Influence of alfalfa meal, as a source of dietary fibre, on growth performance, development, pH of gastrointestinal tract, blood biochemical profile, and meat quality of broilers

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ABSTRACT
In this study, 192 female broilers (21 days old) were allocated to four dietary treatments: a control diet (CT), and three experimental diets containing 25 g/kg alfalfa meal [AM25], 50 g/kg alfalfa meal [AM50], and 75 g/kg alfalfa meal [AM75]. Final body weight and average daily feed intake of birds were significantly higher in alfalfa supplemented birds, with a linear response to alfalfa inclusion seen for average daily gain (P < 0.05). Serum triglycerides were comparatively lower in AM50 group than in CT group (P < 0.05). The total weight and gastrointestinal tract indices were higher in AM75 and AM50 and sequentially lower in AM25 and CT (P < 0.05). The AM75 and AM50 treatments resulted in significantly lower pH in the duodenum and caecum than in the CT (P < 0.05). The yield percentage of half-eviscerated AM75 chickens was significantly higher than that of CT (P < 0.05). However, breast muscle percentage was significantly lower in AM75 than in CT (P < 0.05). AM diets resulted in an increased yellowness of the muscles. It was concluded that AM improved the growth performance, development of the intestine, and meat quality, and reduced the pH of the intestine and serum triglycerides.

1. Introduction
Alfalfa meal (AM) is an important feed resource for ruminants. AM contains high protein and well-balanced amino acids (Ensminger 1992; Sen et al. 1998; Ponte et al. 2004; Markovic et al. 2007), vitamins (Jiang et al. 2012), and various microelements (Kindschy 1991; Sen et al. 1998). The AM also contains biologically active substances, such as saponins (2–3% of dry matter). Some studies have shown that saponins have anti-inflammatory, anticarcinogenic, hypcholesterolemic, and antioxidant properties (Klita et al. 1996; Rao and Gurjinkel 2000; Francis et al. 2002). However, AM is also high in fibre and low in metabolizable energy (Donelson et al. 2005). Considering the growth performance of poultry, AM has not attracted considerable attention in the context of poultry nutrition during the early growth stages. Early data suggested that when more than 5% AM is added to poultry diets, it has an adverse effect on performance (Lepkovsky et al. 1950), and the growth-suppressing effect of AM may vary depending on its level in the diet. Given these indications, to maintain performance in the starter phase, alfalfa meal should be used in a limited quantity in the diet (Guenthner et al. 1973). This limitation on the use of AM in broiler diets is largely due to its high dietary fibre content (Dansk 1971; Guenthner et al. 1973). Recent evidence has confirmed that an appropriate amount of dietary fibre may benefit the development of the gut, the gut microecosystem, growth performance, and welfare of poultry (Jiménez-Moreno et al. 2010; Guo et al. 2016). AM has attracted attention as a high-quality fibre source in poultry nutrition. Some studies have examined the effect of adding AM to laying hens (Güçlü et al. 2004; Mourao et al. 2006; Olgun and Yildiz 2015), while few other studies have systematically investigated the addition of AM to broiler chick diets (Carrasco and Bellof 2013), especially in terms of its effects on body measurements, development and pH of the gastrointestinal tract, and meat quality. Currently, supplementing poultry diets with forages, and their derivative meals, to improve growth performance, GI tract development, and meat quality is attracting increasing interest. Alfalfa is a commonly used forage material in ruminant diets, but its application in broilers has not been well studied. To date, there have been few studies with inconsistent results investigating the effects of AM in broiler diets. Therefore, the objective of this present study was to systematically investigate the effect of the dietary AM on the development and pH of the GI tract, blood biochemical profile, carcass yield characteristics, meat quality, and growth performance from 22 to 84 days of age in broilers.

2. Materials and methods
2.1. Alfalfa meal preparation and analysis
Alfalfa (Medicago sativa) was harvested from Ningxia Province, China, at the beginning of flowering stage. After sun drying for...
5 h, alfalfa was dried at room temperature (15–25 °C). The dried alfalfa was then ground through a 1.0 mm screen. The nutrient composition of AM was as follows: dry matter, 91.99%; crude protein (CP), 21.51%; aether extract (EE), 1.37%; crude fibre (CF), 28.21%; ash, 11.01%; calcium, 1.46%; total phosphorous, 0.50%; insoluble dietary fibre (IDF), 32.24%; and soluble dietary fibre (SDF), 25.35%. The neutral detergent fibre (NDF) (36.70%) and acid detergent fibre (ADF) (25.00%) were analysed according to Van Soest et al. (1991). The IDF and SDF of AM were determined according to the method recommended by Gómez-Ordóñez et al. (2010). Other chemical composition analyses were based on the guidelines of AOAC (2012).

