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Effects of Dietary Inclusion of *Angelica gigas* Nakai root extract on the Growth Performance, Hematological and Serum Biochemical Parameters in Broilers

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ABSTRACT

The study aimed to evaluate the impact of dietary supplementation with *Angelica gigas* Nakai (AGN) root extract on growth performance, hematological indices, and serum biochemical parameters in broiler chickens. A total of 320 straight-run Cobb broiler chicks from a commercial hatchery were distributed among four treatment groups: Basal diet (BD) as the Control; Treatment 1 (T1): BD + 2 g/kg AGN; Treatment 2 (T2): BD + 4 g/kg AGN; and Treatment 3 (T3): BD + 8 g/kg AGN), each comprising eight replicates with 10 birds per replicate. The supplementation of AGN resulted in dose-dependent improvements ($P < 0.05$) in body weight, gain, and feed efficiency. On both day 21 and day 35, increasing AGN dosage in the diet led to a significantly higher ($P < 0.05$) values of red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), and packed cell volume (PCV). By day 21, AGN supplementation dose-dependently decreased ($P < 0.05$) serum alkaline phosphatase (ALP), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), urea, and creatinine levels. Meanwhile, AGN dose escalation correlated with a notable increase ($P < 0.05$) in serum total protein (TP), albumin, and globulin levels. On day 35, increasing AGN levels led to a significant reduction ($P < 0.05$) in serum AST and ALT activity, along with lowered serum glucose, cholesterol, triglycerides, urea, and creatinine levels. In conclusion, AGN supplementation enhanced growth performance and positively influenced hematological indices and serum biochemistry profiles in broiler chickens. The study confirms the safe and effective utilization of AGN at an 8 g/kg (0.8 %) feed additive dosage to optimize broiler performance. These findings provide valuable insights into the potential benefits of AGN in poultry nutrition.

Keywords: Angelica gigas Nakai, Blood values, Broiler chicken, Feed additive, Serum biochemistry

Introduction

The ban on the inclusion of antimicrobial growth promoters (AGPs) in animal feed has spurred a surge in research aimed at discovering viable alternatives. A growing interest has emerged in the exploration of phytobiotics as potential substitutes for AGPs (Dibner and Richards, 2005; Mountzouris *et al.*, 2011). Phytobiotics have gained popularity as feed additives due to their positive impact on gut health, immunity, and growth. Phytobiotics offer benefits such as stimulating feed intake, enhancing enzyme secretion, boosting immunity, and exerting antibacterial, coccidiostatic, anthelmintic, antiviral, or anti-inflammatory effects (Beford, 2000).

Angelica gigas Nakai (AGN), a traditional medicinal plant native to Korea, Japan, and China (Shehzad *et al.*, 2018), has been extensively utilized for treating conditions like amenorrhea, dysmenorrhea, infections, menopause, pain, injuries, articular rheumatism, and migraine headaches (Fontamillas *et al.*, 2019). Notable coumarin compounds found in AGN include decursin (D), decursinol angelate (DA), nodakenin, umbelliferon, and marmesin (Ko *et al.*, 2020). AGN exhibits pharmacological properties including anticancer (Zhang *et al.*, 2012), antioxidant (Lee, 2021), anti-inflammatory (Cho *et al.*, 2015), and neuroprotective (Sowndhararajan and Kim, 2017) effects. Numerous studies have suggested application of herbal plants as feed additives to increase health, productivity, and/or high-quality product in livestock. However, despite the beneficial activity of AGN, limited in vivo studies have been conducted on the effects of AGN on broilers.

Given its diverse range of effects, researchers have proposed AGN as a promising natural feed additive, offering a potentially safe alternative to antibiotic growth promoters in poultry production. This study aims to assess the impact of varying levels of AGN extract on the growth performance, as well as on hematological and serum biochemical indicators in broiler chickens.

