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# Effect of pine (*Pinus densiflora*) needle and Korean mistletoe (*Viscum album* var. *coloratum*) powder on male broiler chicken growth, serum cholesterol profiles, and meat quality

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ABSTRACT

Pine (*Pinus densiflora)* needle leaf (PNL) and Korean mistletoe (KM) (*Viscum album* var. *coloratum*) were compared as dietary supplements for male broiler chickens, and their effects on broiler growth, serum cholesterol profiles, and meat quality were investigated**.** A total of 240 one-day-old male broilers (40~45 g/body weight) were randomly assigned to 4 dietary groups (control, 0.5% PNL, 1% PNL, and 1% KM) of 60 birds each (3 replicates per group) in a completely randomized design. PNL and KM dietary supplements did not affect broiler growth performance, but mortality was reduced in the 1% KM-treated group. Serum cholesterol profiles indicated that total cholesterol and low-density lipoprotein cholesterol level (LDLC) decreased significantly while high-density lipoprotein cholesterol (HDLC) level increased in treatment groups compared to levels in the control group. Decrease in lightness and redness color values in thigh meat of broilers fed PNL and KM powder were observed. However, no significant differences were observed among the groups with regard to triglyceride and glucose levels, pH, and yellowness of the meat. In conclusion, 1% KM dietary supplement can reduce mortality, which may be due to the immune-regulating effects of KM, as well, it can reduce lipid oxidation and improve meat color stability via an antioxidant action in broiler thigh muscles.

**Key words**: Growth performance, Korean mistletoe, Meat quality, Pine needle, Serum cholesterol

In the European Union, antibiotic use in animals has been limited to growth-promoting treatments that are not used in human medicine. Imposition of that limitation is due to the need to surveil for residues in animal products and to avoid the transfer of resistant pathogens from animals to humans (Feizi *et al*. 2014). From a health point of view, research interest in natural antibiotic alternatives has increased in the past decade due to the potential antioxidant effects of plant-based medicines. In particular, livestock producers have been encouraged to use plant and plant extracts as feed additive alternatives to antibiotics.

*Pinus densiflora* (Japanese red pine), an evergreen tree with needle-shaped leaves, is indigenous to the mountainous regions of Asian countries (Cheong and Lim 2010). Pine needles have been used as an anti-hypertension medicine in traditional medicine (Park *et al.* 2008, Kim *et al.* 2010), have antioxidant and reactive oxygen species scavenging activities and reduce total lipid and liver cholesterol levels (Lee 2003, Hsu *et al.* 2005). Terpenoids and phenolic compounds are the main bioactive constituents in pine needles, and such compounds may contribute to their diverse medicinal benefits.

Mistletoe is a hemiparasitic plant that typically grows on different kinds of trees including pine, poplar, apple, and locus trees (Vica *et al.* 2012). Although there are American (*Phorandendron serotinum*), European (*Viscum album L.*), and Korean (*Viscum album L. coloratum*) mistletoe varieties, European mistletoe is the variety that has been most commonly used in a number of studies (Vica *et al.* 2012). Mistletoe has been used as a medicinal agent to treat many diseases and has demonstrated pharmacological effects in human and animal studies (Kim *et al.* 2007, Hossain *et al.* 2012). It can enrich animal diets in drought areas and has been used as a mineral-rich forage source for animals (Madibela *et al.* 2000). In addition, it exhibits immunomodulatory (Onay-Ucar *et al.* 2006) and pharmacological effects (Ekhaise *et al.* 2010). The major active constituents associated with the antioxidant activity of mistletoe include lectins, phenolic acid, and the flavonol quercetin (Haas *et al.* 2003).

Studies on the effects of *P. densiflora* needle leaf (PNL) and Korean mistletoe (KM) powder on growth and meat quality in chicken broilers are limited. Therefore, investigating the use of these two natural materials in poultry diets is important since these plants may improve immune system function and enhance animal growth. Our study aimed to compare the effects of PNL and KM when used as dietary supplements and to investigate their effects

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on growth, serum cholesterol profiles, and meat quality in male broiler chickens.

