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Full Length Research Paper

Eosungcho (*Houttuynia cordata)* with multi strain probiotics as alternative to antibiotic for broiler production

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A study was conducted with 140 "Ross" broilers (1-day old) having four treatments to know the potentiality of Eosungcho, *Houttuynia cordata* with probiotics (HCP) and suitable level in replacing antibiotic. The groups were control (basal diet), antibiotic (basal diet + 0.05% OTC) and HCP 0.5 and 1.0% with basal diet. The birds were arranged in a completely randomized design having 5 replications with 7 chicks per replication in wire cage. Addition of feed additives in the diet did not show negative effect on growth and feed conversion efficiency in broilers. Significantly, highest protein percentage in broiler meat fed 1.0% HCP was recorded compared to control ($p<0.05$). Crude fat significantly reduced in 1.0% HCP like antibiotic compared to control group ($p<0.05$). The thiobarbituric acid (TBA) value of meat in antibiotic and HCP groups showed significantly lower value compared to control ($p<0.05$). No mortality was observed in 1.0% HCP added diet compared to other groups. Although, no difference in internal organs among the groups were observed except large intestine weight. Significantly, lowest large intestinal weight was found in antibiotic and 1.0% HCP addition diet were found ($p<0.05$). Considering the findings, addition of 1.0% Eusungcho (*Houttuynia cordata)* with multi strain probiotics can be replaceable to antibiotic for broiler production.

Key words: Eusungcho probiotics, oxytetracycline (OTC), crude protein, thiobarbituric acid (TBA).

INTRODUCTION

Due to the outbreaks of resistant bacteria and residues of antibiotics in poultry products, using antibiotics is regulated by Korean Government (Yu et al., 2004). There are several kinds of antibiotics alternative developed and used currently, among which readily memorable natural substances for customers and medicinal plants with excellent physiological activities are getting attention by researchers (Hernandez et al., 2004). Some examples of medicinal plants are green tea, artemisia, acanthopanax and other plants (Yang et al., 2003; Kwon et al., 2005). Phytogenic feed additives are plant-derived products used in animal feeding to improve the performance of agricultural livestock. They are also called phytobiotics or botanicals which improve the productivity of livestock through amelioration of feed properties, promotion of animal production performance and improving the quality of food derives from those animals (Windisch et al., 2008). *Houttuynia* (H.) *cordata* is a well known traditional Chinese medicinal plant widely used in China, Japan and Korea. It possesses a variety of pharmacological functions including anti-bacterial, anti-microbial, anti-inflammatory and immunomodulatory effects due to the nine bioactive components (Meng et al., 2009) (Figure 1). It also acts as an antioxidant and anti-mutagenic agents (Chen et al., 2003). Nuengchamnong et al. (2009) reported that antioxidants in the aqueous extract of *H. cordata* mainly consisting of chlorogenic acids and its derivatives, catechin and procyanidin B were characterized using on-line LC–MS coupled with DPPH assay for the first time.

Probiotics with medicinal plants are being suggested as effective antibiotics alternatives in calves (Sarker et al., 2010a), in broiler chicken (Sarker et al., 2010b, c).

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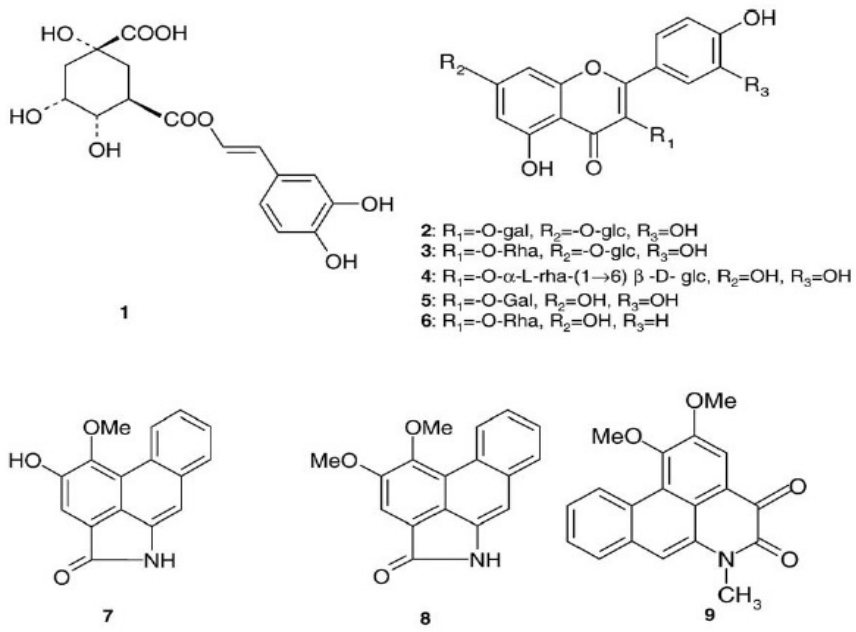