Materials and Methods

Plant material collection, extraction and preparation

The powdered roots of *Angelica gigas* Nakai (AGN) were obtained from a Korean Oriental Herb store in Anseong City, South Korea. The hot water extract was prepared regarding methods by Park *et al.* (2015) as follows: One kilogram of the dried AGN powdered root was soaked in 10 liters of water and heated to 90℃ for 4 hours, with mixing every 30 minutes. The mixture was then cooled to room temperature, and the suspension was filtered. The filtered aqueous extract was subjected to freeze-drying to obtain a concentrated AGN root extract powder.

Experimental birds and diets

The experimental procedures and animal handling protocols were approved by the Institutional Animal Care and Use Committee of Hankyong National University. A total of 320 mixed sex day-old straight-run Cobb broiler chicks were procured from a commercial hatchery and then randomly allocated into four treatment groups with Completely Randomize Design (CRD), each comprising eight replicates with 10 birds per replicate. The birds were raised in conventional housing and were exposed to environmental temperature and relative humidity ranging from 26.4 to 35.2°C and 55 to 88%, respectively. All groups received the same booster diet with *ad libitum* access to feed and water. The 4 groups include Basal diet (BD) as the control; treatment 1 (T1): BD + 2 g/kg AGN; treatment 2 (T2): BD + 4 g/kg AGN; and treatment 3 (T3): BD + 8 g/kg AGN. Starter diets were given from day 11-21 and finisher feeds were fed from day 22-35. Diets were formulated to contain 21% and 19% CP and metabolizable energy (ME) of 3050 and 2950 kcal/kg, for broiler starter and broiler finisher diets, respectively (Table 1). The basal diets were formulated based on NRC recommendations (NRC, 1994).

Growth performance

The birds' initial body weights were recorded upon arrival (40.0 ± 0.4 g), and subsequently, their body weight (BW) and feed intake (FI) were measured weekly throughout the entire experiment. This data was used to calculate weight gain (WG) and feed conversion ratio (FCR). Incidents of mortality were recorded as they occurred.

Blood indicators and biochemical indices

On days 21 and 35, eight broiler chickens were randomly selected from each experimental group. Blood samples were collected from each bird via the brachial vein to analyze hematological indicators and serum biochemical indices. For the analysis of hematological blood indicators, blood samples were collected in K3 EDTA tubes (BD Vacutainer®, Franklin Lakes, NJ, USA). Within 2 hours of collection, hematological parameters including red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) were measured using a hematology analyzer (ABC Vet®, ABX Diagnostics, Montpellier, France).

Table 1. Ingredients and nutrients composition of starter and finisher diets

Ingredients (%)	Starter	Finisher
Corn	55.0	60.0
Soy bean meal	28.0	24.0
Fish and bone meal	6.0	5.0
Palm kernel cake	10.3	15.3
NaCl	0.25	0.25
Premix	0.25	0.25
Methionine	0.1	0.1
Lysine	0.1	0.1
Nutrients		
Metabolizable energy (Kcal/kg)	3050	2950
Crude protein (%)	21.41	19.85

Premix for Starter diet (per kg diet): Vitamin A 15,000 I.U, Vitamin D3 13,000 I.U, thiamin 2 mg, Riboflavin 6 mg, pyridoxine 4 mg, Niacin 40 mg, cobalamine 0.05 g, Biotin 0.08 mg, choline chloride 0.05 g, Manganese 0.096 g, Zinc 0.06 g, Iron 0.024 g, Copper 0.006 g, Iodine 0.014 g, Selenium 0.24 mg, Cobalt 0.024 mg and antioxidant 0.125 g.

For serum biochemical analyses, blood samples were collected in plain tubes and centrifuged at 3000×g for 15 minutes to obtain serum, which was then stored at −20°C until further analysis. The serum was assessed for biochemical indices, including aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), urea, creatinine, triglycerides, cholesterol, glucose, total protein (TP), albumin, and globulin. Additionally, serum electrolytes, including sodium (Na), chloride (Cl), and potassium (K), were measured using commercial kits and an autoanalyzer (Microlab 300 Semiautomated Biochemical Analyzer).

Statistical analysis

The data were analysed using the General Linear Model with $P \leq 0.05$. The collected data were subjected to Analysis of Variance (ANOVA). Means of the treatment groups were compared using the Tukey's Honest Significant Difference Test.