2.2. Birds and diets

All experimental procedures were approved by the Academic Committee of Southwest Forestry University. One hundred and ninety-two 21-day-old female Guangxi-Tiejiaoma broiler chickens were housed in an environmentally controlled room. The birds were randomly divided into 4 groups; each group had 4 replicates (12 birds per replicate, fed within a single pen). All birds were kept in wired cages for 63 d. The dietary treatments included the control (0 g alfalfa meal /kg diet, CT), 25 g alfalfa meal /kg diet (AM25), 50 g alfalfa meal /kg diet (AM50), and 75 g alfalfa meal /kg diet (AM75) treatments (11, 16, 22, and 27 g of crude fibre per kg diet, respectively). The diets were formulated according to the Chinese Feeding Standard of Chicken (MAC 2004) and the National Research Council (NRC 1994) recommendations (Table 1).

Table 1. Ingredient composition of the experimental diets (as fed basis).

| Items          | CT          | AM25        | AM50        | AM75        |
|----------------|-------------|-------------|-------------|-------------|
| Corn (g/kg)    | 746.0       | 728.3       | 699.4       | 665.9       |
| Corn protein concentrate (g/kg) | 161.1       | 160.0       | 160.0       | 155.0       |
| Fish meal (g/kg) | 39.0        | 40.0        | 37.2        | 40.0        |
| Alfalfa meal (g/kg) | 0.0         | 2.50        | 50.0        | 75.0        |
| Soy oil (g/kg)  | 0.0         | 5.0         | 12.0        | 23.0        |
| L-Lys-HCl (g/kg) | 5.8         | 3.8         | 4.4         | 4.6         |
| DL-Met (g/kg)   | 0.3         | 0.3         | 0.3         | 0.0         |
| Limestone (g/kg)| 14.0        | 9.8         | 12.0        | 12.0        |
| Calcium phosphate dibasic (g/kg) | 15.0        | 14.0        | 10.9        | 10.9        |
| Sodium chloride (g/kg) | 3.8         | 3.8         | 3.8         | 3.8         |
| Vitamin and mineral premix (g/kg) | 10.0        | 10.0        | 10.0        | 10.0        |
| Calculated nutrient content |               |             |             |             |
| ME (MJ/kg)     | 13.09       | 13.05       | 12.97       | 12.97       |
| Crude protein (%) | 20.00       | 20.04       | 20.13       | 20.22       |
| Aether extract (%) | 3.37        | 3.81        | 4.43        | 5.42        |
| Lysine (%)     | 1.13        | 0.95        | 1.00        | 1.02        |
| Methionine (%) | 0.47        | 0.47        | 0.46        | 0.43        |
| Calcium (%)    | 1.02        | 0.90        | 0.91        | 0.96        |
| Available phosphorous (%) | 0.49       | 0.49        | 0.44        | 0.45        |
| Acid detergent fibre % | 1.08       | 1.62        | 2.15        | 2.68        |
| Neutral detergent fibre % | 8.46       | 9.16        | 9.80        | 10.36       |
| Acid detergent fibre % | 3.38       | 3.91        | 4.43        | 4.92        |

\*CT = Control (0 g/kg AM supplement); AM25 = 25 g/kg AM supplement; AM50 = 50 g/kg AM supplement; AM75 = 75 g/kg AM supplement.

\*The premix supplied the following per kilogram of diet: VA (vitamin A acetate) 15000 IU, VB, 0.75 mg; VB3, 3 mg; VB6, 2.23 mg; VB12 (cyanocobalamin) 0.21 mg, VD3 (cholecalciferol) 2750 IU, VE (dl-alpha tocopherol acetate) 62.5 IU, VK1 (Menadione) 0.65 mg, biotin 2 mg, folic acid 3.63 mg, D-pantothenic acid (D-calcium pantothenate) 2.23 mg, nicotinic acid 3.63 mg, Choline (Choline chloride) 1000 mg, ethoxyquin, 150 mg, butylated hydroxytoluene 75 mg, Cu (as CuSO4·5H2O) 8 mg, Fe (as FeSO4·7H2O) 70 mg, Mn (as MnSO4·H2O) 60 mg, Zn (as ZnSO4·7H2O) 40 mg, I (as KI) 0.35 mg, Se (as Na2SeO3) 0.15 mg.

2.3. Bird management

All birds were vaccinated against Marek’s disease, Newcastle disease, infectious bronchitis and infectious bursal disease prior to the commencement of the study. All birds had free access to feed in mash form and water throughout the trial. The lighting schedule consisted of 24 h of light throughout the trial. The temperature was set at 25 ± 0.5 °C during the first week of experimentation and lowered by 1–2 °C each week thereafter, until a final temperature of 20 ± 0.5 °C on the third weekend, at which point it was maintained until the end of the study.