Figure 1. Chemical structure of the compounds (1) chlorogenic acid. (2) querecetin-3-0-β-D-galactophyranosyl-7-0- β-D-glucopyranoside.(3)quercetin-3-0-α-l-rhamnopyranosyl-7-0-β-D-glucopyranoside. (4)rutin. (5) hyperin (6) quercitrin. (7) piperolactam A. (8)aristolactam. (9) cepharadione B.

Probiotics maintains intestinal microbial balance and helps gut mucosa development, improving digestion and absorption rate and thus improving production (Mohan et al., 1996).It has been reported in several studies that single and mixed probiotics increases poultry production (Mohan et al., 1996; Jin et al., 1998). Generally, the digestive organ of healthy animal is dominated by anaerobic organisms and the upper part of it is dominated by *Lactobacillus acidophilus*, the middle part by *Bacillus subtilis* and the lower part by *Streptococcus faecium*. Considering this fact, the effect of probiotics may be improved using mixed probiotics (Yoon et al., 2004). It is reported that supplementation of 0.1 and 0.2% of mixed probiotics containing *L. acidophilus, B. subtilis* and *Saccharomyces cerevisiae* improve production of broilers and indirect immunity (Kim et al., 2002).

Lipid oxidation is one of the primary mechanisms of quality deterioration in meat products through adverse changes in flavor, color, texture and nutritive value (Ura et al., 2008). Chicken meat enriched with polyunsatuarated fatty acids (longer fatty acids) with a high number of double bonds, which increases the susceptibility of meat to oxidation (Maraschiello et al., 1999; Ruiz et al., 1999; Grau et al., 2001a, b). One of such product is malondialdehyde (MDA), which has long been considered as an index of oxidative rancidity. Among many methods proposed for assessing MDA, the 2-thiobarbituric acid (TBA) has been widely adopted as a sensitive assay method for lipid oxidation in animal tissues. In practice, meat is stored and cooked for consumption. As lipid oxidation is one of the primary mechanisms of quality deterioration in meat products through adverse changes in flavor, color, texture and nutritive value, so it's a great concern of the health conscious consumers. The purpose of this study was to assess possibility of *H. cordata* with probiotics to be used as broiler feed additives for substituting antibiotic and their effect on productivity, meat quality and organ development.

MATERIALS AND METHODS

Animals and experimental design

One hundred and forty one-day old "Ross" broiler chicks were considered for this study. The chicks were housed in a close, ventilated caged-broiler house in which they were raised for 24 h of daily light. From 1 to 14 days of age, supplemental heat was provided by electric heater which placed inside the chicken house, thereafter the room temperature was kept at 22±2ºC through a supplemental heating system. The birds were assigned to 4 treatments with 5 replications following completely randomized design (CRD). There were four dietary treatments, control, antibiotic (basal+0.05% oxytetracycline, OTC) and eosungcho (basal+0.5% and basal+1.0%). The feed and drinking water were provided *ad libitum.*

Experimental diets and feeding

Experimental diets were divided into two phases; starter 0 to 3 weeks and finisher 4 to 5 weeks of age. Diets were formulated

Table 1. The species and strain of microflora used in *Houttuynia cordata* probiotics with chemical composition.