Results and Discussion

Growth parameters

The growth parameters observed in broiler chickens are summarized in Table 2. On day 21, the treatment groups showed a significantly higher weight gain and improved FCR compared to the control group ($p < 0.05$), while feed intake remained unaffected ($p > 0.05$) by AGN supplementation. By day 35, the group supplemented with 8g/kg AGN

Table 2. Effect of different levels of AGN on broiler chicken growth performance

Production days	C	T1	T2	T3
Body weight				
0	40.5 ± 0.22	40.53 ± 0.35	40.44 ± 0.41	40.40 ± 0.36
10	302.53 ± 5.14	303.45 ± 5.12	305.35 ± 5.12	304.04 ± 4.17
21	906.48 ± 3.48^{c}	936.03 ± 6.84^{b}	944.38 ± 10.22^{ab}	958.75 ± 9.05^{a}
35	1697.85 ± 9.24^{d}	1735.6 ±7.63^{c}	1777.6 ± 14.82^{b}	1815.8 ± 13.02^{a}
Feed intake				
1-10	304.23 ± 10.71	303.7 ± 6.54	303.38 ± 5.99	303.45 ± 7.65
11-21	974.65 ± 10.68	980.13 ± 9.9	978.38 ± 8.13	976.12 ± 8.41
21-35	1977.38 ± 11.53	1978.63 ± 10.97	1980.38 ± 12.5	1979.63 ± 13.3
1-35	3255.65 ± 14.69	3261.53 ±4.74	3260.73 ± 21.77	3259.88 ± 27.9
Weight gain				
1-10	262.03 ± 5.12	262.92 ± 5.23	264.91 ± 5.08	263.64 ± 8.25
11-21	603.95 ± 8.21^{b}	632.58 ± 10.33^{a}	639.03 ± 10.31^{a}	654.71 ± 11.65^{a}
21-35	791.37 ± 12.16^{c}	799.57 ± 10.48^{c}	833.22 ± 12.61^{b}	857.05 ± 13.73^{a}
1-35	1657.35 ± 11.23^{d}	1695.07 ± 9.73^{c}	1737.16 ± 11.56^{b}	1775.4 ± 13.49^{a}
FCR				
1-10	1.16 ± 0.05	1.15 ± 0.04	1.14 ± 0.05	1.15 ± 0.4
11-21	1.61 ± 0.08^{c}	1.54 ± 0.03^{b}	1.53 ± 0.05^{b}	1.49 ± 0.05^{a}
22-35	2.49 ± 0.07^{c}	2.47 ± 0.03^{c}	2.37 ± 0.08^{b}	2.30 ± 0.06^{a}
1-35	1.96 ± 0.05^{d}	1.92 ± 0.05^{c}	1.87 ± 0.07^{b}	1.83 ± 0.08^{a}
Mortality (%)	7.5	3.75	1.25	1.25

^{a,b,c,d} Values within the same row with different superscripts are significantly different (P < 0.05).
FCR: feed conversion ratio; C: control; Basal diet; T1: basal diet+ AGN 2 g/kg; T2: basal diet+ AGN 4 g/kg; T3: basal diet+ AGN 8 g/kg.

exhibited a significant increase in weight gain and lower FCR compared to the control and other treatment groups (P < 0.05). Feed intake, however, showed no significant changes (P > 0.05) due to AGN supplementation. When considering the overall growth performance from day 1 to 35, the T3 group demonstrated the highest weight gain (p < 0.05) and the lowest FCR (P < 0.05) compared to the control and other treatment groups. Additionally, there was a linear increase in weight gain (P < 0.05) and a linear decrease in FCR (P < 0.05) with increasing AGN supplementation on day 35 and for the overall growth performance period.

Supplementation of AGN enhanced the growth performance of broiler chickens. The addition of AGN in the treatment groups significantly enhanced the weight gain and FCR of broilers at day 21, 35 and during the whole duration of the experiment. Aroche *et al.* (2018) and Iwinski *et al.* (2023) reported that the dietary inclusion of natural substances has beneficial effects on broilers which can improve production parameters like body weight gain and FCR.

