained. Useful ethanol extracts were concentrated at 45 °C in water using a vacuum rotary evaporator (Eyela N-1000, Rikakikai Co., Tokyo, Japan) and stored at –20 °C. As a result of the chemical analysis of larvae extracts, the existence of a 5 kDa antibacterial peptide has already been reported (Yoon et al. 2008; Park et al. 2010). HLE additives (HLE 1 000 mg/kg, HLE 1 000 mg per zeolite kg, w/w) were prepared as follows. HLE was dissolved in ethanol (1 000 mg of HLE to 100 ml of EtOH, w/v), mixed well while spraying evenly on 1 kg of zeolite, and then air dried.

Experimental design

All experimental procedures including animal experiments were conducted in accordance with scientific and ethical regulations provided by EC Directive of 1986, 86/609/EEC. This study was carried out with the approval of Kangwon National University Institutional Animal Care and Use Committee (KNU-015017). A total of 1 800, one-day-old male Ross 308 broilers were randomly divided into six groups with six replicates each, and each replicate included 50 broilers. After chicks were transferred on the farm, initial body weight was recorded. Environmental temperature was 32 °C and decreased by 20 °C each week until the end of the experiment. Nutrient levels of basal diets (Table 1) were formulated to meet NRC (2022). The experiment was divided into day 1–21 and day 22–35. The experiment was divided into starter phase (0 to 21 days) and finisher phase (22 to 35 days). The compositions of experimental diets are listed in Table 1. Treatment groups consisted of: CON (control without HLE), PC (positive

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Table 1. Ingredients and chemical composition of the experimental diets

Ingredient	Day 1 to 21	Day 22 to 35
Yellow corn grain	52.34	51.30
Soybean oil meal (48% protein)	33.84	25.05
Corn gluten meal	4.70	5.70
Wheat grain	0	9.31
Soybean oil	4.82	4.34
Limestone	0.93	0.93
Dicalcium phosphate	1.70	1.70
Choline chloride	0.20	0.20
Salt	0.37	0.37
DL-Methionine-50%	0.30	0.30
L-Lysine hydrochloride-78%	0.30	0.30
Mineral premix[1]	0.34	0.34
Vitamin premix[2]	0.16	0.16
Chemical composition (%)		
Metabolizable energy (kcal/kg)	3 100	3 150
Crude protein	22.00	20.00
Lysine	1.32	1.15
Methionine	0.52	0.50
Calcium	1.00	0.90
Available phosphorous	0.45	0.40

[1]Provided per kg of diet: Cu 200 mg, Fe 80 mg, Zn 180 mg, Mn 13 mg, I 0.4 mg, Co 0.15 mg, Se 0.35 mg. [2]Provided per kg of diet: vitamin A 20 000 IU, vitamin D_3 4 000 IU, vitamin E 75 IU, vitamin K_3 12 mg, vitamin B_2 4 mg, vitamin B_6 1 mg, vitamin B_{12} 60 µg, pantothenic acid 50 mg, niacin 120 mg, biotin 0.08 mg

control, 8 ppm of avilamycin), HLE50 (diet containing 50 ppm of HLE), HLE100 (diet containing 100 ppm of HLE), HLE150 (diet containing 150 ppm of HLE), and HLE200 (diet containing 200 ppm of HLE). This study used the antibiotic "avilamycin" as a substance challenge agent for measuring antimicrobial effect/immune system. Feed and water were provided *ad libitum*. Every week during the 35-day experimental period, individual broilers were weighed and feed intake was determined in each pen. Feed conversion was calculated as the feed to gain ratio.

Blood lipid profiles and immune organ

At the end of the experiment, eight chicks were randomly selected from each replicate pen and 4 ml of whole blood were collected from the wing vein using a 5-ml syringe. The blood was placed into a serum separator tube (SST tube; BD Falcon, Franklin Lakes, NJ, USA) and collected blood was left at room temperature for 40 minutes. Then the serum was centrifuged at 1 500 g for 15 min at 4 °C and stored at –20 °C until subsequent analysis. The levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were analysed with diagnostic kits (Sigma Chemical Co., St. Louis, MO, USA). After blood sampling, chicks were slaughtered and immune organ weight was calculated.

Serum immunoglobulin

The level of serum immunoglobulin (IgG) was measured using a chicken immunoglobulin ELISA kit (Bethyl Laboratories Co., Montgomery, TX, USA) following the manufacturer's protocol. The level of immunoglobulin was calculated by measurement of the absorbance with a precision microplate reader (Molecular Devices Inc., San Jose, CA, USA) at 450 nm and calculated by comparison with a concentration curve of the standards.