Species of microflora	Strain	Composition of SHP (%)	
Lactobacillus acidophilus	KCTC 3111	Moisture	37.05
Lactobacillus plantarum	KCTC 3104	Crude protein	9.35
Bacillus subtilis	KCTC 3239	Crude fat	2.73
Saccharomyces cerevisiae	KCTC 7915	Crude fiber	9.36
		Crude ash	13.94
		NFE	27.57

NFE: Nitrogen free extract, KCTC: Korean collection of type culture.

Table 2. Effects *Houttuynia cordata* probiotics on growth performance of broiler.

Items	Control	Antibiotics (0.05% OTC)	*Houttuynia cordata* probiotics	
			0.5%	1.0%
Initial weight (g)	45.00	45.00	45.00	45.00
Final weight (g)	1908.57±67	1854.86±29	1869.14±34	1855.43±47
Weight gain (g)	1863.57±66	1809.86±29	1824.14±34	1810.43±47
Feed intake (g)	2922.00±116	2868.54±48	2894.00±27	2790.48±81
FCR(Feed/Gain)	1.57±0.02	1.59±0.01	1.59±0.03	1.54±0.02

Values are mean ± Standard error (n=4). Means within the row are not significantly difficult (p>0.05).

following NRC (1998) and the chemical compositions of experimental diets are analyzed by AOAC (1990) method. The probiotics (bacteria and yeast) used in the formulation of *H. cordata* probiotics (HCP) are given in Table 1.

Parameters studied

Body weight gain, feed intake, feed conversion ratio and mortality

Body weights were measured on weekly basis from initial day to the final day of the experiment. Feed intake had been determined by measuring feed residue on weekly basis since the beginning of the experiment. Feed conversion ratio was obtained by dividing the feed intake to body weight gain. Dead birds were recorded and accordingly adjusted the feed intake.

Carcass composition and organ development

At the end of the experiment, 16 chickens were slaughtered and samples were collected from breast and thigh muscle. The chemical composition of the carcass was determined according to common method of AOAC (1990). The organs development was measured by taking weight and length of the broilers after slaughtering and calculated in respect of body weight.

Lipid oxidation determination

For this analysis, 10 g of thigh and breast meat mixture (equal amount of each) was blended at full speed for 1.5 min in chilled stainless watering blender cup with 25 ml of extracting solution containing 20% trichloroacetic acid (TCA) in 2 M phosphoric acid. The resulting sediment was transferred quantitatively to 50 ml volumetric flask with 20 ml distilled water and diluted by shaking and homogenized. A 25 ml portion was filtrated through Whatman No.1 filter paper. Then 5 ml filtrate was transferred to a test tube and 5 ml of 2-thiobarbituric acid (0.005 M in DW) was added. The solution was shaken in a water bath at 80°C for 30 min. After cooling, the color development was measured at 530 nm in a spectrophotometer, biochrom, Libra S22 (Biochrom Ltd., Cambridge, England). Thiobarbituric acid (TBA) reactive substance values were expressed as micromole of malondialdehyde (MDA) per hundred gram of meat.

Statistical analysis

The data obtained from this study were analyzed by general linear models (GLM) of SAS Package Program (1990) to estimate variance components for a completely randomized design. Duncan's multiple comparison tests (1955) were used to examine significant differences between treatment means. Differences were statistically assessed at $p<0.05$.

RESULTS AND DISCUSSION

Growth performance

The results in Table 2 stated that body weight gain, FCR (feed conversion ratio) and feed intake of broiler chicks were not significant among the treatment groups.

Kim et al. (2002) reported that it was effective to

Table 3. Effects of *Houttuynia cordata* probiotics on the carcass composition of broiler (%).

Treatments	Control	Antibiotics (0.05% OTC)	*Houttuynia cordata* probiotics	
			0.5	1.0%
Moisture	75.33±0.32	75.44±0.70	74.66±0.73	73.90±0.25
Crude protein	22.33^{b}±0.30	22.60^{ab}±0.60	22.93^{ab}±0.55	23.84^{a}±0.22
Crude fat	1.07^{a}±0.11	0.69^{b}±0.07	0.99^{ab}±0.15	0.71^{b}±0.04
Crude ash	1.27±0.03	1.28±0.12	1.42±0.11	1.26±0.06

Values are mean ± Standard error (n=4). Means with different letters within the row are not significantly difficult (p<0.05).

