Effects of dietary supplementation with fermented *Chenopodium album* L. on growth, nutrient digestibility, immunity, carcase characteristics and meat quality of broilers

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**ABSTRACT**

Here, we investigated the effects of fermented *Chenopodium album* L. (FCAL) on growth performance, apparent total tract nutrient digestibility, serum immunity, carcase characteristics and meat quality of broilers. Arbour Acres broilers (160, 1-d-old) were randomly allocated into four treatment groups with five replicates of eight birds each. The birds were fed a corn–soybean meal basal diet supplemented with 0 (control [CON]), 2, 4 and 8 g/kg FCAL. During the starter period (days 1–21), 4 and 8 g/kg FCAL significantly increased the average daily gain (ADG), apparent digestibility of dry matter (DM), crude protein (CP) and ether extract (EE), moreover; 8 g/kg FCAL significantly decreased the feed conversion ratio (FCR). During the finisher period (days 22–42), 2 g/kg FCAL significantly increased ADG and apparent digestibility of DM, CP and EE but significantly decreased FCR. During the whole period (days 1–42), dietary supplementation of 4 and 8 g/kg FCAL significantly increased ADG. Compared with the CON group, in the FCAL groups, the serum interleukin (IL)-1\(\beta\) and IL-8 levels were lower, while insulin-like growth factor-1, immunoglobulin (Ig) A (IgA) and IgM levels were higher. The serum IL-10 content was lower in the 2 and 4 g/kg FCAL groups. The breast muscle percentage significantly increased with 8 g/kg FCAL. FCAL significantly decreased muscle drip loss (breast), shear force (breast), water loss rate and lightness (leg). FCAL had positive effects on growth, nutrient digestibility, immunity, carcase characteristics and meat quality of broilers, thus it could be a reliable and phytogenic feed additive for promoting growth and maintaining health of broilers.

**HIGHLIGHTS**

- Fermented *Chenopodium album* L. (FCAL) can improve growth performance and apparent nutrient digestibility of broilers.
- FCAL can promote immune responses and enhance carcase characteristics and meat quality of broilers.
- FCAL could be a reliable and potential phytogenic feed additive to promote growth and maintain the health of broilers.

**Introduc**

Currently, many countries, including China, have banned the utilisation of antibiotics as a growth promoter in animal feed to avoid resistant bacteria emergence and residues in animal-derived products (Ye et al. 2020; Mohebodini et al. 2021; Wang et al. 2021). Before the development of antibiotics, medicinal herbs were used for health and medical purposes for almost 60,000 years (Kuralkar P and Kuralkar SV 2021). In recent years, natural herbal products (HPs) have been found to possess various biological activities, such as antimicrobial, anthelmintic, antioxidative, growth promotion and immune modulation (Upadhaya and Kim 2017). Considering the safety and efficacy of HPs, they have been widely used as feed additives. In poultry diets, it has been reported that HPs have positive effects on growth performance (Ashour et al. 2020; Redoy et al. 2021). Additionally, HPs could improve the apparent digestibility of broilers by stimulating saliva and bile secretions and enhancing digestive enzyme activity (Lee et al. 2003; Platel and Srinivasan 2004). The pathogen load-reducing effect of HPs in...
the gut of broilers was also investigated by Long et al. (2020) and De Lange et al. (2010). Additionally, HPs improve the dressing percentage (Al-Kassie 2010) and meat quality of broilers, as indicated by increased pH, redness and water holding capacity and decreased lightness and shear force (Rahman and Kim 2016).

