Effect of dietary pine needles powder supplementation on growth, organ weight and blood biochemical profiles in broilers

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ABSTRACT
A total of eighty 21-days old broilers with similar body weight were randomly allocated to four dietary treatments with five replicates of four chickens per replicate. Birds were fed diets containing 0% PNP (Control, CT), 1% PNP (PNP1), 3% PNP (PNP3) and 5% (PNP5) respectively. The results showed that the final body weight and body weight gain had no significant differences between groups. The PNP supplementation increased the weight of gizzard and rectums, but decreased small intestine weight (P < 0.05). Overall the weight indices of gizzards and rectums increased in the PNP treatments, while the weight indices of small intestine decreased compared with the CT. Broilers receiving 3% PNP had higher serum superoxide dismutase activity compared with CT. The serum malondialdehyde content was lower in PNP5 treatment compared with CT. The serum triglyceride contents were lower in the PNP3 and PNP5 treatments compared with CT. Total serum cholesterol of PNP1 and PNP3 treatments was lower than CT. In conclusion, this study showed that PNP supplementation reduced the serum cholesterol and triglycerides in broilers and it may improve the development of the digestive tract and antioxidant functions of broilers without altering growth performance.

1. Introduction
Pine needles are traditionally consumed as a food and dietary supplement to promote human health in Asia, specifically in China and Korea (Chung et al. 1996; Lee et al. 2013). Pine needles have been used in Chinese traditional medicine to treat diseases, such as wind-cold-dampness arthralgia, traumatic injury, sleeplessness, eczema and oedema. Previous literature suggested that the medicinal properties of pine needles may be related to their bioactive substances such as carotene, terpenoids, phenolic compounds, tannins and alkaloids (Chung et al. 1996; Kim et al. 2002). The bioactive substances in pine needles may provide protection against oxidative DNA damage in non-cellular and cellular systems (Jeong et al. 2009). Earlier in vitro studies reported that the pine needles had anti-inflammatory and anti-bacterial abilities against E. coli, S. aureus and B. subtilis (Rohdewald 2002; Zeng et al. 2011). Moreover, phenolic extracts from pine trees were found to be a potent food source of natural phenolic anti-oxidants, which may improve anti-oxidant status in human and animals (Kähkönen et al. 1999; Touriño et al. 2005).

Currently, there is a growing public interest in the application of natural feed additives to improve poultry health and production. Pine needles powder (PNP) is one such natural feed additive that has potential to improve poultry production according to some preliminary research being conducted over the last decade. Kim (2011) provided initial evidence showing that PNP may improve poultry meat quality without reducing meat production and feed conversion rate (FCR), when up to 0.9% of PNP was included in the diet. Further, Kim et al. (2012) showed 2% supplementation of PNP in the poultry diet reduced the total serum cholesterol and increased phenols by 13% and 12%, respectively. The beneficial effect of PNP supplementation on broiler immunity is still a relatively new area for research and development. A recent study showed that supplementation of 1% PNP in the diet resulted in no change in immunological organ weights (i.e. spleen) in broilers (Park and Kim 2013). Nonetheless, the majority of research on PNP supplementation for poultry production was carried out with less than 2% of PNP in the diet. To the best of our knowledge, there is limited research investigating more than 2% PNP supplementation on broiler meat production, immunological organ weight indices, anti-oxidant and immunological status within one study. Therefore, in this study, 0%, 1%, 3%, 5% PNP supplementations were used to evaluate effect on growth performance, development of the digestive tract and blood biochemical profiles in broilers.

2. Materials and methods
2.1. Birds and diets
Eighty 21-day-old female Guangxi-Tiejiawma broiler chickens were purchased from a commercial hatchery (Kunming, China). The birds were randomly divided into four groups,
where each group had five replicates (four birds per replicate and fed within a pen). All birds were raised in wired cages for 35 days. The dietary treatments consisted of the basal diet as control (CT), 1% PNP supplement (PNP1), 3% PNP supplement (PNP3) and 5% PNP supplement (PNP5) (Table 1). The diets were formulated in accordance with National Research Council (NRC 1994) and Chinese Feed Standard of Chicken (CFSC 2004) recommendations. The dietary formulation was divided into two stages (3–6 weeks of age and >6 weeks of age) as shown in Table 1.