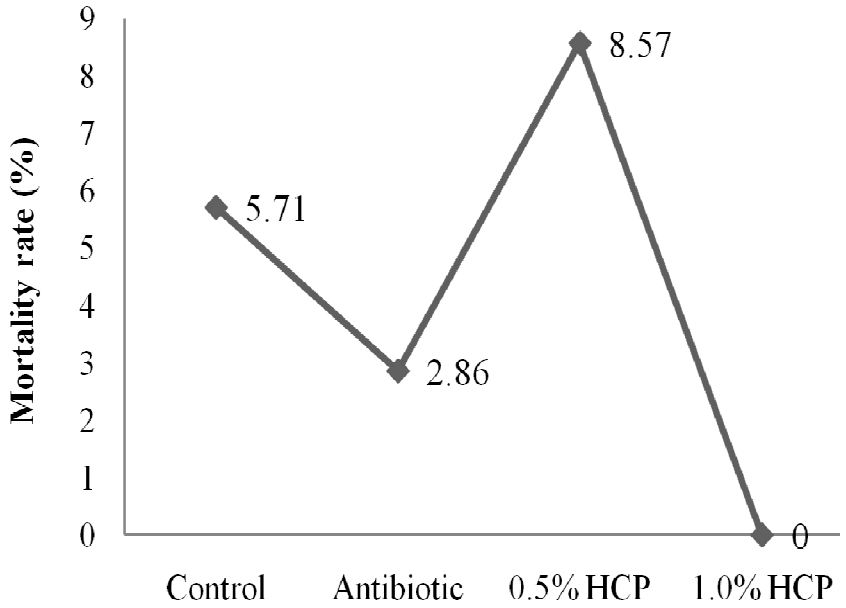

Figure 2. Effect of feed additives on on mortality rate (%) in broiler chicks. 2, 1 and 3 chicks were died in control, antibiotic and and 0.5% *Houttuynia cordata* probiotics (HCP) among 35 chicks in each group except 1.0% HCP. Data with different superscripts are significantly different (p<0.05). MDA: Malondialdehyde.

improve weight gain and feed conversion ratio when 0.1 to 0.5% probiotics with *Lactobacillus* sp., *Bacillus* sp. and Yeast was fed to broilers. According to Ryu et al. (2003), supplementation of 0.1 to 0.3% mixed probiotics containing *L. acidophilus*, *B. subtilis*, *S. cervisae* to broiler chicks fed diets improved weight gain and feed conversion ratio as well as feed intake. Although, the similar species of probiotics were used in current study but differs with the findings of those researchers, the reason might be addition of *H. cordata* with the probiotics. Uuganbayar (2004) reported that 0.5 to 1.5% green tea supplement in broiler diet had effect to reduce body weight gain and feed intake of the chicks. Sarker et al. (2010c) found that the growth performance between 0.5 and 1.0% addition of both green tea and fermented green tea probiotics groups did not show any statistical difference.

Carcass composition

The meat composition of broilers is shown in Table 3. The crude protein content was increased significantly after addition of *H. cordata* probiotics compared to the other treatment (p<0.05).

Figure 2 shows the trend of mortality in the broiler chicks in different feed additives groups. There were no dead birds recorded in addition of 1.0% *H. cordata* probiotics group except other treatments (Figure 2). The trend of mortality revealed the potentiality of the *H. cordata* in the diet, the immunomodulatory effect of this medicinal plant was observed by Meng et al. (2009). The crude fat contents were found reduced in antibiotic and probiotics groups compared to control (p<0.05). The crude ash in broiler meat did not affect after addition of antibiotic or HCP groups (0.5 and 1.0%) compared to the

Table 4. Effect of *Houttuynia cordata* probiotics in the broiler meat (µmol MDA[1]/100g).

