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ANIMAL

Effects of a mixture of essential oils and organic acid supplementation on growth performance, blood profiles, leg bone length, and intestinal morphology in broilers

Shu Dong Liu[1,†], Min Ho Song[2,†], Won Yun[1], Ji Hwan Lee[1], Seung Yeol Cho[3], Gok Mi Kim[4], Hyeun Bum Kim[5,*], Jin Ho Cho[1,*]

[1]Division of Food and Animal Science, Chungbuk National University, Cheongju 28644, Korea
[2]Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 34134, Korea
[3]Eugenebio, Suwon 16675, Korea
[4]Department of beauty art, Yonam college, Cheonan 31005, Korea
[5]Department of Animal Resource and Science, Dankook University, Cheonan 31116, Korea

*Corresponding author: hbkim@dankook.ac.kr, jinhcho@chungbuk.ac.kr
† Those authors contributed equally to this work.

Abstract

This study was done to evaluate the effects of a mixture of essential oils and organic acid supplementation on growth performance, blood profiles, leg bone length and intestinal morphology in Ross broilers. A total of 40 Ross 308 broilers (1140 ± 80 g) were randomly allocated to 2 groups, a basal diet (CON) and a basal diet + 0.05% Avi-protect® (AVI, Mixture of 25% citric, 16.7 sorbic, 1.7% thymol, and 1.0% vanillin), with 20 replicates for every group and 1 chicken per replicate per cage. The broilers were raised in a temperature-controlled room maintained at 24 ± 1°C and 50 ± 5% humidity. The body weight ($p < 0.05$) and weight gain ($p < 0.05$) of the broilers were increased in the AVI group compared with the CON group. The triglyceride ($p < 0.05$) and low density lipoprotein (LDL) ($p < 0.05$) contents were significantly decreased in the AVI group compared with the CON group. There was no significant difference in the leg bone length between the AVI and CON groups ($p > 0.05$). The villi height ($p < 0.05$) and goblet cell count ($p < 0.05$) were significantly increased in the AVI group compared with the CON group. In conclusion, Avi-protect® as a feed additive improved the growth performance and lipid metabolism and promoted the development of the intestinal morphology of broilers.

Keywords: essential oil, growth performance, intestinal morphology, lipid metabolism organic acid

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Introduction

Along with the development of economy, our life has undergone enormous changes, and more and more people are focus on the safety of animal production. As we all know, antibiotics are widely use in animal feed industry due to they have some advantages, such direct sterilization. Because of its side effects on animal, the use of antibiotics as a general feed additive has been largely banned in some country and union, so finding new substance replaces or combination with antibiotics become a research hotspot. Most of essential oils and organic acid are of plant origin, so they are generally regarded as safe material.

Essential oils are compounds that include alcohols, aldehydes, phenols, acetone and terpenes, and it has been shown that they have antimicrobial and anti-inflammatory actions in laboratory study (Cowan, 1999; Ultee et al., 2002; Abe et al., 2003; Faleiro et al., 2003; Park et al., 2016). Organic acids enhance to intestinal microbial composition and affect digestibility and growth performance (Franco et al. 2005; Hansen et al., 2007). Taking these factors into consideration, we decided to design the present experiment to analyze and evaluate the effects of supplemental a commercial blend (containing essential oils and organic acid) on growth performance, blood profiles, leg bone length and intestinal morphology in broilers.

Material and Methods

The experimental protocols describing the management and care of animals were reviewed and approved by the Animal Care and Use Committee of Chungbuk National University.

Experimental animals and treatment

40 healthy mixed sex 21-day-old Ross 308 broiler chickens (initial body weight: 1140 ± 80 g) were obtained from YangJi Company (Cheonan, Korea) and were randomly allocated to 2 treatments, each of treatment consisted of 20 replicate cages with 1 broiler per cage (every treatment included 10 male and 10 female birds). Broilers were raised in a temperature-controlled room maintained at 24 ± 1℃. The relative humidity was approximately 50 ± 5%. The 2 treatments are as follows: CON: basal diet, AVI: basal diet + 0.05% Avi-protect®. The Avi-protect® is a commercial blends containing 25% citric, 16.7 sorbic, 1.7% thymol and 1.0% vanillin. The basal diet was a kind of commercial available complete formula powder feed which was supported by AT feed Company (Cheongju, Korea) and meted or exceeded the recommending nutrient contents according to NRC (1994) for broiler (Table 1).