Chenopodium album L. (CAL), a common wild herbal medicinal plant, is widely distributed globally and withstand harsh soil and climatic conditions, such as freezing, high salt concentration and drought (Chen and Jia 2002; Chen 2006; Jan et al. 2017). CAL plays an important role in the prevention and treatment of diseases (Singh et al. 2015; Rashmi 2016; Sikarwar et al. 2017), owing to its antibacterial (Korcan et al. 2013), antioxidant, anti-inflammatory (Amodeo et al. 2019) and anthelmintic properties (Jabbar et al. 2007), which are realised by secondary metabolites. CAL has been found to contain several secondary metabolites, such as polyphenols, polysaccharides, flavonoids and other nutritional substances (Cutillo et al. 2004; He et al. 2018; Arora and Itankar 2018). Furthermore, a previous study showed that polyphenols, flavonoids and saponins from CAL can inhibit NF-κB protein expression, consequently relieving joint inflammation and significantly returning the body weight of arthritic rats to normal levels (Arora et al. 2014). Sikarwar et al. (2017) also found that feeding 0.4 g/kg aqueous extract of CAL to rats affected by kidney stones for 28 d prevented body weight loss and resulted in the regaining of the relative body weight to near-normal levels. Free-range animals (chickens, ducks, geese, pigs, sheep and cattle) often freely feed on CAL in rural areas. However, Fairley et al. (2012) indicated that excessive intake of CAL could result in hypocalcaemia, allergic symptoms, and even death. The palatability, nutrient absorption and utilisation of CAL are affected by its high level of antinutritional factors, such as pectin (0.93–1.52 mg/g), phytic acid (7.4–8.1 mg/g), saponins (2.1–2.7 mg/g) and tannins (1.15–1.21 mg/g) (Dongowski et al. 1992; Pachauri et al. 2017). Exogenous enzymes, such as pectinase and phytase, have been proven to degrade antinutritional factors of medicinal herbs and prevent growth inhibition, allergic symptoms and death in animals (Kerovuo et al. 1998; Gupta et al. 2005; Pachauri et al. 2017).

It has been proven that the fermentation of medicinal herbs with probiotics, such as Bacillus subtilis, could eliminate anti-nutritional factors (Hmani et al. 2017) and degrade macromolecular materials into micromolecular materials (Tanasković et al. 2021), which are beneficial for digestion and absorption in the broiler gastrointestinal tract. However, enzyme production during microbial fermentation is lower and insufficient for degrading anti-nutritional factors. Mao et al. (2019) reported that fermentation by probiotics supplemented with exogenous enzymes could resolve this problem, illustrated by increased efficiency of degrading anti-nutritional factors and improved quality of fermented materials. Furthermore, more secondary metabolites, such as polyphenols and flavonoids, are released after fermentation by probiotics supplemented with exogenous enzymes (Xie et al. 2020).

Thus, we hypothesised that CAL fermented by compound probiotics and pectinase might be a potential feed additive in poultry diets. To address this hypothesis, we assessed the effects of dietary fermented CAL (FCAL) supplementation on growth performance, apparent nutrient digestibility, serum immune responses, carcass characteristics and meat quality of broilers.

Materials and methods

Materials

Fresh CAL was collected from April to November in Hohhot (Inner Mongolia, Hohhot, China). The aerial parts of CAL were obtained and dried at 25°C in the shade, then ground to a fine powder. B. subtilis (CGMCC 1.0892), Lactobacillus plantarum (CGMCC No. 1.12934) and Saccharomyces cerevisiae (CGMCC No. 2.1190) were purchased from the China General Microbiological Culture Collection Centre (Beijing, China). Pectinase was obtained commercially (Beijing Solarbio Technology Co., Ltd, Beijing, China). Maize meal and ground cinnamon were obtained from a local market.

Fermentation of CAL

The compound probiotics were prepared by mixing B. subtilis, L. plantarum and S. cerevisiae at a ratio of 1:1:1. The CAL was mixed with corn meal, ground cinnamon and pectinase at a ratio of 16.5:3.0:1.0:0.4 (4 kg in total), blended with 50% distilled water (w/v) and was inoculated with 0.1% compound probiotics. The fermentation was conducted at 30°C for 24 h. Fermentation of CAL was conducted in multi-layer polythene bags (5 kg capacity) equipped with a gas pressure opening valve. After fermentation, the extract was dried at 45°C and ground with a hammer mill. The obtained FCAL was stored at −20°C until mixing in the diets. The polyphenol and polysaccharide contents of CAL before and after fermentation were...
17.81–27.32 mg/g and 141.96–229.56 mg/g, respectively, in this study.