## MATERIALS AND METHODS

*Birds, diets and experimental design:* The PNL and KM were obtained from a local herbal market in Daegu, South Korea. Leaves and stems of both plants were dried on a mat under sunlight at 30–35°C for 10 h. Samples were dried further in a drying oven at 50°C for 8 h and then finely ground three times with a grinder for 2 min each. To prepare the powders, samples were screened with a fine mesh sieve and stored at 4°C until used.

All live animal procedures followed the farm animal care guidelines of Daegu University (Approval no: DUIACC-2016-016-1012-008). A total of 240 one-day-old male broilers were divided into 4 treatment groups of 60 birds each in a completely randomized design. Three replicate pens (20 birds/pen) were allocated starter (0–21 d) followed by finisher (22–35 d) diets (Table 1).

Dietary treatment groups received a basal diet (control) or 0.5% PNL (T1), 1% PNL (T2), or 1% KM (T3) supplemented diets. Two feed additives were used in the 0.5% PNL, 1% PNL, and 1% KM treatments as reference levels, according to the methods reported in previous studies (Kim *et al.* 2012, Kim and Choi 2014). Each pen (0.09 $m^2$/bird) contained a hanging tube feeder and an automatic bell drinker. Throughout the trials, the birds were raised on rice hulls that were top-dressed with new bedding and were given *ad lib.* access to feed and water. Ambient pen temperature was initially maintained at 35±1°C for 7 d and then gradually reduced to 24°C at 35 d. All birds lived under a standard light-dark cycle (14 h light, 10 h dark) with automatically regulated air ventilation. Body weight of the birds and feed intake in each pen were recorded at 1 d and 35 d of age, whereas mortality was recorded daily. At the end of each experiment, the feed conversion ratio was calculated by dividing feed intake by body weight gain.

Table 1. Ingredients and chemical composition of the basal diet fed to broilers (as-fed basis)

| Ingredient | Starter (1 to 21 d; %) | Finisher (22 to 35 d; %) |
|---|---|---|
| Corn | 54.624 | 62.612 |
| Soybean meal CP46% | 35.480 | 30.220 |
| Fish meal | 1.000 | |
| Soya oil | 4.960 | 3.620 |
| Dicalcium phosphate | 1.940 | 1.520 |
| Limestone | 1.190 | 1.270 |
| Salt | 0.300 | 0.300 |
| DL-methionine | 0.160 | 0.112 |
| Choline 50% | 0.080 | 0.080 |
| Etoxyquine 30% | 0.066 | 0.066 |
| Vitamin premix[1] | 0.100 | 0.100 |
| Mineral premix[2] | 0.100 | 0.100 |
| Total | 100.000 | 100.000 |
| Calculated analysis (%) | | |
| ME (MJ/kg) | 12.97 | 12.97 |
| Crude protein (%) | 21.5 | 19 |
| Methionine (%) | 0.5 | 0.38 |
| Lysine (%) | 1.1 | 1 |
| Ca (%) | 1 | 0.9 |
| 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, 0.11 mg; thiamine, 2.2 mg; ethoxyuin, 125 mg. [2]Mineral premix provides the following (per kilogram of diet): Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.17 mg; I, 0.46 mg; Ca, min: 150 mg, max: 180 mg.

*Collection of meat and blood samples:* At the end of the trial (day 35), all birds were fasted for 6 h. Twelve birds per group were processed under commercial conditions; birds were electrically stunned, slaughtered by neck cutting, and exsanguinated. Thigh muscle samples were obtained by removing subcutaneous fat and visible connective tissues and stored in a sealed plastic bag at 4°C for subsequent analysis (Approval no: DUIACC-2016-016-1012-008).