While numerous studies have highlighted the growth-promoting effects of phytobiotics, the precise mechanisms underlying their role as animal growth promoters remain incompletely understood. Valenzuela-Grijalva *et al.* (2017) proposed key mechanisms through which phytobiotics may induce growth promotion: enhancing feed status and consumption through improved flavor and palatability; enhancing nutrient digestion and absorption by augmenting intestinal functions; and exerting direct and indirect anabolic activity on target tissues through the activation of endocrine and antioxidative defense systems. Dehydropyranocoumarins, decursin and decursinol angelate are principal secondary metabolites in AGN, and the amounts exceed 3%, 2.5% of the dried root, respectively (Ahn *et al.*, 2008) may enhance nutrient digestion and absorption by augmenting intestinal functions. The components within AGN extract may contribute to these effects, as evidenced by the evident growth-promoting effect observed in broilers supplemented with AGN. Additional research is needed to gain a more comprehensive understanding of the precise mechanisms behind AGN's growth-promoting effects in broiler chickens.

Haematological blood indicators

Table 3 and 4 present data showcasing the effects of varying AGN doses on the haematological blood indicators of broilers on day 21 and day 35, respectively. On day 21, all AGN-supplemented groups showed significant improvements (P < 0.05) in RBC counts compared to the control group. Additionally, PCV, Hb, and WBC counts were also significantly higher in the AGN-supplemented groups compared to the control group. However, the dietary supplementation of AGN did not have a significant effect (P > 0.05) on MCV, MCH, and MCHC.

On day 35, birds supplemented with 8g/kg AGN showed significant increases (P < 0.05) in RBC, PCV, Hb, and WBC compared to the other groups. However, AGN supplementation did not have a significant influence (P > 0.05) on MCV, MCH, and MCHC values in the experimental broiler chickens.

Table 3. Effect of different levels of AGN on haematological blood indicators of broilers at day 21

Parameters	C	T1	T2	T3
RBC (x 10^6)	2.33^{d}	2.54^{c}	2.77^{b}	2.98^{a}
PCV (%)	29.18^{b}	32.14^{a}	32.96^{a}	32.11^{a}
Hb (g/dL)	9.27^{b}	10.47^{a}	10.65^{a}	10.51^{a}
MCV (fL)	127.33	123.32	126.04	125.11
MCH (pg)	38.14	38.17	39.22	38.53
MCHC (%)	30.02	31.22	33.06	32.58
WBC (x 10^6)	21.15^{b}	22.03^{b}	24.11^{a}	24.63^{a}

^{a,b,c,d} Values within the same row with different superscripts are significantly different (P < 0.05).
RBCs: red blood cells; PCV: packed cell volume; Hb: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBCs: white blood cells; C: control; basal diet; T1: basal diet+ AGN 2 g/kg; T2: basal diet+ AGN 4 g/kg; T3: basal diet+ AGN 8 g/kg.

Table 4. Effect of different levels of AGN on haematological blood indicators of broilers at day 35

Parameters	C	T1	T2	T3
RBC (x 10^6)	2.47^c	2.62^b	2.66^b	2.84^a
PCV (%)	31.04^b	33.32^a	33.73^a	33.57^a
Hb (g/dL)	9.71^c	10.65^c	11.03^b	12.65^a
MCV (fL)	126.55	125.04	126.18	124.48
MCH (pg)	38.11	37.65	38.02	37.59
MCHC (%)	30.33	31.47	32.06	31.74
WBC (x 10^6)	22.82^c	23.09^c	24.15^b	25.41^a

[a,b,c] Values within the same row with different superscripts are significantly different (P < 0.05).

RBCs: red blood cells; PCV: packed cell volume; Hb: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; WBCs: white blood cells; C: control; basal diet; T1: basal diet+ AGN 2 g/kg; T2: basal diet+ AGN 4 g/kg; T3: basal diet+ AGN 8 g/kg.