Sample collection

Body weight was recorded every week. Feed intake was recorded weekly and feed conversion ratio was calculated by dividing feed intake by weight gain. 10 broilers (5 male and 5 female) were randomly selected from each group on the ending of experiment. Blood samples were collected from the wing vein into a sterile syringe, at the time of collection, blood samples were collected into both no heparinized tubes and vacuum tubes containing tripotassium-ethylene-diamine-tetraacetic-acid (K3EDTA, Becton, Dickinson and Company, Franklin Lakes, USA) to obtain serum and whole blood, respectively. Red blood cell (RBC) and white blood cell (WBC) concentrations were analyzed using an automatic blood analyzer (ADVIA, Bayer, Tarrytown, USA) and the concentration of cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) were analyzed using an automatic biochemical analyzer (RA-1000, Bayer Corp., Tarrytown, USA).

After blood collection, the same broilers were euthanized by an intravenous injection of pentobarbital, with cervical dislocation to confirm death. During necropsy of 10 broilers from every treatment, the legs were removed from carcasses, the meat was separated from the bones. Then, these bone samples were dried at 80℃ for at least 24 hours and leg bone lengths were measured with a vernier caliper. The gastrointestinal tract was removed and duodenum (corresponding to the portion of the small intestine situated between the pylorus and the end of the duodenal loop). Segments 1 cm long were taken from the centre of this part and fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 3 μm and stained with haematoxylin-eosin. Histological sections were examined with a Nikon phase contrast light microscope coupled with a Microcomp integrated digital imaging analysis system (Nikon Eclipse 80i, Nikon Co., Tokyo, Japan). Images were viewed using a 4x EPlan objective (40×) to measure morphometric parameters of intestinal architecture. For this purpose, three favourably orientated sections cut perpendicularly from villus enterocytes to the muscularis mucosa were selected from every animal and measurements were carried out as follows: villous height (VH) was estimated by measuring the vertical distance from the villous tip to villous-crypt junction level for 10 villi per section.

Table 1. Compositions of basal diets (as-fed basis).

Items	Ingredient (g/kg)
Corn	408.00
Wheat	200.00
Soybean meal, CP 48%	254.80
Rape seed meal	35.00
Tallow	60.10
Limestone	11.70
Dicalcium phosphate	18.40
L-Lysine·HCl, 78.4%	2.00
DL-Methionine, 88%	4.10
Threonine, 98.5%	1.20
Vitamin premix[y]	0.30
Trace Mineral premix[z]	1.50
Salt	2.90
Total	1,000.00
Calculated composition (g/kg)	
ME (kcal/kg)	3,180
Analyzed composition (%)	
CP	18.50
Lysine	1.10
Met + Cys	0.93
Ca	1.00
Available P	0.50
Ether-extract	7.96
Crude Fibre	3.25

[y] Vitamin premix provided per kg of complete diet: 15 000 IU of trans-retinol, 3750 IU of cholecalciferol; 37.50 mg of tocopherol; 2.55 mg of menadione; 3.00 mg of thiamine; 7.50 mg of riboflavin; 4.50 mg of pyridoxine; and 24.00 μg of cobalamin; 51.00 mg of niacin; 1.50 mg of folic acid; 126.00 mg of biotin; and 13.5 mg of pantothenic acid.
[z] Provided per kg of complete diet: Zn (as $ZnSO_4$), 37.50 mg; Cu (as $CuSO_4{\cdot}5H_2O$), 26.00 mg; Mn (as MnO_2), 137.50 mg; Fe (as $FeSO_4{\cdot}7H_2O$), 37.5 mg; I (as KI), 0.83 mg; Se (as $Na_2SeO_3{\cdot}5H_2O$), 0.23 mg and choline, 1408 mg.