2.2. Management of birds

The experiment was conducted at the animal nutrition laboratory, Southwest Forestry University, Yunnan Province, China. All experimental procedures were conducted according to the Management Regulation for Laboratory Animals of China. The birds were vaccinated against Newcastle disease, Marek’s disease and infectious bursal disease prior to the study. Birds were kept in a deep litter housing system with concrete floors and pens covered with wood shavings to a depth of 5 cm. Feed and water were offered ad libitum throughout the study. Room temperature was maintained at 25 ± 0.5°C during the first week of the study and gradually decreased to 20 ± 0.5°C by the end of the third week, and then maintained at the temperature until the end of the study.

2.3. PNP preparation

Pine needles were collected locally from Pinus yunnanensis (Yunnan province, China). PNP was prepared following the procedures outlined by Kim et al. (2012), except with a lower drying temperature. Pine needles were stripped from the stems and then cut into pieces, thinly spread on a mat and air-dried at room temperature (15–25°C). The air-dried pine needles were then ground to powder and sieved through a 1.0-mm sieve. The chemical composition of pine needle (P. yunnanensis) were crude protein (7.56%), ether extract (6.58%), crude fibre (39.23%), ash (2.32%), nitrogen-free extract (37.54%), calcium (0.24%), phosphorous (0.13%) and gross energy (19.30 MJ/kg), which were determined by a method described in AOAC (Association of Official Analytical Chemists 1990). The PNP was added to feed according to dietary treatment; it was then mixed in a mixing machine before feeding to the birds.

2.4. Growth performance

During the experimental period, birds were fed ad libitum. The leftover feed was collected the next morning before feeding, air-dried and then weighed and subtracted from the weight of the feed provided on the previous day to calculate feed intake (FI). Body weight (BW) was taken at the beginning of the experiment and then on a weekly basis, in the morning at 08:00 hours before the feed was offered. The FI and BW per pen (i.e. four birds) were determined for calculation of average daily body weight gain (ADG) and FCR at weekly intervals. The FCR was calculated by dividing the total FI per pen by the total BW gain of the birds over the period of 35 days (21–56 days of age).

2.5. Blood sampling and analysis

For analyses of blood parameters, five birds were selected from each treatment (i.e. one bird per replicate) at the end of the experiment (on day 55). Blood samples were collected (5 mL per bird) from the wing vein. The serum was

Table 1. Ingredient composition of the experimental diets.