Before slaughtering, blood samples were collected from a wing vein using sterilized syringes and needles (Becton Dickinson Vacutainer Systems, Franklin Lakes, USA). Approximately 5 mL of whole blood was sampled per bird. The samples were centrifuged at 3,000 × *g* for 20 min for serum extraction. Serum samples were aliquoted and stored at –20°C until assays were performed (Kim 2014).

*Analytical procedures:* The samples were analyzed for total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), triglycerides (TG), and glucose (G) levels using an automated analyzer (Hitachi 747; Hitachi, Tokyo, Japan) and direct enzymatic kits (Boehringer Mannheim, Germany) (Kim 2014).

To determine muscle pH, 10 g of thigh muscle was added to 90 mL of distilled water and homogenized in a blender (HM-3000; Hyundai Electronics Industry, Incheon, South Korea); thereafter, the pH was immediately measured using a pH meter (691 pH meter; Metrohm, Switzerland)(Lee *et al.* 2010) .

Thiobarbituric acid reactive substances (TBARS) in broiler thigh muscle were quantified to examine lipid oxidation (Witte *et al.* 1970). TBARS values were measured as milligrams of malonaldehyde per kilogram of meat.

Meat color was determined using a Minolta colorimeter calibrated with a standard white tile with a lightness (L*) of 96.16, redness (a*) of 0.10, and yellowness (b*) of 1.90. Meat color parameters, expressed as CIE L*, a*, and b* values, were determined by calculating the average of three colorimetric readings from different points on each sample (Kim 2014).

*Statistical analysis:* All measurements were conducted in triplicate. Data were analyzed as representative of a completely randomized design with 4 groups and were subjected to analysis of variance (ANOVA) using the General Linear Models procedure in SAS software (SAS 2002). Each broiler pen was considered an experimental unit. Duncan's multiple range tests (Duncan 1955) with statistical significance at the 0.05 level were conducted to measure the differences among groups.

## RESULTS AND DISCUSSION

*Growth performance and mortality:* Weight gain, feed intake, and feed conversion rates were not significantly different among the groups (P>0.05). However, birds fed 1% KM (T3) powder had significantly lower mortality than those fed the control diet, or diets supplemented with 0.5% PNL (T1) or 1% PNL (T2) powder (Table 2).

*Serum cholesterol levels:* Dietary supplementation with 1% KM, 0.5% PNL, and 1% PNL decreased TC and LDLC levels significantly (P<0.05) and increased the HDLC levels significantly (P<0.05) from those of the control (Fig. 1) in which TC was 170.60 mg/dL, LDLC was 41.63 mg/dL, and HDLC was 103.32 mg/dL. However, there were no differences in TG or G levels (range in control and treatment groups, 118.93–120.85 mg/dL and 226.60–238.73 mg/dL, respectively) among the 3 treatment groups (P > 0.05). TC, LDLC, and HDLC levels in 1% KM and 1% PNL treatment groups were similar, but the results for the 0.5% PNL group were different. This is not supported by the results in Fig. 1, in which no significant differences are identified among the 3 treatments.

*Thigh muscle pH or meat color:* No effect of 1% KM, 0.5% PNL, or 1% PNL powder was observed on thigh muscle pH or meat color b* values (Table 3). However, thigh muscle TBARS values and meat color L* and a* values were significantly different among the groups (P< 0.05). The pH, TBARS, and all meat color values were similar for the 0.5% and 1% PNL treatments; however, among the groups, significantly high a* values and significantly low TBARS and L* values were observed in the 1% KM group.

This study demonstrated that dietary supplementation of PNL and KM did not enhance growth in chickens. These results are similar to those of other studies by Kim *et al.* (2012) in which broiler chickens were fed 1% and 2% PNL powder. Kim and Choi (2014) reported no significant differences in growth performance for broilers fed diets supplemented with 0.5%, 1%, or 2% KM. Of interest in our results, however, was the large reduction in mortality with a 1% KM powder supplement, followed by that in the 1% PNL, 0.5% PNL, and control groups. Egbewande *et al.* (2011) observed no mortality in broiler chickens when fed 5%, 10%, or 15% levels of leaf meal of African mistletoe (*Tapinanthus bangwensis*) throughout their feeding trial. One possible explanation for our results is that PNL and KM may have some immune-regulatory functions (Onay-Ucar *et al.* 2006).