2.4. Growth performance and body measurements

Every morning, before feeding the chickens, the leftover feed was collected, air-dried, weighed, and subtracted from the weight of the feed provided the previous day, to calculate the average daily feed intake (ADFI) throughout the trial period. The body weight (BW) of the chickens was determined at the beginning (22 days of age) and at the end of the experiment (84 days of age). Additionally, we determined the average daily gain (ADG) and feed conversion rate (FCR). The ADFI and BW per pen (12 birds) were determined for the calculation of ADG and FCR. The FCR was calculated by dividing the total feed intake per pen by the total BW gain of the birds over the 63-day period (22–84 days of age). Body measurements (shank length, fossil bone length, breast width, breast depth, and body slope length) of the birds were taken at 3, 6, 9, and 12 weeks of age according to the methods recommended by the Chinese National Poultry Breeding Committee (CNPBC 1984).

2.5. Development and pH of the intestine and organ indices

At the end of the experiment, 12 birds representing the main body weight of each treatment (three birds per replicates) were selected and raised separately in the prepared pens. The feed was withdrawn overnight prior to slaughter. The birds were weighed and sacrificed by severing their jugular vein until complete bleeding. Then, the pH of the gastrointestinal tract was measured in each segment using a digital pen-type pH metre. The fine tip of the glass electrode was sterilized and the pH of the gastrointestinal tract was recorded twice in the intestinal segments (duodenum, jejunum, ileum, caecum, and rectum). The empty parts of the intestinal segments (proventriculus, gizzard, duodenum, jejunum, ileum, caecum, and rectum) and fresh organs (thymus, bursa of Fabricius, spleen, heart, liver, and pancreas) were dried on desiccant paper and weighed. The GI tract and organ indices were expressed as the ratio of the organ or intestinal segment weight (g) to the live BW (kg).

2.6. Carcase yield characteristics and meat quality

At the end of the experiment (84 days of age), three chickens from each replicate (12 birds per group) were randomly selected and kept separately in pre-slaughter pens. The
chickens were then sacrificed by severing the jugular vein and wait until the total bleeding was completed. The birds were de-feathered manually, eviscerated, and cut up using standard methods to assess slaughter performance by the Chinese National Poultry Breeding Committee (CNPBC 1984; Jiang et al. 2018). The dressing percentage, semi-eviscerated proportion, eviscerated proportion, breast muscle, thigh muscle, and abdominal fat, expressed relative to BW, were calculated according to the standard methods suggested by the CNPBC (1984).

Afterward, the left breast and thigh muscles were used for meat quality analysis. The meat colour of breast and thigh muscles was determined at 45 min post-mortem, using a chroma metre (CM-2300d, Minolta Co. Ltd., Japan) [lightness (L*), redness (a*), yellowness (b*)]. Three readings, each measured in triplicate and then averaged, were taken from different locations on the surface of each sample. The pH of the breast and thigh muscles was measured 45 min and 24 h post-mortem at 1-cm depth using a portable pH metre (HI-99163, Hanna Instrument Co. Ltd., Italy). The average pH was determined by obtaining three measurements from the same area, and the approaches used for determination were the same for all meat samples. The meat samples were stored at 4 °C before measuring the pH at 24 h post-mortem. The method recommended by Christensen (2003) was used to determine the drip loss of breast and thigh muscles. Cooking loss was determined according to the method recommended by Chen et al. (2013). The crude protein and aether extract contents of the breast and thigh muscles were determined according to AOAC (2012) methods, and expressed as dry matter percentages.

2.7. Blood biochemical parameters

To analyse the blood parameters, we selected 12 birds from each treatment, at the end of the experiment (84 days of age). Blood samples (10 mL) were collected from the wing vein of each bird. The serum was separated by centrifugation (3,000 rpm, 10 min) and immediately stored at −20 °C until further analysis. Albumin (ALB), total protein (TP), glucose (GLU), triglycerides (TG), total cholesterol (TC), uric acid (UA), blood urine nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT) levels were determined using an automatic chemistry analyser (Chemray-240, Rayto Life and Analytical Sciences Co. Ltd., China).

2.8. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, NY, USA). One-way ANOVA was used to analyse the experimental data. Significant differences between treatment groups were further analysed using Duncan’s multiple range test. The results are presented as the means. Variability in the data was expressed as the SE of the means, and a probability level of P < 0.05, was considered statistically significant.

3. Results

The effects of different levels of AM inclusion in broiler diets on body weight gain, feed intake, and FCR are shown in Table 2. The final BW and ADFI were significantly higher (P < 0.05) in the AM diet group than in the CT group. The ADG was also linearly higher in the three AM groups than in the CT group. Although there were no significant differences between the FCR of the AM and CT groups, there was a declining trend in FCR from the CT to the AM75 group.