| Items                | Treatmenta (3–6 weeks of age) | Treatmentb (>6 weeks of age) |
|----------------------|-------------------------------|-------------------------------|
| Ingredient, %        | CT   | PNP1 | PNP3 | PNP5 | CT   | PNP1 | PNP3 | PNP5 |
| Corn                 | 63.39| 62.80| 61.50| 60.30| 66.82| 66.20| 64.90| 63.60|
| Soybean meal         | 23.46| 23.20| 22.80| 22.30| 22.96| 22.70| 22.30| 21.90|
| Fish meal            | 6.70 | 6.60 | 6.50 | 6.40 | 3.00 | 3.00 | 2.90 | 2.90 |
| Soybean oil          | 3.00 | 3.00 | 2.90 | 2.90 | 3.50 | 3.50 | 3.40 | 3.30 |
| PNP                  | 0    | 0    | 0    | 0    | 0    | 1.00 | 3.00 | 5.00 |
| Lysine               | 0    | 0    | 0    | 0    | 0.09 | 0.10 | 0.10 | 0.10 |
| Methionine           | 0.09 | 0.10 | 0.10 | 0.10 | 0.16 | 0.20 | 0.20 | 0.20 |
| CaHPO4               | 1.00 | 1.00 | 1.00 | 1.00 | 1.10 | 1.10 | 1.10 | 1.10 |
| Limestone            | 0.98 | 1.00 | 0.90 | 1.80 | 0.98 | 1.00 | 1.00 | 0.90 |
| Salt                 | 0.38 | 0.40 | 0.30 | 0.30 | 0.38 | 0.38 | 0.30 | 0.30 |
| Premixc              | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Total                | 100.00| 100.00| 100.00| 100.00| 100.00| 100.00| 100.00| 100.00|
| Calculated nutrient level, % |     |     |     |     |     |     |     |     |
| ME, MJ/kg            | 12.80| 12.73| 12.59| 12.46| 12.99| 12.91| 12.77| 12.63|
| Crude proteind       | 20.01| 19.88| 19.62| 19.37| 18.00| 17.89| 17.68| 17.47|
| Crude fibre          | 2.40 | 2.67 | 3.20 | 3.71 | 2.42 | 2.70 | 3.23 | 3.74 |
| Methionine           | 0.77 | 0.76 | 0.74 | 0.73 | 0.76 | 0.75 | 0.74 | 0.73 |
| Lysine               | 1.10 | 1.09 | 1.07 | 1.05 | 0.99 | 0.99 | 0.97 | 0.95 |
| Calcium              | 0.95 | 0.94 | 0.93 | 0.91 | 0.82 | 0.81 | 0.80 | 0.79 |
| Available phosphorus | 0.50 | 0.49 | 0.49 | 0.48 | 0.41 | 0.41 | 0.40 | 0.39 |

CT = control diet (0% PNP supplement); PNP1 = 1% PNP supplement; PNP3 = 3% PNP supplement; PNP5 = 5% PNP supplement.

The premix supplied the following per kilogram of diet: Vitamin A, 11023 IU; Vitamin D3, 1653.45 IU; Vitamin E, 5.51 mg; Vitamin K3, 41 mg; Vitamin B1, 0.55 mg; Vitamin B2, 1.1 mg; Folic acid, 0.28 mg; Pantothenic acid, 8.81 mg; Vitamin C, 8 mg; Manganese, 60 mg; Zinc, 40 mg; Iron, 80 mg; Copper, 8 mg; Iodine, 0.35 mg; Selenium, 0.15 mg.

Calculated on the basis of actual analyses of the raw materials.
separated using centrifugation at 3000 rpm for 10 min. The supernatant was gathered into sterilized Eppendorf tubes and then immediately stored at −20°C until further analysis. Albumin (ALB), triglyceride (TG), total cholesterol (TC), superoxide dismutase (SOD) and malondialdehyde (MDA) in the serum were determined using standard assay kits (Nanjing Jiancheng Bioengineering Institute, China).

### 2.6. Digestive tract measurements

At 55 days of age, 1 bird representing the mean BW of each replicate (5 birds per treatment) was selected and kept separately in temporarily prepared pens. Feed was withheld overnight before slaughter. They were then killed by severing the jugular vein to allow complete bleeding. The birds were de-feathered and eviscerated manually and cut into carcase parts in accordance with the procedures as outlined by Kim et al. (2012). The empty weights of proventriculus, gizzard, duodenum, small intestine, pancreas, caecum and rectum were recorded. In addition, the weight of the gizzard, duodenum, caecum and rectum were recorded and expressed as a percentage of slaughtered weight (i.e. weight indices).

### 2.7. Statistical analysis

The data were analysed using one-way analysis of variance with SPSS 11.5. The statistical model included the level of PNP supplementation as treatment effect. The averaged per pen data for all measurements were used for analysis and significant ($P < 0.05$) treatment means were separated by Duncan’s multiple range test.

#### 3. Results

The effects of PNP supplementation on the growth performance of broilers over 35 days of study are shown in Table 2. The FI and FCR were higher ($P < 0.05$) for the PNP3 and PNP5 treatments compared with CT. The final BW and BW gain had no significant differences between the groups.