Storage time	Control	Antibiotics (0.05% OTC)	*Houttuynia cordata* probiotics	
			0.5%	1.0%
Fresh	1.74±0.12[a]	1.44±0.06[b]	1.50 ±0.04[b]	1.38 ±0.02[b]
1st	6.07±0.82[a]	4.06±0.31[b]	3.95 ±0.09[b]	3.06 ±0.25[b]
2nd	15.19±2.13[a]	10.40±2.02[b]	6.26 ±0.77[bc]	4.04 ±0.39[c]
3rd	20.09±4.24[a]	10.38±2.25[b]	12.34 ±3.07[b]	8.86 ±1.47[b]

Data: means±standard error. [a,b] Means with different superscripts within same raw are significantly different ($p<0.05$). [1]MDA = Melondialdehyde,

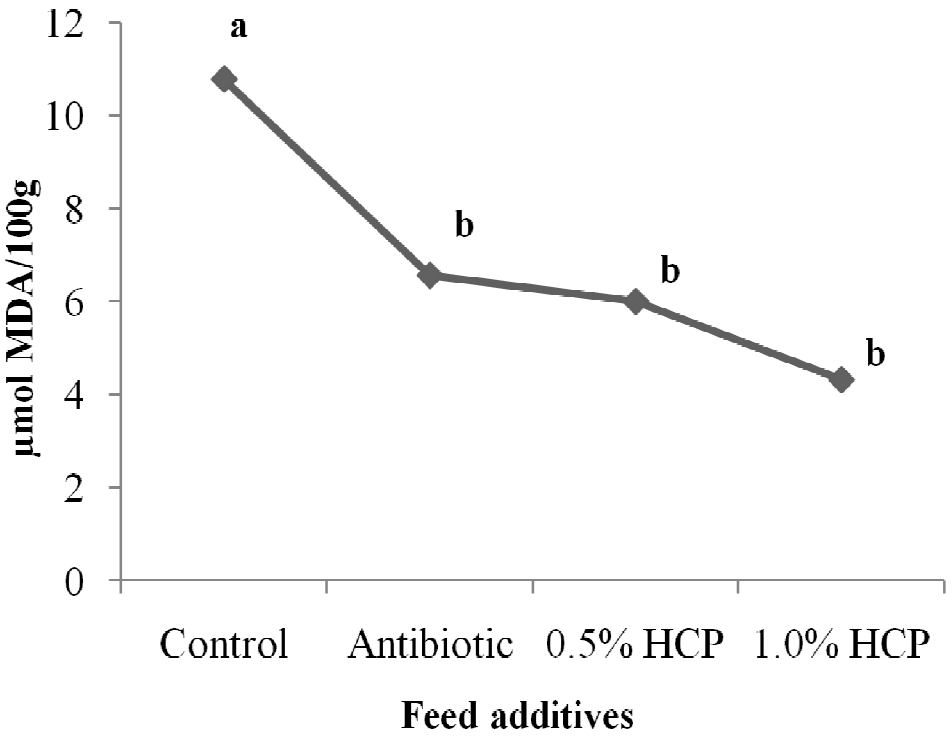

Figure 3. Effect of feed additives on thiobarbituric acid (TBA) value in broiler meat. Data with different superscripts are significantly different ($p<0.05$). MDA: Malondialdehyde.

control ($p>0.05$). Davis et al. (1975) reported that the crude protein content and the crude fat of meat are negatively correlated to each other. In other words, if the crude fat content is higher, the crude protein content tended to lower which is in agreement with our study.