In this study, PNL and KM powder addition led to a reduction in serum cholesterol levels. That decrease in serum cholesterol could be attributed to a reduction or inhibition in cholesterol absorption and synthesis as an antioxidant (Miyake and Shibamoto 1997, Shahidi 2000, Kim 2014). A similar result was observed by Kim *et al.* (2012), wherein 1% and 2% PNL powder decreased total

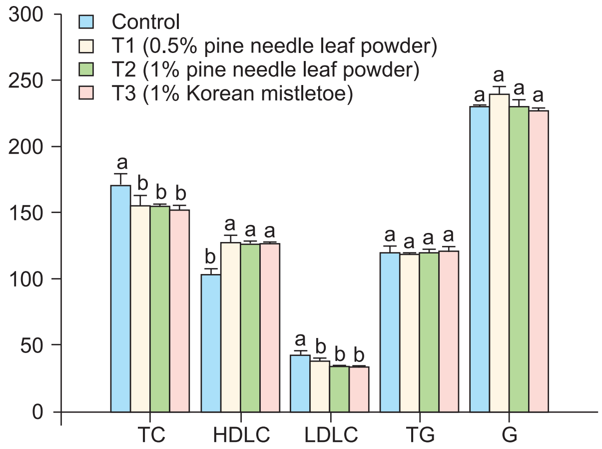


Fig. 1. Effect of dietary supplementation of PNL and KM powders on the serum cholesterol of male broilers. Results are expressed as mean±SEM. [a-b]Bars with same letters are not significantly different at P<0.05. TC, total cholesterol; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; TG, triglycerides; G, glucose.

Table 2. Effect of dietary supplementation of PNL and KM powder on male broiler growth performance and mortality

| Parameter | Group | | | | SEM | P |
|---|---|---|---|---|---|---|
| | Control | T1 | T2 | T3 | | |
| Initial body weight (g, day 1) | 41.12±0.41 | 40.45±0.17 | 40.98±0.16 | 41.38±0.39 | 0.22 | 0.1615 |
| Final body weight (g, day 35) | 1870.74±18.51 | 1877.58±21.19 | 1870.77±17.94 | 1880.76±13.36 | 15.38 | 0.6737 |
| Weight gain (g, day 1 to 35) | 1829.63±19.28 | 1837.14±21.35 | 1829.79±18.10 | 1839.38±13.75 | 8.37 | 0.8380 |
| Feed intake (g, day 1 to 35) | 3164.82±48.94 | 3180.95±47.50 | 3136.60±53.27 | 3156.22±51.93 | 17.35 | 0.3114 |
| Feed conversion (feed:gain ratio, day 1 to 35) | 1.73±0.05 | 1.73±0.05 | 1.73±0.05 | 1.72±0.04 | 0.02 | 0.9505 |
| Mortality (%) | 3.21±0.14[a] | 2.44±0.04[b] | 1.81±0.02[c] | 1.60±0.08[c] | 0.13 | 0.0006 |

[a-c] Values within a row with different superscripts differ significantly (P<0.05). T1, 0.5% pine needle powder; T2, 1% pine needle powder; T3, 1% Korean mistletoe powder.