Haematology blood indicators in experimental animals play a crucial role in assessing the potential toxic effects of supplemented compounds or plant extracts. These tests are invaluable tools for understanding the physiological and pathological conditions of organisms (Oloruntola *et al.*, 2016). In this study, the haematological blood indicators were observed to fall within normal ranges (Thrall *et al.*, 2012). Normal range of haematological blood in broiler are RBC 2.5 – 3.5 (x 10^6); PCV 35 - 55%; Hb 10 – 14 g/dL; MCV 104 – 140 fL; MCH 33 – 47 pg; MCHC 30.2 – 36.2% (Baudouin *et al*., 2021). These normal values suggest that the broiler chickens supplemented with AGN were adequately nourished and displayed a robust immune status.

The present study revealed significant elevations in RBC and WBC counts, as well as in Hb and PCV values. These findings align with the results of Reis *et al.* (2018), who observed increased erythrocyte counts and haemoglobin levels in broiler chickens supplemented with phytobiotics compared to the control group. Similarly, Krauze *et al.* (2020), reported enhanced immune system function and improvements in RBC and Hb parameters in broiler chickens when supplemented phytobiotics. Another study by Gilani *et al.* (2018), explored the use of organic acids and flavonoid-rich phytobiotics as alternatives to AGPs in poultry feed, and observed significant increases in RBC and WBC counts, along with elevated PCV levels in broiler chickens.

The current study did not yield statistically significant results (P > 0.05) for MCV, MCH, or MCHC in the experimental broiler chickens. These outcomes are in line with the findings of Oghenebrorhie and Oghenesuvwe (2016), who similarly reported non-significant results for MCV, MCH, and MCHC in broilers supplemented with Moringa oleifera leaf meal (MOLM). The data indicates that AGN supplementation in broilers led to improvements in RBC, WBC, PCV, and Hb levels, suggesting enhanced utilization of dietary nutrients and an improved immune status.

Biochemical indicators

The data in Tables 5 and 6 illustrate the impact of varying doses of AGN on different biochemical indicators in broilers on day 21 and day 35, respectively. On day 21, there was a notable decrease (P < 0.05) in AST and ALT activity in the serum of birds with increasing levels of AGN supplementation, compared to the control group. Conversely, the inclusion of AGN in the diet did not affect the serum ALP activity (P > 0.05). However, there was a significant rise (P < 0.05) in total protein, albumin, and globulin levels with AGN supplementation in the broiler diet. Additionally, the addition of AGN led to a significant decrease (P < 0.05) in serum levels of cholesterol, triglycerides, potassium, urea, and creatinine compared to the control.

Table 5. Effect of different levels of AGN on biochemical indicators of broilers at day 21

Parameters	C	T1	T2	T3
ALP (U/L)	1661.6	1659.7	1660.4	1661.1
AST (U/L)	225.5^a	205.3^b	198.7^b	195.3^b
ALT (U/L)	8.24^a	6.85^b	6.87^b	5.65^c
Total Protein (g/dL)	2.421^c	2.470^c	2.667^b	2.784^a
Albumin (g/dL)	1.175^b	1.261^a	1.251^a	1.257^a
Globulin (g/dL)	1.231^b	1.237^b	1.407^a	1.454^a
Glucose (mmol/L)	15.62	15.47	15.52	15.55
Cholesterol (mmol/L)	3.43^a	2.93^b	2.89^b	2.67^c
Triglycerides (mmol/L)	1.09^a	0.95^b	0.94^b	0.94^b
Na (mmol/L)	133.1	133.7	134.2	132.6
K (mmol/L)	5.44^a	4.63^b	4.38^b	4.42^b
Cl (mmol/L)	115.8	116.6	109.5	115.4
Urea (mmol/L)	0.48^a	0.40^b	0.35^c	0.34^c
Creatinine (mmol/L)	31.22^a	30.05^a	24.77^b	24.16^b

[a,b,c] Values within the same row with different superscripts are significantly different (P < 0.05).

ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; C: control; basal diet; T1: basal diet+ AGN 2 g/kg; T2: basal diet+ AGN 4 g/kg; T3: basal diet+ AGN 8 g/kg.

