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Full Length Research Paper

Effect of dietary mugwort (*Artemisia vulgaris* L.) and pine needle powder (*Pinus densiflora*) on growth performance, serum cholesterol levels, and meat quality in broilers

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The effects of dietary mugwort and pine needle powder supplementation on growth performance, serum cholesterol, and meat quality of broilers were evaluated in a 35 days feed trial. 200 one day old broilers were randomly allocated to five dietary treatments (0, 1 and 2% mugwort or 1 and 2% pine needle powder) with four replicate pens of 10 birds per treatment. During the experimental period, growth performance did not differ among treatments ($P > 0.05$). The additives with mugwort and pine needle showed lower crude fat content of thigh muscle compared with the control ($P < 0.05$); however, no significant differences were detected for moisture, crude protein and crude ash content of thigh muscle. Compared with the control, total phenol content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity were significantly increased ($P < 0.05$) with the inclusion of mugwort and pine needle powder level in the broilers' diets, whereas pH values decreased ($P < 0.05$). Serum cholesterol and triglyceride concentrations were significantly decreased ($P < 0.05$) by the increased mugwort and pine needle powder level in the diet, except for high density lipoprotein (HDL) cholesterol concentrations. For thigh meat color, inclusion of mugwort and pine needle powder decreased L* and b* values and increased a* values in thigh muscle of broilers compared with the controls ($P < 0.05$). Overall, the present study indicates the beneficial effect of using 1 or 2% mugwort and pine needle powder in reducing serum cholesterol and improving meat quality.

Key words: Mugwort, pine needle powder, serum cholesterol, meat quality.

INTRODUCTION

Mugwort (*Artemisia vulgaris* L.), a perennial weed growing wild and abundantly in temperate and cold regions (Europe, Asia, northern Africa and Alaska), has been used for a variety of medicinal purposes or as edible plants for centuries (Terra et al., 2007). It is known that the main chemical compounds in mugwort consist of isocoumarin, coumarin, diterpenelactone and flavonoid (Kang, 1995). In the orient, mugwort has been employed as an analgesic and antimicrobial agent and in conjunction with acupuncture therapy and muxibustion to cure asthma and hepatitis (Tan et al., 1999; Yoshikawa et al., 1996; Lee et al., 2000). Experimental studies (rats) on various biological functions of mugwort have also shed light on the effects of antioxidant and antimicrobial activity (Kwon et al., 1993; Lee and Lee, 1994), anticancer effect

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Table 1. Ingredient composition and calculated analysis of the basal diets (as fed basis).

Item	Starter (1 to 21 days)	Finisher (22 to 35 days)
Corn	59.66	63.55
Soybean meal	27.02	30.11
Wheat bran	10.00	3.50
Dicalcium phosphate	1.19	1.12
Limestone	1.40	1.07
Salt	0.40	0.40
DL-methionine	0.13	0.05
Vitamin Premix[1]	0.10	0.10
Mineral Premix[2]	0.10	0.10
Total	100	100
Calculated composition		
Metabolizable energy (MJ/kg)	12.97	12.97
Crude protein (%)	21.50	19.00
Methionine (%)	0.50	0.38
Lysine (%)	1.10	1.00
Ca (%)	1.00	0.90
Available P (%)	0.45	0.35

[1]Vitamin premix provides the following (per kg of diet): Vitamin A, 5,500 IU; Vitamin D_3, 1,100 IU; vitamin E, 10 IU; riboflavin, 4.4 mg; vitamin B_{12}, 12 mg; nicotimic acid, 44 mg; menadione, 1.1 mg; biotin, 0.11mg; thiamine, 2.2 mg; ethoxyquin, 125 mg. [2]Mineral premix provides the following (per kilogram of diet): Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10mg; Se, 0.17 mg; I, 0.46 mg; Ca, min: 150 mg, max: 180 mg.

(Sun et al., 1992), anticoccidial and hepato protective activity (Gilani et al., 2005). In addition, other studies have suggested that the antimalarial activity in teas or the leaves of this plant as a treatment for fever and malaria was found to be related to artemisin (Klayman, 1985). As a result, the application of mugwort in broiler may be necessary and useful to improve quality and self-life of meat-type chickens during storage (Kim, 2006a).