Lipid oxidation of meat

The thiobarbituric acid (TBA) reactive substance test is the most widely used method for quantifying lipid oxidation development in meat and meat products. The TBA test determines the amount of malondialdehyde (MDA), a major secondary byproduct of lipid oxidation, in an oxidized lipid. In Table 4, TBA value of fresh broiler meat was significantly lower in 1.0% HCP group (1.38 µmol/100 g), while control was significantly high (1.74 µmol/100 g) ($p<0.05$). In case of one to three weeks of storage, TBA values of broiler meat were significantly higher in control group compared to the other treatments ($p<0.05$). The average TBA value of 0 to 3 weeks of storage at 4°C, the TBA value of meat in all feed additives group showed the lowest value than control (Figure 3). In both the levels of HCP (0.5 and 1.0%) showed statistically similar value with antibiotic fed birds (Figure 2). Lipid oxidation causes loss of nutritional and sensory values as well as the formation of potentially toxic compounds that compromise meat quality and

Table 5. Effects of *Houttuynia cordata* probiotics on relative organ weight in broiler.

Internal organs (%)	Control	Antibiotics (0.05% OTC)	*Houttuynia cordata* probiotics	
			0.5%	1.0%
Crop wt.	0.28±0.05	0.48±0.12	0.33±0.04	0.28±0.03
Heart wt.	0.50±0.03	0.51±0.01	0.52±0.04	0.43±0.02
Liver wt.	1.60±0.06	1.73±0.02	1.86±0.18	1.84±0.15
Gizzard wt.	0.88±0.09	1.04±0.18	1.14±0.12	0.97±0.10
Pancreas wt.	0.17±0.01	0.17±0.01	0.19±0.02	0.18±0.02
Cecum wt.	0.58±0.07	0.74±0.10	0.63±0.10	0.47±0.08
Kidney wt.	0.64±0.04	0.64±0.04	0.59±0.07	0.62±0.03
Small intestine wt.	2.57±0.24	2.70±0.26	2.73±0.25	3.01±0.57
Large intestine wt.	0.17^{b}±0.02	0.11^{c}±0.01	0.23^{a}±0.01	0.11^{c}±0.01
Abdominal fat wt.	2.12±0.28	1.84±0.40	2.22±0.12	2.49±0.24
Proventriculus wt.	0.54±0.13	0.67±0.17	0.58±0.11	0.49±0.14

Values are mean ± Standard error (n=4). Means with different letters within the row are not significantly difficult ($p<0.05$).

reduce its shelf life. The storage time also had an effect on these values and the highest TBA occurred at the end of the storage period. This result is agreed with the findings of Muhammet et al. (2005). The anti-oxidative property of *H. cordata* with probiotics in current study revealed and agreed with other researchers (Chen et al., 2003; Nuengchamnong et al., 2009).

Sarker et al. (2010b, c) showed a similar trend in reducing lipid oxidation with fed broilers medicinal plants (hamcho, green tea) with the same probiotics and same level. Uuganbayar (2004) reported that 0.5 to 1.5% green tea supplement in broiler diet had effects to reduce the TBA value on broiler meat compared to the control and Yang et al. (2003) also reported that the TBA value of broiler meat was decreased significantly when broilers were fed diets with 0.5 to 2.0% green tea by-products supplement diet. Mountney (1976) reported that the rancidity of broiler meat arises faster than the red meat of pork and beef because of more unsaturated fatty acid contained. It can be conclude that the green tea supplementation in broiler diet may reduce the rancidity of the broiler meat.

Weight of relative internal organs

In Table 5, the results showed that the large intestine weight was significantly reduced in broilers fed diets containing 0.5% HCP compared to the other treatment groups ($p<0.05$). The abdominal fat weights were not changed statistically for broilers fed diets. Broilers fed 1% HCP showed reduced large intestine weight like antibiotic fed birds compared to control ($p<0.05$). Some other researcher fed medicinal plant to broilers and got similar result. Uuganbayar (2004) reported diets containing 0.5% green tea showed a significant weight loss of the small intestine compared to the control diet, which is similar to our study. Kim et al. (2006) also expressed similar opinion in reducing small intestine weight if the plant when probiotics is used. Other internal organs showed in the Table 5, did not show any adverse effect after addition of medicinal plants mixed with probiotics and are similar with the findings of our previous study (Sarker et al., 2010b).

Conclusion

Considering the parameters, diets containing 1% *H. cordata* (eosungcho) with multi strain probiotics, can be substituted instead of antibiotic (oxytetracycline) for broiler production.

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