Caecal bacteria

After slaughtering, the caecum was removed by the anaerobic method and it was kept under anaerobic conditions in sealed anaerobic jars (Oxoid, Basingstoke, UK) equipped with AnaeroGen sachets (Oxoid, Basingstoke, UK). The caecum was homogenized with sterilized saline solution (phosphorus buffered saline; PBS 0.1 M, pH 7.0) and diluted 10 times (1 : 9, wt/vol), then serial dilutions were diluted consecutively from 10^{-2} to 10^{-8}. To culture, a total of 100 µl of each diluted solution was divided into sterilized media six times repeatedly, and the plates were cultured at 37 °C for 48 h for bacterial counts. Bifidobacteria (Modified Columbia agar; Thermo Fisher Scientific Inc., Rockford, IL, USA), *Lactobacillus* (MRS agar; Thermo Fisher Scientific Inc., Rockford, IL, USA), *E. coli* (McConkey purple agar, Difco, USA), and coliform bacteria (violet red bile agar, Difco, USA) were cultured. A microbial colony count was taken

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as the common logarithm of colony forming units/g of wet caecal content.

Short-chain fatty acids

The levels of short-chain fatty acids (SCFA) were measured using a gas chromatography (GC) system (Model GC 15A, Shimadzu Corp., Kyoto, Japan) and SCFA were determined using the method by Zhang et al. (2003). After mixing approximately 0.5 g of caecal content was obtained from each animal with 5 ml of distilled water in a screw cap tube and homogenizing the mixture, it was centrifuged at 1 500 g for 10 min at 4 °C. The supernatant was stored at –20 °C before analysis. After that 1 ml of the supernatant liquid was transferred to an ample bottle and left in an icebox for 30 min, 0.2 ml of metaphosphoric acid solution was added, and the mixture was centrifuged at 1 500 *g* for 10 min before the GC analysis. The GC with a flame ionization detector and a polyethylene glycol column (Hewlett Packard, 30 m × 320 µm × 0.50 µm; Dallas, TX, USA) analysed the liquid at 100 to 150 °C using highly purified N_2 (1.5 ml/min) as the carrier gas. The following parameters were used: 1 µl of sample inject, 250 °C injector temperature, 265 °C detector temperature, 1 ml/min of helium carrier gas flow. The fatty acid levels are percentages of total peaks determined in each sample. The percentage of total peak levels was measured in each sample of fatty acids.

Statistical analysis

Statistical analyses for all data were performed using IBM SPSS Statistics v17.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA and Duncan's multiple range tests were performed. Data are expressed as mean values and standard errors. *P*-values lower than 0.05 (95% confidence level) were considered statistically significant.

RESULTS AND DISCUSSION

Growth performance

Broiler bodyweight (BW) gain, feed intake (FI) and feed conversion ratio (FCR) are presented in Table 2. The PC, HLE150, and HLE200 groups had significantly ($P < 0.05$) greater BW gain, and better FCR during the starter phase (1 to 21 days) and overall (1 to 35 days) periods compared with the CON group. No significant ($P > 0.05$) differences in BW gain and FCR were observed between the PC, HLE150, and HLE200 groups during the whole ex-

Table 2. Effect of feeding a diet containing housefly larvae extracts (HLE) on body weight (BW), feed intake (FI) and feed conversion ratio (FCR) in broiler chickens

	CON	PC	HLE50	HLE100	HLE150	HLE200	SEM	*P*-value
1 to 21 days								
Initial BW (g)	38.6	38.6	38.7	38.7	38.7	38.6	0.163	0.960
BW gain (g)	705^{b}	875^{a}	727^{b}	730^{b}	832^{a}	847^{a}	24.21	< 0.01
FI (g)	1 141^{b}	1 325^{a}	1 184^{ab}	1 176^{ab}	1 271^{ab}	1 305^{a}	34.40	0.005
FCR	1.62^{a}	1.52^{b}	1.63^{a}	1.61^{a}	1.53^{b}	1.54^{b}	0.016	< 0.01
22 to 35 days								
BW gain (g)	1 009	979	1 003	1 000	998	985	15.42	0.887
FI (g)	2 131	2 060	2 133	2 203	2 092	2 068	37.95	0.125
FCR	2.11	2.11	2.13	2.20	2.10	2.10	0.030	0.166
1 to 35 days								
BW gain (g)	1 714^{c}	1 854^{a}	1 730^{bc}	1 731^{bc}	1 829^{ab}	1 832^{ab}	24.66	< 0.01
FI (g)	3 271	3 385	3 317	3 379	3 363	3 373	32.97	0.199
FCR	1.91^{a}	1.83^{b}	1.92^{a}	1.95^{a}	1.84^{b}	1.84^{b}	0.016	< 0.01