The effects of AM on the body measurements of broilers are shown in Table 3. Compared to the CT treatment, the AM75 treatment increased the fossil bone length and shank length at 9 and 12 weeks of age, respectively. The breast width was comparatively higher in the AM50 group than in the CT group at 12 weeks of age (P < 0.05). The breast depth and body slope length did not exhibit any noticeable changes in either the AM or CT groups at 12 weeks of age.

The effects of AM on GI tract development in broilers are shown in Table 4. Compared to the CT group, the AM groups had increased proventiculus and gizzard weights. Total GI tract weights were higher in the AM50 and AM75 groups and sequentially lower in the AM25 and CT (P < 0.05) groups. In the case of different GI tract segments, significant differences were observed between the ileum weight and ileum indices (P < 0.05); however, no significant differences were found among the gizzard, jejunum, and caecum indices (P > 0.05). The effects of AM addition on the GI tract pH are shown in Figure 1. Compared to the CT group, the AM groups exhibited decreasing pH levels. The lowest and highest duodenal pH were obtained in the AM75 and CT groups, respectively (P < 0.05). The caecum pH was significantly lower in the AM75 and AM50 groups than in the CT group (P < 0.05), whereas no difference was found between the AM25 and CT groups. The rectal pH of the AM75 group was significantly lower than that of the AM25 and CT (P < 0.05) groups. The thymus weight was comparatively higher in the AM75 group than in the CT group (P < 0.05). Dietary inclusion of AM had no effect on the weight and indices of the bursa of Fabricius, spleen, pancreas, and liver (Table 5).

As shown in Table 6, chickens fed the AM75 diet had a significantly higher percentage of half-eviscerated yields than the CT group (P < 0.05). However, there were no significant differences among the AM25, AM50, and CT groups. The dressing percentage, eviscerated yield, thigh muscle percentage, and abdominal fat percentage did not differ significantly among the AM25, AM50, AM75, and CT groups. However, the breast muscle percentage was significantly lower in the AM75 group than in the CT group (P < 0.05).

Table 7 shows the effect of AM on the blood biochemical profiles of broilers. The TG was comparatively lower in the AM50 group than in the CT group (P < 0.05), while the AM75 and AM25 groups did not show any obvious changes. The lowest AST value was found in AM25, followed by AM50, while there were no significant differences between AM75 and CT groups. Other than those for TC, AST, GLU, TP, ALB, BUN, UA, GGT, and ALT, there were no other biochemical profile changes worth mentioning.
Table 2. Effects of alfalfa meal on growth performance of broilers.

| Item                        | CT            | AM25          | AM50          | AM75          |
|-----------------------------|---------------|---------------|---------------|---------------|
| Initial BW (g)              | 235.30±7.49   | 238.40±5.58   | 237.45±2.74   | 232.55±6.70   |
| Final BW (g)                | 1345.95±64.68 | 1535.35±96.40 | 1542.50±19.10 | 1593.60±64.70 |
| ADFI (g/d)                  | 69.56±2.94    | 75.99±2.58    | 76.90±0.85    | 79.22±1.22    |
| ADG (g/d)                   | 17.63±1.02    | 20.59±3.12    | 20.71±0.31    | 21.60±1.03    |
| FCR                         | 3.95±0.14     | 3.75±0.50     | 3.71±0.08     | 3.67±0.13     |

CT = Control (0 g/kg AM supplement); AM25 = 25 g/kg AM supplement; AM50 = 50 g/kg AM supplement; AM75 = 75 g/kg AM supplement.

Table 3. Effects of alfalfa meal on body measurements of broilers.