Pine needle *(Pinus densiflora),* an evergreen needle-leafed tree, is widely distributed in Korea, Japan, and China and considered as an important folk remedy or as food to improve health (Chung et al., 1996; Park et al., 2004). In general, the chemical composition of pine needle contains phenolic compounds, terpenoids and tannin (Chung et al., 1996; Kuk et al., 1997; Kim et al., 2002). According to several reports, biological activity of pine needle can be divided into several categories: anti-microbial, antimutagenic, antioxidant antitumoral and cholesterol effects (Choi et al., 1997; Choi et al., 2002; Kwak et al., 2006). Lee and Choi (2000) reported that in rats fed with a high cholesterol diet, pine needle powder has potential to decrease total serum cholesterol levels and liver thiobarbituric acid reactive substance (TBARS).

Currently, there is growing public interest in the application of natural compounds as feed additives or anti-oxidants in poultry industry. Thus, we assumed that mugwort and pine needle can benefit animal health and production. Apart from this, scientific reports or information on broiler production, the nutritional values (thigh muscle) and the use of mugwort and pine needle for broilers as feed additives were less clear. Therefore, this study was undertaken to evaluate the effects of mugwort and pine needle powder on growth performance, serum cholesterol, and meat quality, including total phenol and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging in broiler chickens.

MATERIALS AND METHODS

Birds and diets

All experimental procedures used in this study were approved by the Animal Ethics Committee of the Gyeognam National University of Science and Technology (Jinju, South Korea). 200 one day old male broilers (Hubbard) obtained from a local commercial hatchery were grown over a 35 days experimental period. Birds were randomly allocated to five dietary treatments: control, 1% mugwort (M1), 2% mugwort (M2), 1% pine needle powder (PNP1) and 2% pine needle powder (PNP2). As industry diets, a starter diet (crumbles) was provided from 1 to 21 days, and a finish diet (steamed pellet) was given from 22 to 35 days (Table 1). All diets and water were offered *ad libitum* and light was provided continuously throughout the experiment. This experiment had a completely randomized design with four replicate pens of 10 birds per treatment.

Each bird had an area of approximately 0.09 m^2. Approximately 5 cm of bedding mixed rice hulls and wood savings was added to each pen (2 × 1 m). Pens were equipped with an automatic bell drinker and one tube feeder. The temperature was maintained at 30±1°C until day 7 and was gradually decreased to 24±1°C by 35 days of age. All broilers and feed were recorded at 1 and 35 days for

Table 2. Proximate composition of dry mugwort (*Artemistia vulgaris* L.) and pine needle (*Pinus densiflora*) powder used in this study.

Item	Mugwort powder	Pine needle powder
Component (g/kg, as-is basis)[1]	-	-
Dry matter	905.4	882.5
Crude protein	172.4	34.1
Crude fat	10.5	25.9
Crude fiber	263.7	503.4
Crude ash	17.9	34.1

[1]Determined in triplicate.

calculation of weight gain, feed intake, and feed conversion (feed:gain ratio). Feed intake was determined as the difference between supplied feed and feed left in each pen. Weight gain was calculated by the difference between initial weight and weight at 35 days of age. Feed conversion was calculated by dividing the total feed intake per pen by the total weight gain of the broilers.

Sample preparations

Mugwort and pine needle were purchased from the Daegu herbal market (Daegu, Korea). Mugwort and pine needle powders were prepared following the procedures. Leaves were stripped from the stems. The materials collected were cut into slices and subsequently thinly spread on a mat under sunlight at 35°C for 1 day. The drying process was conducted in a well-ventilated place and kept away from urban traffic to protect the mugwort and pine needle from environmental pollution. The air-dried mugwort and pine needle were then ground to a powder. Proximate composition of dry mugwort and pine needle powder is shown in Table 2.

Measurements and analysis

At the end of the experimental period (day 35), 5 birds from each pen were fasted for 6 h before slaughter and transferred to the slaughterhouse. After slaughter, birds were eviscerated to obtain thigh muscles and used for analyzing proximate composition, pH, total phenol, DPPH-radical scavenging assay, and thigh meat color. Before determination for different quality parameters, all skins that include subcutaneous fat and visible connective tissues were eliminated from the thigh muscles.