CON = control without HLE; HLE50 = diet containing 50 ppm of HLE; HLE100 = diet containing 100 ppm of HLE; HLE150 = diet containing 150 ppm of HLE; HLE200 = diet containing 200 ppm of HLE; PC = positive control, 8 ppm of avilamycin

$^{a–c}$Mean values within the same row with no common superscripts differ significantly ($P < 0.05$)

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periment or between all the treatment groups during the finisher phase starting from day 22. The PC and HLE200 groups had significantly ($P < 0.05$) higher FI than the CON group in the starter phase. However, there were no significant ($P > 0.05$) differences in FI between treatments during the finisher phase and overall period. The current experiment was conducted to determine the optimal levels of house fly larvae extracts in broiler feed. Supplementation of 150 to 200 ppm of housefly larvae extracts increased BW and improved the feed efficiency of broilers to the level achieved by addition of 8 ppm avilamycin.

Serum IgG and immune organs

The weight of immune organs expressed on per kg BW basis and levels of serum IgG are presented in Table 3. The PC group had significantly ($P < 0.05$) greater weight of the bursa of Fabricius, spleen, and thymus than the CON, HLE50, and HLE100 groups, but it had similar ($P \leq 0.05$) weight of the immune organs to the HLE150 and HLE200 groups. In addition, the HLE150 and HLE200 groups had significantly ($P < 0.05$) heavier spleen than the CON, HLE50 and HLE100 groups. No significant ($P > 0.05$) differences in serum concentrations of IgG and IgM were noticed between treatments. Weight of immune organs was significantly ($P < 0.05$) higher in broilers fed diets containing 150 to 200 ppm housefly larvae extracts or 8 ppm avilamycin when compared with the CON group. Park and Park (2015) reported that the antimicrobial peptides in housefly larvae extract increased the weights of lymphoid organs such as thymus and spleen. The growth of the lymphoid organs is the basis of the immune system function (Makkar et al. 2014; Park and Park 2015; Jozefiak and Engberg 2017).

Blood lipid profiles

The blood lipid profiles are shown in Table 4. Serum concentration of triglyceride (TG) in the PC, HLE150, and HLE200 groups was significantly ($P < 0.05$) lower than in the HLE100 group. Birds in the HLE150 and HLE200 groups had a significantly ($P < 0.05$) lower concentration of TC compared with the CON, HLE50, and HLE100 groups, but they had a similar ($P \leq 0.05$) serum TC level relative to the PC birds. The PC, HLE150, and HLE200 groups had a significantly ($P < 0.05$) higher concentration of HDL-C and lower LDL-C when compared with the CON, HLE50, and HLE100 groups. The present study showed that supplementation of 150 to 200 ppm housefly larvae extract or 8 ppm avilamycin could reduce blood concentrations of TG, TC and LDL-C and increase the level of HDL-C in broilers. The decreases in lipid levels by dietary supplementation of the housefly larvae extract are in agreement with previous findings in rats (Park and Park 2012; 2015), which is estimated to be attributed to the bifidogenic effect from antimicrobial peptides in housefly larvae extract (Patterson and Burkholder 2003; Park and Park 2015; Jozefiak and Engberg 2017). This study found that housefly larvae extracts reduced levels of TG, TC, and LDL-C. These results may be attributed to the bifidogenic effect from bifidobacteria and *Lactobacillus* growth by the antimicrobial peptides in housefly larvae extract (Ratcliffe et al. 2011; Makkar et al. 2014; Jozefiak and Engberg 2017). The bifidogenic effect is bioactive, improving the growth and immune response of the host through a selective increase in bifidobacteria, which is a beneficial intestinal microflora in the caecum of animals (Patterson and Burkholder 2003; Lomax et al. 2012; Park and Park 2015).