The PNP supplementation increased gizzard and pancreas weights, but decreased small intestine weight ($P < 0.05$). Little change was observed in the duodenum weight among treatments ($P > 0.05$). The digestive tract weight indices of the gizzards and rectums increased with PNP supplementation ($P < 0.05$) (Table 3).

The ALB was comparatively higher in the PNP5 treatment than CT ($P < 0.05$). Diet inclusion of PNP3 increased SOD, while PNP1 and PNP5 treatments did not exhibit any noticeable changes. The MDA was comparatively lower in the PNP5 compared with CT ($P < 0.05$). The TG was significantly decreased ($P < 0.05$) in the PNP3 and PNP5 treatments compared with the CT treatment. The TC of the PNP1 and PNP3 were significantly lower than in the CT (Table 4).

#### 4. Discussion

### 4.1. Effects of PNP supplementation on broiler growth performance

BW gain results from this study were in agreement with the findings of Kim et al. (2012), who reported no differences in BW gain when 2% PNP was supplemented to broiler diets compared with no PNP supplementation. On average, a 969.3 g BW gain was achieved during the 35 days of study.

### Table 2. Effect of dietary PNP supplementation on growth performance of broilers (mean ± SE).

| Item                           | CT    | PNP1 | PNP3 | PNP5 |
|--------------------------------|-------|------|------|------|
| Initial BW (at 21 days, g/bird)| 250.8 ± 1.96 | 249.8 ± 7.21 | 252.8 ± 4.32 | 249.3 ± 5.50 |
| Final BW (at 56 days, g/bird)  | 1273.5 ± 33.65 | 1222.2 ± 32.86 | 1191.0 ± 24.99 | 1193.8 ± 42.24 |
| BW gain (21–56 days, g/bird)   | 1022.8 ± 34.12 | 972.4 ± 33.68 | 938.3 ± 27.41 | 944.6 ± 39.21 |
| FCR (21–56 days, g/g)          | 2.5 ± 0.08b | 2.6 ± 0.09b | 2.8 ± 0.07b | 2.8 ± 0.09b |

aData are the means of five replicates of four birds per pen. CT = control diet (0% PNP supplement); PNP1 = 1% PNP supplement; PNP3 = 3% PNP supplement; PNP5 = 5% PNP supplement.

### Table 3. Effect of the PNP supplementation on GIT weight and weight indices of broilers (mean ± SE).

| Item                        | CT    | PNP1 | PNP3 | PNP5 |
|-----------------------------|-------|------|------|------|
| Proventriculus, g           | 5.5 ± 0.27b | 4.6 ± 0.19b | 4.2 ± 0.12b | 6.1 ± 0.43b |
| Proventriculus of BW, %     | 0.5 ± 0.04b | 0.5 ± 0.03b | 0.5 ± 0.02b | 0.6 ± 0.04b |
| Gizzard, g                  | 16.6 ± 0.50b | 16.8 ± 1.44b | 17.8 ± 1.33b | 21.5 ± 1.01b |
| Gizzard of BW %             | 1.6 ± 0.05b | 1.7 ± 0.15b | 1.9 ± 0.14b | 2.1 ± 0.15b |
| Duodenum, g                 | 6.7 ± 0.56 | 8.0 ± 1.50 | 6.5 ± 0.32 | 6.6 ± 0.65 |
| Duodenum of BW, %           | 0.65 ± 0.05 | 0.82 ± 0.17 | 0.69 ± 0.04 | 0.63 ± 0.05 |
| Small intestine, g          | 36.7 ± 1.74b | 32.0 ± 2.44b | 32.5 ± 1.56b | 30.4 ± 1.06b |
| Small intestine of BW, %    | 3.6 ± 0.17b | 3.2 ± 0.21b | 3.5 ± 0.25b | 2.9 ± 0.07b |
| Pancreas, g                 | 3.1 ± 0.19b | 2.8 ± 0.29b | 2.8 ± 0.34b | 3.8 ± 0.30b |
| Pancreas of BW, %           | 0.3 ± 0.02 | 0.3 ± 0.03 | 0.3 ± 0.03 | 0.4 ± 0.03 |
| Cecum, g                    | 5.1 ± 0.24b | 5.4 ± 0.46b | 5.1 ± 0.29b | 6.4 ± 0.48b |
| Cecum of BW, %              | 0.5 ± 0.02 | 0.5 ± 0.06 | 0.5 ± 0.04 | 0.6 ± 0.03 |
| Rectum, g                   | 1.7 ± 0.25d | 4.9 ± 0.33b | 3.6 ± 0.10b | 2.5 ± 0.15b |
| Rectum of BW, %             | 0.17 ± 0.03d | 0.49 ± 0.03b | 0.38 ± 0.01b | 0.24 ± 0.02b |