Statistical analysis

All data were analyzed using the t-test of SAS. The GLM procedures of the SAS program ver 9.5 (SAS Institute Inc., Cary, USA) were used for linear regression about relationship between temperature and body weight gain. Statements of statistical significance were based on $p < 0.05$.

Results and Discussion

Growth performance

The effect of mixture of essential oils and organic acid supplementation on growth performance in broilers are showed in Table 2. At the end of experiment, BW and weight gain significantly increased in the AVI group compared with the CON group ($p < 0.05$). the average daily feed intake and water intake did not have any difference between the CON and AVI group ($p > 0.05$). The growth performance of broilers always has been and will continue to be very important to the poultry industry, and many factors can affect it, such as genetic factors, feed nutrition levels and quality, the breeding environment and animal diseases. Many studies have reported that essential oils or organic acid as dietary supplements which can affect the growth performance of broilers (Ciftci, 2005; Khattak, 2014; Agboola et al., 2015). This study is consistent with them that mixture of essential oils and organic acid significantly increased the live weight gain and improved the growth performance in broilers. It could be that essential oils and organic acid has a function of anti-bacteria and anti-inflammatory, which can regulate the intestinal flora and improve the intestinal development of broilers promoting the absorption and utilization of nutrients.

Blood profiles

The effect of mixture of essential oils and organic acid supplementation on blood profiles in broilers are given in Table 3. The concentration of triglyceride and LDL significantly ($p < 0.05$) decreased in the AVI compared with the CON. The cholesterol ($p > 0.05$), HDL ($p > 0.05$), WBC ($p > 0.05$), and RBC ($p > 0.05$) did not have difference between the CON and the AVI. Total cholesterol reflects the extent that the absorption and metabolism of lipids in body. the LDL and HDL have separately function that the former is used for transport of cholesterol, the latter is responsible for decomposing cholesterol into bile acid. In the present study, the mixture of essential oils and organic acid supplementation improved lipid metabolism

Table 2. Effect of supplementa tion of mixture of essential oils and organic acid on growth performance in broilers.

Items	Treatment		S.E.	p-value
	CON	AVI		
Initial BW (g)	1,125.73	1,168.56	-	-
Final BW (g)	1,737.60b	1,916.64a	15.33	< 0.05
Weight gain (g/d)	611.87b	748.08	5.15	< 0.05
Feed intake (g/d)	144.25	1,554.17	9.74	0.38
Water intake (g/d)	292.00	294.78	9.74	0.79

CON, basal diet; AVI, basal diet + 0.05% Avi-protect ®; BW, body weight; S.E., standard error.
a, b: Means in the same row with different superscript s differ ($p < 0.05$).

in the broilers, it might be due to the essential oils improve the enzyme activity of lipase (Hashemipour et al., 2013), and then reduce the content of cholesterol and triglyceride in blood.

Leg bone length

The effect of mixture of essential oils and organic acid supplementation on leg bone length in broilers are given in Table 4. The length of tibia ($p > 0.05$) and femur ($p > 0.05$) did not have difference between the CON and the AVI. Shank length is often considered as a parameter for monitoring growth, because shank length is so closely correlated with body weight (Leeson and Caston, 1993). According to several studies about poultry, bone growth is affected by various factors such as age, feeding methods, genotype, feed and environment (Hester, 1994; Skinner and Waldroup, 1995). In the current study, there has no significant differences on length of tibia and femur among groups, this result means that feeding broiler with mixture of essential oils and organic acid supplementation did not affect leg bone growth and bone length of broilers.