Table 3. Effect of feeding a diet containing housefly larvae extracts (HLE) on levels of blood immunoglobulin (mg/ml) and immune organ weight (g/kg body weight) in broiler chickens

Item	CON	PC	HLE50	HLE100	HLE150	HLE200	SEM	*P*-value
IgG	5.81	5.87	5.81	5.78	5.81	5.82	0.045	0.635
IgM	2.93	3.00	2.99	2.97	2.91	2.93	0.063	0.860
Bursa (F-sac)	0.94^{bc}	1.20^{a}	0.90^{c}	0.94^{bc}	1.14^{ab}	1.15^{ab}	0.033	< 0.01
Spleen	1.60^{b}	1.85^{a}	1.57^{b}	1.59^{b}	1.80^{a}	1.83^{a}	0.050	< 0.01
Thymus	0.91^{b}	1.22^{a}	0.95^{b}	0.97^{b}	1.12^{ab}	1.10^{ab}	0.056	0.005

CON = control without HLE; HLE50 = diet containing 50 ppm of HLE; HLE100 = diet containing 100 ppm of HLE; HLE150 = diet containing 150 ppm of HLE; HLE200 = diet containing 200 ppm of HLE; PC = positive control, 8 ppm of avilamycin

$^{a–c}$Mean values within the same row with no common superscripts differ significantly ($P < 0.05$)

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Table 4. Effect of feeding a diet containing housefly larvae extracts (HLE) on levels of blood triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in broiler chickens

Item	CON	PC	HLE50	HLE100	HLE150	HLE200	SEM	*P*-value
TG	130.2^{ab}	111.3^{b}	128.9^{ab}	131.4^{a}	111.5^{b}	111.6^{b}	4.608	0.002
TC	119.5^{a}	104.6^{bc}	121.9^{a}	117.6^{ab}	102.9^{c}	101.2^{c}	3.592	< 0.01
HDL-C	27.71^{b}	35.99^{a}	30.66^{b}	29.84^{b}	39.59^{a}	40.01^{a}	1.031	< 0.01
LDL-C	65.77^{a}	46.33^{b}	65.48^{a}	61.45^{a}	41.01^{b}	38.67^{b}	3.158	< 0.01

CON = control without HLE; HLE50 = diet containing 50 ppm of HLE; HLE100 = diet containing 100 ppm of HLE; HLE150 = diet containing 150 ppm of HLE; HLE200 = diet containing 200 ppm of HLE; PC = positive control, 8 ppm of avilamycin

$^{a–c}$Mean values within the same row with no common superscripts differ significantly ($P < 0.05$)

Caecal bacteria

Effect of housefly larvae extracts on the caecal bacteria is presented in Table 5. Supplementation of housefly larvae extracts or antibiotics significantly ($P < 0.05$) increased bifidobacteria counts. An increase in bifidobacteria counts in HLE50 and HLE100 groups was significantly ($P < 0.05$) lower than in PC, HLE150 and HLE200 groups. The *E. coli* counts in HLE50 and HLE100 groups were significantly ($P < 0.05$) lower compared with the CON, HLE50, and HLE100 groups, except that the *E. coli* counts were similar ($P \leq 0.05$) in HLE100 and HLE150 groups. There were significant ($P > 0.05$) differences in the counts of the caecal bacteria tested between the PC, HLE150, and HLE200 groups. The counts of *Lactobacillus* and *E. coli* in the caecal digesta were similar ($P \leq 0.05$) in CON, HLE50, and HLE100 groups. As shown in Table 5, dietary housefly larvae extracts affected bacteria in caecal contents. The PC, HLE150 and HLE200 groups increased the concentration of bifidobacteria and *Lactobacillus* in the caecum compared with the CON group. The increased bifidobacteria and *Lactobacillus* counts in the caecum when the bifidogenic effect selectively stimulates the beneficial microflora, thereby inhibiting the growth of harmful bacterial strains. Antibacterial peptides increase the counts of bifidobacteria and *Lactobacillus* (Sanchez et al. 2010; Park and Park 2014; Jozefiak and Engberg 2017). The relationship of bifidobacteria with a decrease in triglyceride and cholesterol concentration by regulating the blood lipid metabolism (Roberfroid 2000) was observed; *Lactobacillus* is the dominant bacterial species in the gut (Zhang et al. 2003; Albazaz and Bal 2014) that can inhibit the growth of pathogens by maintaining microbial balance and acidic environment in the gut (Zhang et al. 2003; Park and Park 2014).