Proximate analysis

Proximate analyses of mugwort and pine needle and thigh muscle were performed according to AOAC standard methods (AOAC, 1999; Method 942.05 for moisture, Method 954.01 for crude protein, Method 920.39 for crude fat, Method 962.09 crude fiber, Method 942.05 for ash). Moisture was determined at a temperature of 11°C for 24 h using drying oven; crude protein was determined according to the Kjeldahl method. Crude fat was evaluated by the ether extract. Crude fiber was determined as the loss of weight on ignition. Crude ash was conducted on the gravimetric loss by heating to 600°C during 2 h.

pH

To measure pH, approximately 10 g of a sample of thigh muscle was homogenized using a homogenizer (Physcotron NS-50, Nichion Irika, Tokyo, Japan) with 90 ml of distilled water and pH value was measured using a digital pH meter (691 pH meter, Metrohm, Swiss).

Total phenol contents

About 5 g of thigh muscle was blended with 100 ml of 80% ethanol and extracted with reflux using a heating mantle at 80°C for 2 h. After cooling, the extracts were filtered through No. 1 filter paper (Whatman Inc., Clifton, NJ) to separate the lipids and concentrated in a vacuum evaporator (Buchi Co., R-124, Germany) at 40°C. Total phenol contents were estimated by the modified method of Slinkard and Singleton (1977) and Singleton et al. (1999) involving Folin-Ciocalteu reagent and gallic acid as standard. 1 ml of extract solution was added to 3 ml of Folin-Ciocalteu reagents in a test tube, and the mixture was kept at room temperature for 30 min. After that, 3 ml of 10% Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h. The absorbance was recorded at 760 nm using ultra-violet/visible (UV/VIS) spectrophotometer (UV-24D, Shimadzu, Tokyo, Japan). Results were expressed as mg of gallic acid equivalent (GAE) per 100 g of meat.

DPPH radical-scavenging assay

DPPH-radical scavenging activity from thigh meat was determined as described by Blois (1958) with minor modification. Briefly, 1 ml of each extract was added to 4 ml of ethanolic DPPH solution (100 μM). The mixture was shaken well and left to stand at room temperature for 30 min. Solution was measured using a UV-Vis spectrophotometer (UV-24D, Shimadzu, Tokyo, Japan) at 517 nm. Concentration of Ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging was calculated using the following equation:

DPPH radical scavenging (%) = [1 - (absorbance of sample solution/absorbance of control)] × 100.

Blood collection, cholesterol and triglycerides analysis

Before slaughter, five birds from each pen were taken for blood samples collection. Blood was collected from the wing vein into a sterile syringe and transferred into vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) immediately. Samples for serum analysis were then centrifuged at 2,000 × *g* for 30 min and serum was separated. After collecting serum, serum samples were maintained at −20°C (for up to 2 days) for determination of serum cholesterol and triglyceride concentration. The concentration of total

Table 3. Effect of dietary mugwort and pine needle powder supplement on the growth performance of broilers during 35 days.

Item[1]	Treatment[1]					
	Control	M1	M2	PNP1	PNP2	Pooled SEM[2]
Initial BW (at 1 day, g)	40.95	41.32	41.08	40.82	40.78	0.45
Final BW (at 35 days, g)	1870.60	1871.77	1858.60	1871.82	1859.57	24.91
Weight gain (1 to 35 days, g)	1829.65	1830.45	1817.52	1831.00	1818.79	25.27
Feed intake (1 to 35 days, g)	3149.37	3137.61	3137.71	3130.44	3141.77	52.82
Feed conversion (feed: gain ratio, 1 to 35 days)	1.72	1.71	1.73	1.71	1.73	0.05

[1]Data are means of 4 replicate pens of 10 birds each. M1 = 1% mugwort; M2 = 2% mugwort; PNP1 = 1% pine needle powder; PNP2 = 2% pine needle powder. [2]PooledSEM is statistical value not treatment.

cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol, and triglyceride in the serum samples were analyzed with an automatic biochemical analyzer (Hitachi 747, Hitachi Co., Tokyo, Japan) according to the colorimetric method and direct enzymatic kits (Boehringer Mannheim, Germany).