**Animals, diet and experimental design**

All experimental procedures of this study were approved by Inner Mongolia University Research Ethics Committee. A total of 160 1-d-old Arbour Acres broiler chicks (purchased from a local hatchery) were individually weighed (45.96 ± 3.55 g) and randomly divided into four groups with five replications of eight birds each, including the control (CON) and FCAL supplementation groups. The CON group was provided with a corn-soybean meal basal diet and the FCAL groups were provided with a basal diet supplemented with 2, 4 or 8 g/kg of FCAL, respectively. Two different basal diets were used: a starter ration from 0 to 21 d and a finisher ration from 22 to 42 d. All basal diets were formulated to meet the nutrient recommendations of the Feeding Standard of Chicken, China (NY/T 33-2004; Chinese Ministry of Agriculture, 2004). The ingredients and chemical composition of the basal diets are presented in Table 1. Diets were supplied in a powder form. Feed and fresh water were provided ad libitum throughout the experiment. The stocking density was 16 birds per m² in the starter period and 9 birds per m² in the finish period. The room temperature was maintained at 33–35 °C for the first week and then reduced by 2–3 °C each week until it reached 20 °C. The lighting schedule was 24 L (24 h of lighting and 0 h of dark per day) for the first 3 d, 23 L:1D for days 4–7 d, and 18 L:6D for days 8–42.

**Growth performances**

Body weight and feed intake were recorded weekly, and the average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of the starter, finisher and whole periods were calculated.

**Apparent nutrient digestibility**

Faecal samples were taken from each replication once daily during the last 3 d of the two periods (19–21 d and 40–42 d, respectively) and pooled per replication. About 600 g of subsamples were taken, dried at 65 °C for 96 h and ground to a fine powder for apparent total tract digestibility analysis. Components of the diets and faecal samples, including dry matter (DM, method 930.15), crude protein (CP, method 976.06) and ether extract (EE, method 920.39) were measured according to the methods of the Association of the Official Analytical Chemists (AOAC 2000). Acid insoluble ash (AIA) was used as an internal marker and determined following the method described by Atkinson et al. (1984). The apparent nutrient digestibility (%) equation was as follows:

\[
1 - \left( \frac{\% \text{ AIA in feed} \times \% \text{ nutrient in feeds}}{\% \text{ AIA in faeces} \times \% \text{ nutrient in faeces}} \right) \times 100
\]

**Serum immune indices**

At 21 and 42 d, one bird from each replicate was randomly selected. Blood samples (5–10 mL) were obtained from the jugular vein. The serum samples were collected by centrifugation at 3000 × g at 4 °C for 10 min and stored at −80 °C for further serum immune indices analysis. The serum concentrations of insulin-like growth factor-1 (IGF-1), cytokines (interleukin [IL]-1β, IL-8, tumour necrosis factor-α [TNF-α], IL-10 and interferon-γ [IFN-γ]) and antibodies (immunoglobulin [Ig] G [IgG], IgA and IgM) were analysed using commercial ELISA kits (Jiangsu Mei Biao Biological Technology Co., Ltd., Jiangsu, China) according to the manufacturer’s protocol.

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**Table 1.** Ingredient and chemical composition of basal diets (% air-dry basis).

| Items                          | Starter (1–21, d) | Finisher (22–42, d) |
|-------------------------------|-------------------|---------------------|
| **Ingredients**               |                   |                     |
| Corn                          | 52.70             | 59.00               |
| Soybean meal                  | 40.00             | 33.80               |
| Soybean oil                   | 3.00              | 3.00                |
| Dicalcium phosphate           | 1.90              | 1.80                |
| Limestone                     | 1.08              | 1.22                |
| Salt                          | 0.37              | 0.37                |
| L-Lysine HCl                  | 0.05              | 0.03                |
| DL-Methionine                 | 0.19              | 0.07                |
| Vitamin and minerals premix   | 0.60              | 0.50                |
| Choline                       | 0.11              | 0.11                |
| **Chemical composition b**    |                   |                     |
| Dry matter                    | 88.62             | 89.47               |
| Crude protein                 | 19.34             | 16.52               |
| Neutral detergent fibre       | 17.83             | 15.33               |
| Acid detergent fibre          | 3.04              | 2.66                |
| Ash                           | 5.72              | 5.98                |
| Calcium                       | 0.72              | 1.2                 |
| Phosphorous                   | 0.52              | 0.53                |
| Lysine                        | 1.34              | 1.15                |
| Methionine                    | 0.55              | 0.40                |
| Cysteine                      | 0.40              | 0.36                |
| Metabolisable energy (MJ/kg)  | 12.20             | 12.33               |

*Provided per kilogram of diet: vitamin A 9000 U, vitamin D₃ 3000 U, vitamin E 26 mg, vitamin K₃ 1.20 mg, vitamin B₆ 3.00 mg, vitamin B₁₂ 8.00 mg, vitamin B₉ 4.40 mg, vitamin B₁ 0.02 mg, nicotinic acid 45 mg, folic acid 0.75 mg, biotin 0.20 mg, choline 1100 mg and calcium pantotenate 15 mg, Fe 100 mg, Cu 10 mg, Zn 108 mg, Mn 120 mg, I 1.5 mg and Se 0.35 mg.