aData are the means of five birds per treatment slaughtered. CT = control diet (0% PNP supplement); PNP1 = 1% PNP supplement; PNP3 = 3% PNP supplement; PNP5 = 5% PNP supplement.

bMeans within a row with no common letters are significantly different ($P < 0.05$).
which was equivalent to a 27.7 g daily BW gain. It is important to note that a higher BW gain and lower FCR have often been reported, compared with the current study (Toghyani et al. 2012), when diets of similar nutrient level was offered to broilers. This difference in growth performance may be due to the specific breed that was used in this study, leading to the differences in FI and nutrient metabolism. For example, when 1% and 2% PNP were offered to Hubbard broilers by Kim et al. (2012), it led to a higher FI and BW gain (3135 and 1825 g/bird, respectively) in contrast to the results of our current study. Though no difference in BW gain was observed between groups, a higher FCR in PNP3 and PNP5 was found compared with CT. This indicates that the PNP3 and PNP5 groups required more feed to achieve the same level of BW gain when compared with CT. The reason for this is unknown, and further study is needed to understand the difference in FCR induced by PNP supplementation in the diet.

### 4.2. Effects of PNP supplementation on the gastrointestinal tract (GIT)

The adoption of crude fibre values to calculate crude fibre content for treatments in the current study indicated that the CT, PNP1, PNP3 and PNP5 groups contained 2.4%, 2.7%, 3.2% and 3.7% crude fibre, respectively. The gizzard was known to respond rapidly to changes in fibre content in the diet (Farner 1960), while dietary fibre has been considered as a diluent in diets (Rougière and Carré 2010) with negative connotations in relation to voluntary FI and nutrient digestibility (Mateos et al. 2002). On the other hand, it has been recently demonstrated that the inclusion of moderate amounts of adequate fibre sources in the diet can improve digestive organ development (González-Alvarado et al. 2007) and increase bile acids and enzymatic secretion. These changes may result in improvements in nutrient digestibility, growth performance and GIT health. Several studies showed that birds responded quickly to changes in dietary fibre content through modifying the intestinal length and weight of the organs, as well as the rate of passage through the different segments of the GIT (Mateos et al. 2012). Jiménez-Moreno et al. (2009) showed that diets with increased fibre content can improve the relative weight gain in the gizzard and gizzard contents (González-Alvarado et al. 2008; Amerah et al. 2009). In general, the inclusion of fibre in the diet may increase the relative weight of different GIT segments, but the effect varies depending on the type and level of fibres, and the segments of the GIT (Jiménez-Moreno et al. 2009).

### 4.3. Effects of PNP supplementation on blood biochemical profile

The SOD and MDA are important anti-oxidative components in serum, which play key roles in the fundamental functions of self-defence mechanisms in animals (Rajput et al. 2013). Zhou et al. (2013) reported that pine needles procyanidine, which was one of the main active components in pine needles extract, can increase SOD activity and reduce blood MDA in rats. SOD is a member of the first defence line in the anti-oxidant system of living cells; it can prevent the production of free radicals. Superfluous free radicals may damage protein and nucleic acids, and induce lipid peroxidation to produce large amounts of MDA. It damages cells and results in disequilibrium of the internal environment and diseases (Liu et al. 2011). Previous studies indicated that the major active ingredients, such as flavonoids and phenolic compounds in pine needles, have anti-microbial, anti-mutagenic, anti-oxidant and anti-tumoural functions (Choi et al. 1997; Choi et al. 2002; Kwak et al. 2006), which helps to explain the increased SOD and reduced MDA levels in this study when PNP was supplemented in the diet.