| Week of age | Body measurements | CT            | AM25          | AM50          | AM75          |
|-------------|-------------------|---------------|---------------|---------------|---------------|
| 3           | Shank length (cm) | 5.30±0.10     | 5.19±0.26     | 5.29±0.17     | 5.24±0.06     |
|             | Fossil bone length (cm) | 5.03±0.08 | 5.02±0.14     | 5.11±0.21     | 5.12±0.15     |
|             | Breast width (cm)  | 5.39±0.75     | 5.83±0.22     | 6.14±0.17     | 6.17±0.18     |
|             | Breast depth (cm)  | 5.74±0.28     | 5.53±0.53     | 5.77±0.54     | 5.36±0.49     |
|             | Body slope length (cm) | 9.31±0.49 | 9.16±0.07     | 8.96±0.11     | 9.05±0.38     |
| 6           | Shank length (cm) | 6.45±0.09     | 5.65±0.17     | 6.67±0.24     | 6.48±0.07     |
|             | Fossil bone length (cm) | 6.49±0.06 | 6.14±0.56     | 6.86±0.21     | 6.80±0.10     |
|             | Breast width (cm)  | 6.94±0.28     | 6.62±0.38     | 6.78±0.12     | 7.36±0.46a    |
|             | Breast depth (cm)  | 6.95±0.14     | 11.10±0.32    | 11.42±0.47    | 10.40±1.24    |
|             | Body slope length (cm) | 11.41±0.38 | 11.01±0.32    | 11.42±0.47    | 10.40±1.24    |
| 9           | Shank length (cm) | 7.61±0.20     | 7.88±0.08     | 8.14±0.15     | 8.08±0.13     |
|             | Fossil bone length (cm) | 8.06±0.34 | 8.25±0.20ab   | 8.32±0.48abc  | 8.75±0.44a    |
|             | Breast width (cm)  | 8.76±0.24     | 9.55±0.58     | 9.16±0.83     | 9.08±0.68     |
|             | Breast depth (cm)  | 8.13±0.07     | 8.46±0.36     | 8.30±0.37     | 8.47±0.39     |
| 12          | Shank length (cm) | 8.74±0.14     | 8.86±0.18     | 9.44±0.66     | 9.47±0.19     |
|             | Fossil bone length (cm) | 9.25±0.17 | 9.63±0.29     | 9.93±0.18     | 9.93±0.22abc  |
|             | Breast width (cm)  | 10.23±0.34    | 10.58±0.53    | 10.90±0.20    | 10.73±0.11ab  |
|             | Breast depth (cm)  | 9.07±0.64     | 9.28±0.16     | 9.59±0.33     | 9.47±0.17     |
|             | Body slope length (cm) | 14.77±0.48 | 15.40±0.66    | 15.39±0.34    | 15.48±0.35    |

CT = Control (0 g/kg AM supplement); AM25 = 25 g/kg AM supplement; AM50 = 50 g/kg AM supplement; AM75 = 75 g/kg AM supplement.

The effects of dietary AM addition on meat quality parameters are shown in Table 8. The thigh muscle CP was lower in the AM group than in CT group (P < 0.05), while there were no significant differences in terms of breast muscle CP. No significant differences were found in terms of EE, cooking loss, pH45min, and pH24h of the thigh and breast muscles. Significant differences were also observed between the meat colour of thigh and breast muscles. The b* of breast muscles were not affected by AM addition. Compared with CT chickens, AM chickens showed an increasing trend of b* in their thigh muscles. The drip losses of the thigh and breast muscles were lower (P < 0.05) in the AM than in CT chickens.

4. Discussion

Over the past few decades, AM has garnered considerable attention, as an alternative feed ingredient for chickens and other poultry diet, with a good balance of amino acids,
Figure 1. Effects of alfalfa meal on digesta pH of broilers at the segments of the gastrointestinal tract. CT = Control (0 g/kg AM supplement); AM25 = 25 g/kg AM supplement; AM50 = 50 g/kg AM supplement; AM75 = 75 g/kg AM supplement.

Table 5. Effects of alfalfa meal on organs weight and indices of broilers.

| Items                  | CT          | AM25       | AM50       | AM75       |
|------------------------|-------------|------------|------------|------------|
| Thymus weight (g/LBW)  | 7.39±2.73b  | 7.28±1.90b | 7.88±2.80ab| 10.17±2.82a|
| Thymus indices (g/kg LBW) | 5.39±1.99  | 4.70±0.96  | 5.38±1.98  | 6.13±1.47  |
| Bursa of Fabriceius weight (g) | 2.51±0.61  | 2.48±0.77  | 3.00±0.59  | 3.24±1.18  |
| Bursa of Fabriceius indices (g/kg LBW) | 1.81±0.46  | 1.62±0.51  | 2.05±0.45  | 1.93±0.59  |

Table 6. Effects of alfalfa meal on slaughter performance of broilers.

| Items                  | CT          | AM25       | AM50       | AM75       |
|------------------------|-------------|------------|------------|------------|
| Dressing percentage (%) | 88.63±1.89  | 87.73±1.40 | 88.10±1.87 | 88.35±0.98 |
| Percentage of half-eviscerated yield (%) | 78.20±2.85a | 78.51±2.19a | 79.99±0.98ab | 80.59±1.34b |
| Percentage of eviscerated yield (%) | 62.66±4.13  | 62.54±2.24 | 63.46±1.05 | 60.02±3.78 |
| Percentage of breast muscle (%) | 20.43±2.30a | 18.80±2.62ab | 17.72±1.62ab | 16.43±4.85b |
| Percentage of thigh muscle (%) | 21.07±3.04  | 20.93±1.92 | 19.71±1.22 | 20.49±1.64 |
| Percentage of abdominal fat (%) | 3.70±1.27  | 4.35±1.27  | 4.44±0.75  | 4.58±1.65  |