Table 3. Effect of dietary supplementation of PNL and KM powders on pH, TBARS, and meat color in broilers

| Parameter | Group | | | | SEM | P value |
|---|---|---|---|---|---|---|
| | Control | T1 | T2 | T3 | | |
| pH | 6.21±0.06 | 6.17±0.04 | 6.12±0.05 | 6.12±0.04 | 0.03 | 0.1715 |
| TBARS (mg MA/kg) | 0.46±0.01[a] | 0.39±0.01[b] | 0.39±0.02[b] | 0.37±0.05[b] | 0.02 | 0.0082 |
| CIE L*(lightness) | 58.50±0.62[a] | 57.36±0.78[ab] | 56.91±0.68[b] | 55.11±0.22[c] | 0.40 | 0.0042 |
| CIE a* (redness) | 11.02±0.83[b] | 11.62±0.75[b] | 11.54±0.53[b] | 12.85±0.48[a] | 0.20 | 0.0036 |
| CIE b* (yellowness) | 8.61±0.16 | 8.52±0.24 | 8.66±0.36 | 8.69±0.42 | 0.14 | 0.8652 |

[a-c]Values within a row with different superscripts differ significantly (P<0.05). T1, 0.5% pine needle powder; T2, 1% pine needle powder; T3, 1% Korean mistletoe powder.

serum cholesterol, LDL cholesterol, and triglycerides but increased HDL cholesterol. Additionally, KM is reported to have an effect on serum cholesterol, but not HDLC, in broilers (Kim and Choi 2014). In general, blood serum profiles are influenced by the organism's current condition, which is influenced by various internal and external factors (Toghyani *et al.* 2011). Moreover, there is evidence that herb and plant extracts exert antioxidant properties owing to the presence of molecules such as phenolic compounds, which have a bioactive effect on animal metabolism (Fernandez-Lopez *et al.* 2005, Kim 2014).

In the present study, 1% KM dietary supplements reduced lipid oxidation (TBARS) and improved meat color to a greater extent than did PNL powder due to the antioxidant activity level of KM. However, despite the presence of PNL and KM in the feed, there was no evidence to show that a decrease in TBARS values via antioxidant effectiveness was chiefly associated with a lower pH. Several previous studies have suggested that pH is one of the primary factors that determine meat quality and antioxidant activity (Fernandez-Lopez *et al.* 2003). In contrast, Kim and Choi (2014) suggested that groups treated with KM (0.5%, 1%, or 2%) had lower tissue pH values than that of the control group; the difference in results may be due to the difference in the KM variety used. Furthermore, it is generally accepted that lipid oxidation, which contributes to the deterioration of meat appearance, color, and flavor, is the primary process leading to meat degradation. Therefore, natural additives with an antioxidant function may be beneficial in maintaining nutritional value and meat quality. In our evaluation of meat color, it was observed that KM had an effect on L* and a* values, demonstrating that KM powder has a stronger antioxidant effect than PNL powder. Our results agree with those of Kim *et al.* (2012), in which a decrease in L* and an increase in a* values in broilers fed mugwort (1% and 2%) and PNL (1% and 2%) powder were observed. Kim and Choi (2014) demonstrated that increasing levels of KM powder were associated with lower L* values in broiler chick thigh muscles, but the treatments had no effect on b* values. This is similar to our results in which there was no significant effect on b* values. In general, L* and a* values are the most important color parameters when evaluating meat oxidation. Previous studies have demonstrated that the rate of oxidation from myoglobin to metmyoglobin increases in a high oxygen atmosphere (Fernández-López *et al.* 2008, McMillin 2008, Bingol and Ergun 2011). Such changes increase L* and decrease a*, which render meat and meat products unacceptable to consumers. Furthermore, Waylan *et al.* (2002) discussed oxidative stability of lipids as it relates to preserving meat color and reported that application of modified tall oil and vitamin E provided natural antioxidants that delayed metmyoglobin formation in meats.

The present study demonstrated that adding 1% KM to a male broiler chicken diet is an effective natural means to decrease broiler mortality and serum cholesterol levels and improve lipid oxidation stability and to maintain meat quality. However, none of the supplements (1% KM, 0.5% PNL and 1% PNL) to the broiler diet improved growth performance or triglyceride or glucose levels.

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