Short-chain fatty acids in the caecal content

The effect of housefly larvae extracts on concentrations of short-chain fatty acids in the caecal contents of broilers is presented in Table 6. The PC, HLE150 and HLE200 groups had significantly ($P > 0.05$) higher concentrations of acetic acid, propionic acid, and total SCFA in the caecal digesta, but lower levels of butyric acid, isobutyric acid, valeric acid and isovaleric acid when compared with the CON, HLE50, and HLE100 groups, except

Table 5. Effect of feeding a diet containing housefly larvae extracts (HLE) on caecal bacterial counts in broiler chickens

Item	CON	PC	HLE50	HLE100	HLE150	HLE200	SEM	*P*-value
Bifidobacteria	6.21^{c}	8.46^{a}	7.81^{b}	7.95^{b}	8.79^{a}	8.83^{a}	0.093	< 0.01
Lactobacillus	6.88^{b}	8.43^{a}	6.83^{b}	7.03^{b}	8.34^{a}	8.65^{a}	0.239	< 0.01
E. coli	7.59^{a}	5.74^{c}	7.50^{a}	7.38^{ab}	6.02^{bc}	5.79^{c}	0.373	< 0.01
Coliform	6.33^{a}	5.11^{b}	6.16^{a}	6.32^{a}	5.29^{b}	5.28^{b}	0.208	< 0.01

CON = control without HLE; HLE50 = diet containing 50 ppm of HLE; HLE100 = diet containing 100 ppm of HLE; HLE150 = diet containing 150 ppm of HLE; HLE200 = diet containing 200 ppm of HLE; PC = positive control, 8 ppm of avilamycin

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valeric acid in HLE100 and isovaleric acid in HLE50 groups. Concentrations of caecal SCFA were not significantly ($P > 0.05$) different between the PC, HLE150, and HLE200 groups or between the CON, HLE50, and HLE100 groups. In the present study differences in concentrations of SCFA between treatment groups were observed. These results showed that caecal acetic acid, propionic acid, and total SCFA concentrations were increased in broilers fed 150 ppm of housefly larvae extract (HLE150), whereas butyric acid, isobutyric acid, valeric acid, and isovaleric acid were decreased compared to those in the control group, although there was no significant difference between HLE150 and antibiotic groups. Also, the results of this study indicate the change of SCFA profiles in the caecum of housefly larvae extract groups, which may be improvement of immunocompetence and adjustment of intestinal microorganism environment in animals (Klasing et al. 2002; Rastall and Gibson 2006; Park and Park 2014; Jozefiak and Engberg 2017). Most organic acids generated from the fermentation of lactic acid bacteria inhibited the intestinal colonization by harmful bacteria (Gong et al. 2002; Park and Park 2015), as support the findings of this study. Based on the results of the study, supplementation of housefly larvae extracts enhanced bifidobacteria and *Lactobacillus* populations, whereas it inhibited *E. coli* and coliform growth.

CONCLUSION

In conclusion, the present study indicated that compared to the antibiotics group the feeding of 150 ppm of housefly larvae extracts containing antimicrobial peptides improved body weight, feed conversion ratio, immune response, blood lipid profiles, and also maintained caecal bacteria and short-chain fatty acid in broiler chickens. Thus, the novelty of this study revealed that the supply of housefly larvae extract containing antimicrobial peptides could be used as a new antibiotic alternative to improve the growth performance of broiler chickens.

Conflict of interest

The author declare no conflict of interest.

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Table 6. Effect of feeding a diet containing housefly larvae extracts (HLE) on levels of caecal short-chain fatty acids (SCFA) in broiler chickens

Item	CON	PC	HLE50	HLE100	HLE150	HLE200	SEM	*P*-value
Total SCFA	132.4^{b}	179.3^{a}	129.3^{b}	125.2^{b}	182.1^{a}	181.6^{a}	8.899	< 0.01
Acetic acid	76.81^{b}	132.9^{a}	76.31^{b}	71.64^{b}	132.8^{a}	133.5^{a}	7.044	< 0.01
Propionic acid	14.30^{b}	24.04^{a}	122.29^{b}	13.71^{b}	26.52^{a}	26.55^{a}	2.083	< 0.01
Butyric acid	15.06^{a}	8.36^{b}	14.98^{a}	15.15^{a}	9.34^{b}	9.36^{b}	1.383	< 0.01
Isobutyric acid	13.16^{a}	6.84^{b}	12.33^{a}	12.31^{a}	6.23^{b}	6.19^{b}	1.039	< 0.01
Valeric acid	7.20^{a}	4.13^{b}	7.39^{a}	6.52^{a}	3.88^{b}	3.90^{b}	0.633	< 0.01
Isovaleric acid	5.83^{a}	3.08^{b}	5.24^{a}	5.88^{a}	3.37^{b}	3.07^{b}	0.519	< 0.01

CON = control without HLE; HLE50 = diet containing 50 ppm of HLE; HLE100 = diet containing 100 ppm of HLE; HLE150 = diet containing 150 ppm of HLE; HLE200 = diet containing 200 ppm of HLE; PC = positive control, 8 ppm of avilamycin

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