*bThe chemical composition was measured, while metabolisable energy was calculated by Tables of Feed Composition and Nutritive Values in China (2018 Twenty-Ninth Edition).
Carcase characteristics

At 42 d, one bird per replicate was randomly taken. All birds were fasted for 12 h and weighed (live weight) and electrically stunned (110 V, 350 Hz). After exsanguination via severing the jugular vein and carotid artery on one side of the neck and defeathering via scalding in a 65–70 °C water 30–60 s, the carcase weight was measured. Eviscerated weight was determined after removing the head, feet, and organs except the lungs and kidneys in the carcase. During evisceration, abdominal fat pads were removed and weighed. Then, breast (including pectoralis major and pectoralis minor) and leg muscles (including thigh and drumstick) were analysed. The indices of carcase, eviscerated, breast muscle, leg muscle and abdominal fat percentages were calculated as follows:

Carcase percentage \(\%\) = \(\frac{\text{carcase weight}}{\text{live weight}}\) × 100

Eviscerated percentage \(\%\) = \(\frac{\text{eviscerated weight}}{\text{live weight}}\) × 100

Breast or leg muscle percentage \(\%\) = \(\frac{\text{ambilateral breast or leg muscle weight}}{\text{eviscerated weight}}\) × 100

Abdominal fat percentage \(\%\) = \(\frac{\text{abdominal fat weight}}{(\text{eviscerated weight} + \text{abdominal fat weight})}\) × 100

Meat quality

The muscles from the breast and thighs were used to determine drip loss, water loss, cooking loss, shear force, pH at 45 min and 24 h after euthanasia (pH\(_{45\text{min}}\) and pH\(_{24\text{h}}\), respectively) and meat colour. The drip loss was measured according to the methods of Honikel (1998). Briefly, the muscle samples were trimmed to a 5 × 2 × 1 cm in size, weighed and then hung in plastic bottles at 4 °C. After 24 h, the excess moisture was wiped off and the samples were weighed again. The drip loss was calculated as the percentage of muscle weight change measured between 0 and 24 h. Water loss was determined using the filter paper press method as described by Farouk et al. (2004). Samples were weighed before and after being subjected to a 35 kg force for 5 min using a pressure instrument (RH-1000, Guangzhou Runhu Instrument Co., Ltd, Guangzhou, China). Determination of cooking loss was made by recording muscle weight loss before and after cooking in an 85 °C water bath for 15 min (Moyo et al. 2020). After the cooking loss was determined, the samples were used to determine the shear force according to the methods of Moyo et al. (2020). The cooked muscle samples were manually trimmed into long strips (3 cm length × 1 cm width × 1 cm thickness) along the direction parallel to the muscle fibre. The strips were sheared perpendicular to the muscle fibre using a texture analyser (C-LM3, Tenico International Co., Ltd, Beijing, China). The test speed was 5 mm/s. Each muscle sample was measured three times, and the average value was taken as the shear force of the samples. The pH values of muscle samples were measured at 45 min (pH\(_{45\text{min}}\)) and 24 h (pH\(_{24\text{h}}\)) after slaughtering via portable pH metre (Corning Glass Works, Medfield, MA) (Shang et al. 2014). The meat colour was evaluated by the measurement of L\(^*\) (lightness), a\(^*\) (redness) and b\(^*\) (yellowness) colour values using a Minolta colour-guide (BYK-Gardener GmbH, Geretsried, Germany).

Statistical analysis

All data are expressed as the means and SEM. Data on growth performance and apparent nutrient digestibility were based on the entire broiler pen, whereas the other data were based on individual broilers. Data were analysed using the GLM procedure of SAS version 9.4 (SAS Institute 2000; Cary, NC). The model used was:

\[ Y_{ij} = \mu + D_i + E_{ij} \]

where \(Y_{ij}\) is the dependent variable, \(\mu\) is the population mean, \(D_i\) is the effect of FCAL supplementation level (0, 2, 4 or 8 g/kg) and \(E_{ij}\) is the random error. The significance of the differences among the treatments was tested using Tukey’s multiple range test. Orthogonal polynomial contrasts were applied to determine the linear (L) and quadratic (Q) effects of increasing the level of FCAL. Differences were considered to be statistically significant when \(p < .05\).