Intestinal morphology

The effect of mixture of essential oils and organic acid supplementation on intestinal morphology in broilers are given in Table 5. The villi height ($p < 0.05$) and goblet cell count ($p < 0.05$) significantly increased in the AVI compared with the CON. Villus height is an important indicator of digestive system in broilers, directly affecting the absorptive capacity of the intestinal mucous membranes. Yamauchi et al. (2006) reported that lower villus height led to lower absorptive capability of the small intestines. Goblet cells can secrete sticky proteins with a protective effect on the intestinal epithelium. Some previous studies have shown that essential oils or organic acid as dietary supplements which can increase villi height and goblet cell counts (Reisinger et al., 2011; Agboola et al., 2015). The villi of the human and chick gut are formed in similar stepwise progressions, wherein the mesenchyme and attached epithelium first fold into longitudinal ridges, then a zigzag pattern, and lastly

Table 3. Effect of supplementa tion of mixture of essential oils and organic acid on blood profile in broilers.

Items	Treatment		S.E.	p-value
	CON	AVI		
Cholesterol (mg/dL)	121.27	113.22	2.96	0.42
Triglycerid e (mg/dL)	62.00a	53.89b	1.80	< 0.05
HDL/C (mg/dL)	82.82	85.67	3.08	0.29
LDL/C (mg/dL)	32.18a	27.11b	0.36	< 0.05
WBC (10^3/μL)	20.33	15.90	1.35	0.11
RBC (10^6/μL)	2.82	2.83	0.10	0.89

CON, basal diet; AVI, basal diet + 0.05% Avi-protect ®; S.E., standard error; HDL/C, high density lipoprotein ; LDL/C, low density lipoprotein cholesterol ; WBC, white blood cell; RBC, red blood cell.
a, b: Means in the same row with different superscript s differ ($p < 0.05$).

Table 4. Effect of supplementation of mixture of essential oils and organic acid on leg bone length in broilers.

Items (cm)	Treatment		S.E.	p-value
	CON	AVI		
Tibia	8.24	8.11	0.16	0.46
Femur	6.41	6.46	0.08	0.35

CON, basal diet; AVI, basal diet + 0.05% Avi-protect®; S.E., standard error.

Table 5. Effect of supplementa tion of mixture of essential oils and organic acid on intestinal morphology in broilers.

Items	Treatment		S.E.	p-value
	CON	AVI		
Villi height (μm)	3,604b	3,884a	85	< 0.05
Goblet cell count	45b	50a	1.5	< 0.05

CON, basal diet; AVI, basal diet + 0.05% Avi-protect ®; S.E., standard error.
a, b: Means in the same row with different superscript s differ (p < 0.05).

individual villi (Shyer et al., 2013). And the growth of intestinal villi depends on the proliferation and differentiation of columnar cell and goblet cell in intestine land. Due to the mixture of essential oils and organic acid increased the goblet cell content, and then improved the villus height of intestine. Some previous studies result indicated that the differentiation of goblet cell is relevant to interactions between transcription factor Hath1, KLF4 and Notch, Wnt/β-catenin signal transduction pathways, especially important is the Notch pathway (Kim and Ho, 2010; Clevers, 2013). The broilers were given the mixture of essential oils and organic acid may be affect the Notch pathway of broilers, and then increase the goblet cell differentiation.

Conclusion

In conclusion, the broilers fed with the mixture of essential oils and organic acid which improved the growth performance and reduced the total cholesterol of blood and triglyceride content, thus we can infer that the Avi-protect® have a positive effect on lipometabolism. The Avi-protect® increased the goblet cell content and villus height in broilers, the results implied that the Avi-protect® improved the intestinal morphology of broilers.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

Authors Information

Shudong Liu, https://orcid.org/0000-0002-7383-4140
Minho Song, https://orcid.org/0000-0002-4515-5212
Won Yun, https://orcid.org/0000-0002-1835-2640
Jihwan Lee, https://orcid.org/0000-0001-8161-4853
Seungyeol Cho, Eugenebio, Chief executive officer
Gokmi Kim, https://orcid.org/0000-0003-1053-4535
Hyeunbum Kim, https://orcid.org/0000-0003-1366-6090
Jinho Cho, https://orcid.org/0000-0001-7151-0778

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