Table 6. Effect of different levels of AGN on Biochemical indicators of Broilers at day 35

Parameters	C	T1	T2	T3
ALP (U/L)	1788.2	1783.3	1822.5	1811.56
AST (U/L)	214.3^a	195.5^b	189.1^c	176.2^d
ALT (U/L)	6.84^a	7.05^a	6.83^a	6.05^b
Total Protein (g/dL)	2.327^d	2.440^c	2.563^b	2.673^a
Albumin (g/dL)	1.114^b	1.231^a	1.221^a	1.237^a
Globulin (g/dL)	1.201^b	1.211^b	1.337^a	1.354^a
Glucose (mmol/L)	14.36	14.24	14.05	13.95
Cholesterol (mmol/L)	3.22^a	2.97^b	2.95^b	2.88^c
Triglycerides (mmol/L)	1.07^a	0.94^b	0.80^c	0.77^c
Na (mmol/L)	127.3^c	133.23^b	137.18^a	137.65^a
K (mmol/L)	5.12	5.03	4.88	4.92
Cl (mmol/L)	105.3	106.1	105.7	105.5
Urea (mmol/L)	0.46^a	0.41^b	0.37^c	0.37^c
Creatinine (mmol/L)	28.52^a	28.35^a	24.27^b	24.06^b

[a,b,c,d] Values within the same row with different superscripts are significantly different (P < 0.05).

ALP: alkaline phosphatase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; C: control; basal diet; T1: basal diet+ AGN 2 g/kg; T2: basal diet+ AGN 4 g/kg; T3: basal diet+ AGN 8 g/kg.

On day 35, increasing AGN supplementation led to a significant decrease (P < 0.05) in AST and ALT activity in the serum. However, AGN supplementation had no significant effect (P > 0.05) on ALP activity in experimental broilers. The serum levels of TP were notably increased (P < 0.05) in AGN supplementation groups compared to the control group. Additionally, the levels of albumin and globulin in the AGN treated groups were significantly elevated (P < 0.05) compared with the control group. Moreover, serum levels of cholesterol, triglycerides, and urea showed a significant decrease (P < 0.05) with increasing AGN supplementation levels compared to the control group. Its affect due to the major constituents of AGN, coumarins (decursin and decursinol angelate) has anti-adipogenic effects and has been reported to possess anti-obesity effects (Park *et al.*, 2020).

Birds supplemented with AGN exhibited lower serum creatinine levels compared with the control group. However, AGN supplementation did not affect (P > 0.05) the serum levels of potassium, chloride, and glucose in the experimental birds. Serum biochemical indicators offer valuable insights into the body's nutrient metabolism and can signal potential changes influenced by both internal and external factors (Liu *et al.*, 2015; Hu *et al.*, 2016). The liver, being one of the largest and most vital organs, plays a crucial role in detoxification, metabolism, and the elimination of both endogenous and exogenous substances (Paul *et al.*, 2016). The activity levels of ALP, AST, and ALT serve as diagnostic indicators for assessing hepatotoxicity. Elevated levels of AST and ALT typically indicate pathological manifestations or toxicity (Toghyani *et al.*, 2011), making them specific indicators of liver injury or dysfunction (Alhidary *et al.*, 2016). In this study, increasing the level of AGN supplementation led to a significant decrease (P < 0.05) in serum AST and ALT activity, underscoring the potential hepatoprotective nature of AGN. This is attributed to the notable concentration of decursin and decursinol angelate in AGN, which is believed to confer hepatoprotective properties (Kim *et al.*, 2020).

Serum proteins are primarily synthesized in the liver, and their concentrations are indicative of hepatocyte function. A decrease in serum protein levels (TP, albumin, and globulin) may be associated with hepatic insufficiency, malnutrition, active inflammation, or recurrent infections leading to immune deficiency (Tothova *et al.*, 2016). These serum protein levels are crucial indicators for assessing the health status of birds. Broiler chickens undergo rapid growth during their short growing period, leading to the rapid accumulation of structural proteins in body tissues. This rapid growth necessitates intensive erythropoiesis and hemoglobin synthesis, potentially resulting in increased globulin production, which can impact serum protein concentrations in growing chickens (Roman *et al.*, 2009; Tothova *et al.*, 2019). In this study, the inclusion of AGN significantly increased TP, albumin, and globulin levels compared to the control group.