Table 7. Effects of alfalfa meal on blood biochemical indexes of broilers.

| Items                  | CT          | AM25       | AM50       | AM75       |
|------------------------|-------------|------------|------------|------------|
| ALT (U/L)              | 17.75±1.4a  | 16.82±0.9  | 16.57±1.0  | 17.13±0.8  |
| AST (U/L)              | 135.8±1.1b  | 135.4±0.6  | 152.9±2.3a | 157.4±2.68a|

Table 8. Effects of alfalfa meal on meat quality of broilers.

| Items                  | CT          | AM25       | AM50       | AM75       |
|------------------------|-------------|------------|------------|------------|
| Breast CP (% of DM)    | 83.84±2.64  | 86.24±8.13 | 82.77±1.27 | 88.30±2.28 |
| EE (% of DM)           | 5.64±1.39   | 5.50±2.19  | 6.63±1.01  | 6.03±2.53  |
| Drip loss (%)          | 3.27±2.16e  | 3.32±3.63a | 2.75±1.14e | 4.13±2.16e |
| Drip loss (%)          | 23.44±2.95  | 21.53±3.80 | 23.36±1.65 | 23.33±1.83 |
| pH 45 min              | 5.78±0.15   | 5.75±0.05  | 5.67±0.07  | 5.66±0.12  |
| pH 24 h                | 5.67±0.22   | 5.69±0.04  | 5.53±0.18  | 5.42±0.44  |
| Lipid (%)              | 52.67±3.20  | 51.39±1.03 | 54.60±3.94 | 50.10±3.39 |
| Lipid (%)              | 18.65±1.91  | 18.74±1.78 | 18.83±1.64 | 18.78±1.56 |
| Lipid (%)              | 13.85±1.1b  | 13.45±0.6b | 15.29±2.33a| 15.74±2.68a|

The results of the present study showed that dietary AM addition resulted in significant improvement in the growth performance of chickens, which is consistent with the results of Tkáčová et al. (2011), who discovered that the addition of 2% AM increased the body weight gain (BWG) of broilers compared to control birds. However, it has also been reported that the addition of 4% AM does not have any effect, while 6% AM has a reducing effect on BWG (Tkáčová et al. 2015). Jiménez-Moreno et al. (2010) reported that the inclusion of fibre (sugar beet pulp and oat hulls) improves the BWG and FCR of broilers from 1 d to 21 d of age, and suggested that broilers require a minimal amount of fibre in their diet to maximize their growth performance and health (González-Alvarado et al. 2007). However, several studies have reported contradictory results (Dansky et al. 1971; Mourao et al. 2006; Jiang et al. 2012). Jiang et al. (2018)
found no significant BWG differences when 4% and 8% AM were added to broiler diets. The fibre in the diet affects the feed intake (FI) of poultry by stimulation of the gizzard with coarse fibre; generally, it is assumed that coarse fibre reduces FI. However, such effects were not observed when adding finely ground and coarsely ground fibre (Itani and Svihus 2019). Sacranie et al. (2012) even showed an increased FI when coarse oat hulls were used instead of ground oat hulls. Furthermore, studies have shown that SDFs such as gum, pectin, alginate, guar gum, and β-glucan, increased satiety in humans, thereby reducing FI (Svihus and Hervik 2019). This is consistent with the recent review of 37 pig experiments by Flis et al. (2017) and concluded that SDF such as inulin, pectin, and citrus pulp also reduced the FI of pigs. However, Flis et al. (2017) also concluded that several IDF increased FI in many cases, such as wheat bran and soybean hulls. Feed intake depression has been mentioned in several studies, with goose, turkey, quail, and broiler diets containing 10% AM or higher, resulting in depressed feed intake (Cheeke et al. 1983). However, the feed intake of 4- and 8-week-old turkey increased when 5% AM was added to their diets (Potter and Shelton 1973). The findings of Mutahar et al. (2011) corroborate our results, as the authors discovered that the addition of 7% AM in laying hen diets requires the use of more feed than in the 2% AM group. In the present study, we observed an increasing trend of AM content in AM25 and AM75, which may influence birds to consume more feed than the CT group. This is may be due to the presence of IDF content in alfalfa meal. Jiménez-Moreno et al. (2010) also showed that 3% cellulose increases the ADFI from 1 d to 15 d of age but not thereafter, which is in agreement with the results of Shakouri et al. (2006) in 1-14-day-old broilers. In contrast, the inclusion of 3% sugar beet pulp reduced the FI considerably compared with the inclusion of 3% oat hulls, which is in agreement with the results of Razdan and Pettersson (1994) on broilers. Cooney et al. (1948) reported that the dietary addition of up to 10% AM did not depress the FI, while the addition of more than 10% AM had a depressive effect on feed intake due to the high fibre content. No significant differences were detected in FCR between the AM and CT groups, which corroborates the results of Jiang et al. (2018) regarding yellow-feathered broilers and Laudadio et al. (2014) regarding laying hens. Svihus and Hetland (2001) also showed that broilers can maintain an adequate BWG when fed diets supplemented with high levels of insoluble fibre (10% oat hull). This is probably because dietary fibre increases the rate of passage of the digesta through the digestive system and the physical capacity of the gut. Body measurements are important parameters that reflect the growth development and flock uniformity of poultry, especially in intensive poultry production systems. Body measurements are not only affected by genetic factors, but also by age, sex, nutritional level, and other factors. The effect of AM on the body measurements of broilers has been scarcely reported. In the present study, supplementing broilers diets with AM did not inhibit their growth and development.