Color measurements

Color measurements of thigh muscle samples were measured with a Minolta chromameter (Minolta CR-300, Osaka, Japan) standardized with the white calibration plate (Y = 93.5; X = 0.3132; y = 0.3198). Areas for measurement, once selected, were done on the surface such that the same meat surface area was used for repeated color measurements over time (Petracci and Fletcher, 2002). The results were expressed as lightness (L*), redness (a*), and yellowness (b*).

Statistical analysis

All data were examined by analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS (SAS Institute, 2002). Pen means were considered as the experimental unit for all the data. Mean differences among treatments were tested with Duncan's multiple range tests at the $P < 0.05$.

RESULTS AND DISCUSSION

Growth performance (weight gain, feed intake and feed conversion)

The effect of dietary mugwort and pine needle powder supplement on the growth performance of broilers during 35 days of study is shown in Table 3. Mugwort and pine needle powder supplements had no effect on growth performance during the experimental period. At present, the exact mechanism of this is not clear. In agreement with the current study, Kim (2006a) reported no differences in body weight between the addition of mugwort and fish oil with 1 and 2% and the control groups. Another study conducted by Jeon et al. (2005) also reported that dietary supplementation with pine needle extracts (1, 2, 4 and 8%) in rat diets significantly decreased body weight gain compared with that of the control diet group. According to Haw et al. (1985), the growing rate of rat groups with 8% or more of mugwort powder were below the control groups because mugwort that contains 19.2% cellulose, 11.8% ash, and 16.15% moisture would hinder digestibility or absorption of nutrients in the body. They recommended that mugwort levels in animal diets not exceed 8%.

Proximate composition

The results of proximate composition of chicken thigh muscle from mugwort and pine needle powder after 35 days are presented in Table 4. The present study show that no differences were noticed for moisture, crude protein and crude ash content; however, in our trial the effects of additives were observed in crude fat contents ($P < 0.05$). Inclusion of mugwort (1% or 2%) and pine need powder (1% or 2%) in the diets decreased crude fat contents of chicken thigh meat compared with the control groups. Also, our observation is that the addition of 2% pine needle powder (PNP2) greatly reduced the crude fat contents. We hypothesized that it may be explained by the fact that mugwort and pine needle powder would decrease fat accumulation in the liver, because of the reduction of fatty acid synthesis (Chang and Johnson, 1980). Similar results were found in pork experiments (Kim, 2006b), where crude fat contents were significantly decreased by the addition of fish oil and mugwort.

pH, total phenol and DPPH radical scavenging

Table 5 shows the changes in pH, total phenol, and DPPH radical scavenging of chicken thigh meat after feeding of mugwort and pine needle powder for 35 days. Compared with the control group, the pH slightly decreased ($P < 0.05$) with the groups with mugwort and pine needle powder. There were significantly differences between treatments with mugwort and pine needle powder and the control regarding total phenol

Table 4. Proximate composition of thigh meat in broilers at 35 days of age fed diets containing different levels of mugwort and pine needle powder.

Item	Treatment[1]					
	Control	M1	M2	PNP1	PNP2	Pooled SEM[2]
Moisture (%)	73.34	73.85	73.89	73.89	74.19	0.18
Crude protein (%)	22.52	22.55	22.50	22.79	22.55	0.08
Crude fat (%)	3.06[a]	2.53[b]	2.52[b]	2.28[c]	2.18[c]	0.10
Crude ash (%)	1.09	1.08	1.10	1.10	1.09	0.02

[1]Data are means of 4 replicates of 5 birds per pen. M1 = 1% mugwort; M2 = 2% mugwort; PNP1 = 1% pine needle powder; PNP2 = 2% pine needle powder. [2]PooledSEM is statistical value not treatment. [a-c]Means within the same row without common superscripts are significantly different (P<0.05).

Table 5. Effect of dietary mugwort and pine needle powder supplementation on pH, total phenol, and DPPH-radical scavenging in chicken thigh meat after 35 days.