In birds, the typical reference range for serum glucose falls within 200 to 500 mg/dL (Thrall *et al.*, 2012). The current study observed that serum glucose concentrations were not significantly affected by AGN supplementation in the experimental chickens; however, numerically higher values were recorded in the control group in comparison to the supplemented groups. These findings align with a study by Abudabos *et al.* (2018) which reported no significant difference in serum glucose levels among experimental broilers supplemented with phytogenic feed additives.

The serum concentrations of cholesterol and triglycerides serve as indicators of lipid metabolism (He *et al.*, 2015). The current study's findings revealed that, on day 21 and 35, AGN supplementation showed notable reductions in the levels of both triglycerides and cholesterol compared to the control group. These findings align with Vispute *et al.* (2019), who reported that phytobiotics, significantly decreased serum triglyceride levels in the final growth phase of broilers. Similarly, our results are supported by Zhang *et al.* (2017), who found that supplementing chickens' diets with Chinese bayberry leaves significantly lowered serum concentrations of triglycerides and cholesterol. Additionally, Zhou *et al.* (2015) and Niu *et al.* (2019) reported that dietary supplementation of broilers with fermented *Ginkgo biloba* rations and fermented *Ginkgo biloba* leaves resulted in significant decreases in serum levels of triglycerides and cholesterol. Lee *et al.* (2019) reported that AGN as a lipolytic supplement showed the down-regulation of proliferation and differentiation in chicken myoblast cells, and affected the metabolic pathways of glycolysis and fatty acids by suppressing fat accumulation followed by amelioration of hepatic steatosis and hyperlipidemia and activating glycolysis.

Maintaining electrolyte balance is crucial for acid-base equilibrium and has a direct impact on the performance of broiler birds. Any disruption in the acid-base balance can lead to dysfunction in biochemical and metabolic pathways, ultimately affecting the physiological well-being of the birds. Minerals such as Na, K, and Cl are essential for maintaining acid-base and osmotic balance, as well as facilitating substance transport across cell membranes. Consequently, they play pivotal roles in the metabolism of living organisms. Any imbalance in these minerals can directly influence acid-base equilibrium, metabolic functions, and consequently, the performance of broiler chickens (Olanrewaju *et al.*, 2007). In the current study, the levels of Na, K, and Cl were within the normal range.

The kidneys are the second target organs susceptible to injury from metabolic dysfunctions. Kidney function serves as a crucial indicator for assessing the potential toxicity of any compound. The state of kidney function can be assessed by monitoring changes in serum levels of urea and creatinine. Elevated creatinine levels are indicative of reduced glomerular filtration, indicating kidney impairment (Rhiouani *et al.*, 2008), while an increased serum urea level may signal cardiac and renal tissue injuries. The current study's findings demonstrated a significant decrease in serum levels of creatinine and urea with increasing AGN dosage. These results suggest that AGN supplementation did not have any detrimental effects on kidney function. Our findings are in line with several studies on phytobiotic supplementation in broiler chickens, including the work by Rubio *et al.* (2019) and Ahmad *et al.* (2018).

Conclusion

The current study revealed that incorporating AGN supplementation in the diet of broiler chickens led to enhanced growth performance, improved hematological blood indicators, and favorable serum biochemical attributes, with no adverse effects. Moreover, broiler chickens fed a diet supplemented with AGN at a rate of 8 g/kg exhibited the most promising outcomes in terms of growth performance, as well as in the tested blood and serum biochemical parameters. Therefore, AGN supplementation of 8 g/kg stands as the appropriate dosage for serving as an alternative feed additive for broiler chickens.

Conflict of interest

We declare that there is no conflict of interest with any financial organization regarding the materials discussed in the manuscript.

Author's contribution

JFC, and SGH conceived and designed the experiments. JFC performed the experiments. All authors analyzed the data. LP and JF writing of the manuscript. All authors critically revised the manuscript and approved the final version.

Ethics approval

All the animal care management and procedures were approved by the Hankyong National University Animal Care and Use Committee.

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