However, growth performance differences may occur owing to the breed or strain (slow-growing broiler and fast-growing broiler) and feeding age, which may be highly tolerant to fibre, leading to variations in BWG and RCR. In recent years, slow-growing chickens have received increasing attention in the United States, Europe, and China. This is because of their higher meat quality, higher price, and lower mortality (Singh et al. 2020). However, compared with fast-growing broilers, studies have shown that slow-growing broilers have lower BWG, higher FI, and FCR (Sarica et al. 2019). Another study showed that the FCR of the commercial slow-growing Cobb-Sasso chicken was 2.14 at day 56 (Cobb-Vantress 2007). This may be related higher maintenance requirements and mobility in slow-growing broilers (Gordon and Charles 2002).

The diets used in the present study induced 50%, 100%, and 150% increases in CF in the AM25, AM50, and AM75 groups, respectively, compared to the CT diet. The AM diets had considerable effects on the total gastrointestinal weight and indices of broilers. The digestive tract responds quickly to dietary fibre adjustments (Farner 1960; Svihus 2011). Studies have suggested that supplementing a moderate amount of dietary fibre in the broiler diet helps to ameliorate GI tract function (González-Alvarado et al. 2007) and stimulate bile acid and enzyme secretion, which induce better nutrient utilization, performance, and physiological function. A rapid and significant increase the size, mass, and muscularity of the gizzard was observed when dietary fibres, such as hulks, wood shavings, or other fibre, were included in the diet (Abdollahi et al. 2018). The gizzard size increases by more than 100% when structural components are added to poultry diets (Svihus 2011). A well-developed gizzard is very important for the digestion of feed by poultry. According to Duke (1986), the gizzard is the ‘pacemaker’ of normal intestinal motility, which is essential for maintaining optimum digestion, absorption, and intestinal health of poultry. A previous study showed that the gizzard is underdeveloped when diets lacking dietary fibres are provided and that dietary fibre deficiency is associated with proventriculitis in growing chickens (O’Dell et al. 1959).

In the present study, gizzard weight of the AM25, AM50, and AM75 groups increased by 16.58%, 22.43%, and 36.55%, respectively, compared to the CT group. The increased weight of the gizzard, proventriculus, and intestines was also supported by Mateos et al. (2012), who found that chickens respond rapidly to changes in the dietary fibre content through intestinal weight and length modification, as well as by adjusting the passage rate through the digestive tract. Better feed utilization in the upper portion of the gastrointestinal tract may consequently diminish the activity of the lower portion, which was the case for the rectum in the present study. Generally, a moderate increase in the dietary fibre content helps improve the gastrointestinal tract function (Håkansson et al. 1978; Amerah et al. 2009), but the impact differs based on the type of fibre, supplement level, and physicochemical properties of the fibre used as well as the sections of the digestive tract (Jiménez-Moreno et al. 2009b; Jiménez-Moreno et al. 2010).

There is limited research on the effects of AM on the pH of the GI tract of broilers. Feeds high in fibre, such as AM, remain longer in the upper portion of the digestive tract. This ultimately increases the gizzard activity, which results in the release of a high amount of hydrochloric acid in the stomach. Refluxes of such acid continue in the duodenum (Hetlandet
et al. 2005); a similar phenomenon was observed in the present study. However, studies have shown that the effect of different fibre types (30 g cellulose /kg diet, 30 g sugar beet pulp /kg diet, and 30 g oat hulls /kg diet) on duodenal pH is not significant (Jiménez-Moreno et al. 2009a). In addition, Jiménez-Moreno et al. (2009a) showed that the use of sugar beet pulp resulted in lower pH in the caecum than in the ileum; an opposite effect was observed owing to the cellulose diet. This could probably be attributed to the fact that the resident anaerobic microflora can ferment sugar beet pulp with more ease than cellulose (Carabano et al. 1997). The fibre is fermented by microorganisms in the hindgut, which produce short-chain fatty acids (SCFAs), predominantly acetate, propionate, butyrate, lactate, and succinate (Liu et al. 2018). The SCFAs decrease the pH of the gut and inhibit the colonization and growth of some pathogens, such as Escherichia coli, Salmonella and some Clostridia (Macfarlane et al. 2006; Lin et al. 2017). It is well-known fact that SCFAs play a very significant role in maintaining the morphology and function of epithelial cells (Koh et al. 2016). The findings of the present study indicate that AM can generate more SCFAs and promote the gut health of broilers. Furthermore, a low pH in the upper gastrointestinal tract increases the solubility and absorption of mineral salts (Guinot et al. 1995) and favours pepsin activity (Jiménez-Moreno et al. 2009b).