In the present study, a significant decrease in serum TG and TC was observed in PNP-supplemented treatments compared with CT. These results were in accordance with the findings of Kim et al. (2012), who reported that levels of total serum cholesterol and low-density lipoprotein cholesterol decreased significantly with PNP supplementation in the poultry diets. Previous studies showed that rats fed with a PNP-supplemented diet had lower TC levels than those fed without PNP (Lee and Choi 2000; Zhang et al. 2008). The consistently observed lower TC and TG from dietary PNP supplementation in animals might partially be explained by the fact that PNP can improve the anti-oxidant capacity, reduce enzymatic activity and suppress oxidative free radicals (Kähkönen et al. 1999; Chowdhury et al. 2002).

### 5. Conclusion

The PNP supplementation increased the relative weight of gizzards and rectums. Supplementation of PNP in broiler diets had a beneficial effect on serum TG and TC. In addition, inclusion of 3% and 5% PNP improved the anti-oxidant functions in broilers. However, the BW and BW gains of broilers were not influenced

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**Table 4. Effect of the PNP supplementation on blood biochemical profile of broilers (mean ± SE).**

| Items             | CT         | PNP1       | PNP3       | PNP5       |
|-------------------|------------|------------|------------|------------|
| Albumin, g/dL     | 1.3 ± 0.05b| 1.5 ± 0.13b| 1.4 ± 0.01b| 1.6 ± 0.11b|
| Total protein, g/dL| 2.3 ± 0.42 | 1.9 ± 0.16 | 1.8 ± 0.15 | 2.4 ± 0.07 |
| Triglyceride, mmol/L| 0.5 ± 0.11b| 0.4 ± 0.03b| 0.2 ± 0.02b| 0.3 ± 0.01b|
| Total cholesterol, mmol/L| 2.1 ± 0.04b| 1.7 ± 0.09b| 1.8 ± 0.08b| 2.1 ± 0.06b|
| Superoxide dismutase, U/mL | 106.8 ± 2.98b| 111.8 ± 5.65b| 142.8 ± 11.58b| 117.1 ± 11.53b|
| Malondialdehyde, nmol/mL | 18.0 ± 0.33b| 18.1 ± 0.14b| 18.2 ± 0.51b| 16.7 ± 0.08b|
| Total protein, g/dL| 2.3 ± 0.42 |
| Albumin, g/dL     | 1.3 ± 0.05b| 1.5 ± 0.13b| 1.4 ± 0.01b| 1.6 ± 0.11b|
| Total protein, g/dL| 2.3 ± 0.42 | 1.9 ± 0.16 | 1.8 ± 0.15 | 2.4 ± 0.07 |
| Triglyceride, mmol/L| 0.5 ± 0.11b| 0.4 ± 0.03b| 0.2 ± 0.02b| 0.3 ± 0.01b|
| Total cholesterol, mmol/L| 2.1 ± 0.04b| 1.7 ± 0.09b| 1.8 ± 0.08b| 2.1 ± 0.06b|
| Superoxide dismutase, U/mL | 106.8 ± 2.98b| 111.8 ± 5.65b| 142.8 ± 11.58b| 117.1 ± 11.53b|
| Malondialdehyde, nmol/mL | 18.0 ± 0.33b| 18.1 ± 0.14b| 18.2 ± 0.51b| 16.7 ± 0.08b|

aData are the means of five birds per treatment. CT = control diet (0% PNP supplement); PNP1 = 1% PNP supplement; PNP3 = 3% PNP supplement; PNP5 = 5% PNP supplement.

bMeans within a row with no common letters are significantly different (P < .05).
by PNP supplementation. This study demonstrated the potential benefit of supplementing PNP in broiler diets on antioxidative and immunological status without negative impact on growth performance. Further research is necessary to strengthen current results and findings with long-term study.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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