Item	Treatment[1]					
	Control	M1	M2	PNP1	PNP2	Pooled SEM[2]
pH	6.27[a]	6.12[b]	6.07[c]	6.13[b]	6.06[c]	0.05
Total phenol (mg GAE/100g)	70.56[c]	73.82[b]	78.54[a]	77.34[a]	79.08[a]	0.87
DPPH-radical scavenging (%)[3]	26.10[d]	28.82[c]	29.41[b]	30.03[ab]	30.95[a]	0.52

[1]Data are means of 4 replicates of 5 birds per pen. M1 = 1% mugwort; M2 = 2% mugwort; PNP1 = 1% pine needle powder; PNP2 = 2% pine needle powder. [2]PooledSEM is statistical value not treatment. [3]DPPH=1-diphenyl-2-picrylhydrazyl. [a-d]Means within the same row without common superscripts are significantly different (P<0.05).

content and DPPH-radical scavenging activity (P < 0.05). This means that the thigh meat from chicken fed the mugwort (1 and 2%, M1 and M2) and pine needle powder (1 and 2%, PNP1 and PNP2) supplements had more total phenol content and DPPH-radical scavenging activity than the thigh meat of chickens fed to the control diets. As shown in Table 5, thigh muscle with low pH had high total phenol content and DPPH-radical scavenging activity, indicating that the antioxidative activity improved with increasing levels of dietary mugwort and pine needle powder.

Consequently, the greatest total phenol and DPPH-radical scavenging was in groups 2% pine needle powder (PNP 2), followed by 1% pine needle powder (PNP 1) or 2% mugwort (M2), and 1% mugwort (M1). For this, there is evidence to suggest that the effectiveness of mugwort and pine needle powder (polyphenols and flavonoids) as antioxidants would be related to the decrease in pH in thigh muscle of chicks (Xiong et al., 1993). Kim et al. (2009) indicated that the pH values were significantly lower (P < 0.05) in groups from gilts fed the 0.5 and 1.5 % mugwort powder supplementation compared with the control groups.

Generally, the antioxidant activity of mugwort and pine needle is well known (Park et al., 2004; Luo et al., 2007). This activity has been mainly attributed to flavonoids in mugwort and phenolic compounds in pine needle. They have not only the ability to act as antioxidants by free radical scavenging but also the ability to chelate transition metals from iron ions (Martin et al., 2002). For example, in systematic comparative study with 26 spices, Shan et al. (2005) verified that some spices with high level of phenolic (clove, cinnamon, oregano, and rosemary) were screened to use as excellent free radical scavengers and potential sources of potent natural antioxidants for commercial exploration.

Serum cholesterol and triglyceride

The changes in serum cholesterol and triglyceride levels in broiler for mugwort and pine needle powder treatments after 35 days are given in Table 6. Levels of total serum cholesterol and LDL–cholesterol decreased significantly (P < 0.05) with inclusion of mugwort and pine needle powder in diets. On the other hand, the HDL-cholesterol levels significantly increased in broiler fed mugwort and pine needle powder. However, with the exception of 2% pine needle powder (PNP2), there were no remarkable differences (P > 0.05) among all treatments in triglyceride. Additionally, no differences were observed for total serum cholesterol between control and 1% mugwort (M1) or among treatments with 2% mugwort (M2), 1 and 2% pine needle powder (PNP1 and PNP2) and for LDL and HDL-

Table 6. Effects of dietary mugwort and pine needle powder supplementation on serum cholesterol and triglyceride levels (mg/dL) in broiler chickens after 35 days.

Item	Treatment[1]					
	Control	M1	M2	PNP1	PNP2	Pooled SEM[2]
Total serum cholesterol	173.60[a]	170.24[ab]	154.21[c]	152.85[c]	151.38[c]	5.97
HDL-cholesterol	109.45[c]	112.59[b]	129.82[a]	131.13[a]	130.35[a]	2.16
LDL-cholesterol	38.37[a]	34.15[b]	32.27[c]	32.92[c]	32.11[c]	1.42
Triglyceride	118.63[a]	117.59[a]	115.62[a]	115.26[a]	108.27[b]	2.92

[1]Data are means of 4 replicates of 5 birds per pen. M1 = 1% mugwort; M2 = 2% mugwort; PNP1 = 1% pine needle powder; PNP2 = 2% pine needle powder. [2]PooledSEM is statistical value not treatment.. [a-c]Means within the same row without common superscripts are significantly different (P<0.05).