The thymus, bursa of Fabricius, and spleen are among the primary immune organs of chickens. According to Yan et al. (2017), the assessment of the weight of immune organs and the estimation of the immune organ weight to total BW ratio constitutes an excellent model for determining the protection rate provided by vaccines against some diseases. In the present study, we reported that supplementing a diet with more than 5% AM resulted in a significant increase in the thymus weight compared to the CT group. However, there were no significant differences in terms of the thymus, bursa of Fabricius, and spleen indices. Liu et al. (2010) showed that supplementation of chicken diets with AM has no significant effect on the development of immune organs, which is consistent with the results of the present study. High saponin levels (approximately 2–3%) were found in the groups supplemented with AM, which are known for hypcholesterolemic, anticarcinogenic, anti-inflammatory, and antioxidant properties (Rao and Gurfinkel 2000; Francis et al. 2002). Liu et al. (2016) found that AM saponins reduce hepatic cholesterol by inhibiting HMG-CoA activity and the formation of insoluble complexes between saponins and cholesterol, which eventually transform into bile acid. The TG decreased in the AM diets compared to the CT diet, which was supported by a previous study on Muscovy ducks (Jiang et al. 2012). The AST or ALT levels of blood are very important as they are indicators of liver disease. Therefore, the levels of AST and ALT in blood are directly related to the extent of tissue damage (Huang et al. 2006). The present study indicated that the AST and ALT levels were similar among the treatment groups, except for AM25 in which the ALT level was the highest and the AST level was significantly lower than that in the other groups. Elevated AST levels can cause serious liver diseases, such as degeneration and necrosis of the liver tissue (Thapa and Anuj 2007); the results of the present study indicate that the use of AM may not be harmful for broiler organs, which is consistent with the results of Lepkovsky et al. (1950).

Generally, carcases with a dressing percentage over 80% and an eviscerated yield percentage over 60% are considered to demonstrate in China (Zhang et al. 2004), which is in agreement with our results. Ayssiwede et al. (2011) showed that the Moringa oleifera leaves meal as a supplement in the diet had no significant effect on the dressing percentage of indigenous Senegalese chickens. However, a declining trend in the percentage of breast muscle was observed in the AM75 group in the present study. This suggests that high AM levels in broiler diets may decrease the breast muscle percentage. Several grass meal types have been examined to improve the meat quality of poultry. The presence of saponins and pigments makes the AM a unique feed ingredient that improves meat quality (Liu et al. 2013). The colour improvement of the breast and thigh muscles was the most influenced characteristic due to dietary AM addition. AM has been reported to be a natural source of xanthophylls (Sauvant et al. 2002). Xanthophylls cause poultry carcases to have a desirable yellow colour (Sen et al. 1998; Ponte et al. 2004). In the present study, significant differences were detected in breast muscle in terms of L∗, this result was also supported by Jiang et al. (2018), who found no significant differences in terms of L∗ among the studied groups. An increasing trend was observed in terms of b∗ in the AM group, which was possibly due to the AM xanthophyll content. An increasing trend of a∗ in thigh muscles was observed in our study; however, there were no significant differences in a∗, which is consistent with the results of Jiang et al. (2018) who did not find significant differences in a∗ using 0%, 4%, and 8% AM in broilers diets. Jiang et al. (2018) reported that dietary supplementation with 80 g/kg AM decreased breast drip loss and increased b∗ post-mortem, possibly owing to the flavonoid and saponin contents of AM. The pH of muscle is a direct reflection of the acid content, which influences the drip loss and meat colour. The absence of significant pH differences at 45 min and 24 h post-mortem in either the breast or the thigh muscles indicated low lipid oxidation owing to the antioxidative properties of AM (Jiang et al. 2018).

5. Conclusions

In conclusion, AM supplementation improved growth performance, development of the intestine, and meat yellowness, and decreased the pH of the GI tract and serum triglycerides in broilers. However, it had little effect on slaughter performance and immune organs. This study demonstrated that as an excellent source of dietary fibre, the use of AM may be feasible and effective for broiler production. Further research and long-term studies are necessary to validate our findings.

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