Table 7. Effects of dietary mugwort and pine needle powder supplementation on meat color of chicken thigh after 35 days.

Item	Treatment[1]					
	Control	M1	M2	PNP1	PNP2	Pooled SEM[2]
Minolta lightness[3] (L*)	58.19[a]	56.73[b]	55.92[b]	56.40[b]	54.92[c]	0.48
Minolta redness[3] (a*)	11.17[c]	11.83[b]	13.05[a]	13.10[a]	13.43[a]	0.43
Minolta yellowness[3] (b*)	9.26[a]	9.07[a]	7.78[c]	8.53[b]	7.70[c]	0.42

[1]Data are means of 4 replicates of 5 birds per pen. M1 = 1% mugwort; M2 = 2% mugwort; PNP1 = 1% pine needle powder; PNP2 = 2% pine needle powder. [2]PooledSEM is statistical value not treatment. [3]Minolta readings were taken 24 h postmortem using a Minolta CR-300 device (Osaka, Japan). [a-c]Means within the same row without common superscripts are significantly different (P<0.05).

cholesterol among 2% mugwort, 1 and 2% pine needle powder.

In the current study, unlike mugwort, inclusion of pine needle powder resulted in the lowest levels of serum cholesterol and triglyceride and the greatest levels of HDL cholesterol. This might partially be explained by the fact that pine needle powder would be expected to have a much higher antioxidant capacity or biological effect than mugwort. Furthermore, the mechanisms by which two additives reduced serum cholesterol and triglyceride levels could be attributed to the reduction in synthetic enzyme activity, which further suppressed oxidative free radicals (Chowdhury et al., 2002) or bio-active components (flavonoides or phenolic compounds) as antioxidant in mugwort and pine needle powder (Miyake and Shibamoto, 1997). This observation is in line with the results of Choi et al. (2010), who reported that increasing the levels of garlic powder and a combination of garlic powder and α-tocopherol significantly decreased total cholesterol and LDL-cholesterol and increased HDL-cholesterol in broiler blood.

Color of thigh muscle

Meat color results from mugwort and pine needle powder are presented in Table 7. Overall inclusion of mugwort and pine needle powder linearly decreased L* and b* values and increased a* values in thigh muscle of broilers when compared with the control groups ($P < 0.05$). These results show that changes in thigh muscle color were affected by the levels of mugwort and pine needle powder. In particular, the lowest L* and b* values and the greatest a* values were found in treatment with 2% pine needle powder (PNP2) compared with the control groups. An increase in lightness and redness with mugwort and pine needle powder as antioxidants might be due to the fact that antioxidants could be delayed metmyoglobin formation in meats (Higgins et al., 1998) or the effectiveness of natural extracts with meatball was associated with meat pH (Fernandez-Lopez et al., 2003). Previously, Chen et al. (2008) showed that adding garlic to pig diets decreased L*, a*, and b*. Jang et al. (2008) reported that using a dietary medicinal herb extract mix (MHEM) in poultry diets resulted in lower L* values of breast at 3 days. In addition, Yin et al. (1993) hypothesized that α-tocopherol retards lipid oxidation directly and OxyMb oxidation indirectly. According to several studies, the changes in meat color are dependent on three major factors; pH, metmyoglobin formation and the oxidation process, and the concentration of heme pigment (Fernandez-Lopez et al., 2005; Chen et al., 2008). Thus, these results obtained from mugwort and pine needle powder can bring expected improvement in

meat color.

Conclusion

In conclusion, feeding mugwort and pine needle powder to broilers had a beneficial effect on serum cholesterol and thigh meat quality. In addition, increasing levels of dietary mugwort and pine needle powder could have a valuable potential as a natural antioxidant agent, which was important for commercial diets or uses. However, the performance of broilers and proximate composition were not greatly influenced by the additions of mugwort and pine needle. Further study in this area is required to investigate the optimal dietary inclusion levels and the mode of action of these products to achieve optimal growth performance in broiler.

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