Results

Growth performance

During the starter period, there was a linear and quadratic influence of FCAL supplementation on ADG and FCR \((p < .05)\). Compared with the CON group, broilers fed 4 and 8 g/kg FCAL had higher \((p < .0001)\) ADG. The FCR of broilers in the 8 g/kg FCAL group was lower \((p = .0014; \text{Table 2})\).
During the finisher period, there was a quadratic influence of FCAL inclusion on ADG (p < .0001) and FCR (p = .0222). Dietary supplementation of 2 and 4 g/kg FCAL significantly increased the ADG of broilers (p < .0001). Broilers fed 2 g/kg FCAL had a lower (p = .0001) FCR than the CON group.

Throughout the study, there were linear (p < .0041) and quadratic (p = .0136) effects of FCAL supplementation on the ADG of broilers. Broilers fed 4 or 8 g/kg FCAL had higher (p < .0001) ADG than the CON group.

**Apparent nutrient digestibility**

During the starter period, there were linear (p < .0001) and quadratic (p < .0001) effects on apparent DM and CP digestibility and a linear (p = .0166) effect on apparent EE digestibility of broilers (Table 3). Broilers fed the 4 or 8 g/kg FCAL had higher (p < .0001) apparent DM, CP and EE digestibility compared with the CON group. Supplementation with 2 g/kg FCAL significantly increased apparent EE digestibility of broilers (p < .0001).

During the finisher period, there was a quadratic (p < .0002) effect of FCAL inclusion on apparent DM digestibility. Compared with the CON group, broilers fed FCAL had higher (p < .0001) apparent DM digestibility. The apparent DM, CP and EE digestibility of broilers fed 2 g/kg FCAL was higher (p < .0001).

**Serum immune indices**

As shown in Table 4, orthogonal polynomial contrast analysis revealed that the serum IGF-1 and IgM content responded in a dose-dependent linear and quadratic manner with FCAL supplementation at 21 d (p < .05). In response to the FCAL dose, the serum IL-1β, IL-8 and IL-10 levels quadratically decreased (p < .05) and the serum IgA level quadratically increased (p < .0068). Compared with the CON group, broilers fed 8 g/kg FCAL had an increased (p < .0318) serum IGF-1 level. Supplementation with 2 and 4 g/kg FCAL significantly decreased (p = .0004) the serum IL-8 concentration, and the 2 g/kg FCAL group had a lower (p < .05) serum IL-10 concentration. FCAL groups had a lower (p < .05) serum IL-1β concentration, while they had a higher (p = .0045 and p = .0007, respectively) serum IgA and IgM concentration.

As shown in Table 5, orthogonal polynomial contrast analysis revealed that the serum IGF-1 and IgA content increased in response to FCAL dose in a

### Table 2. Effects of fermented Chenopodium album L. (FCAL) supplementation on growth performance of broilers.

| Items                        | FCAL levels (g/kg) | SEM | p Value |
|------------------------------|-------------------|-----|---------|
|                              | 0                 | 2   | 4       | 8     | Treatment | Linear | Quadratic |
| Initial body weight          |                   |     |         |       |           |        |           |
| Starter period (1–21, d)     |                   |     |         |       |           |        |           |
| Average daily gain           |                   |     |         |       |           |        |           |
| Average daily feed intake    |                   |     |         |       |           |        |           |
| Feed conversion ratio        |                   |     |         |       |           |        |           |
| Finish period (22–42, d)     |                   |     |         |       |           |        |           |
| Average daily gain           |                   |     |         |       |           |        |           |
| Average daily feed intake    |                   |     |         |       |           |        |           |
| Feed conversion ratio        |                   |     |         |       |           |        |           |
| Whole period (1–42, d)       |                   |     |         |       |           |        |           |
| Average daily gain           |                   |     |         |       |           |        |           |
| Average daily feed intake    |                   |     |         |       |           |        |           |
| Feed conversion ratio        |                   |     |         |       |           |        |           |

**Values with different superscripts indicate a significant difference (p < .05).**

### Table 3. Effects of fermented Chenopodium album L. (FCAL) supplementation on apparent nutrient digestibility of broilers.

| Items                        | FCAL levels (g/kg) | SEM | p Value |
|------------------------------|-------------------|-----|---------|
|                              | 0                 | 2   | 4       | 8     | Treatment | Linear | Quadratic |
| Dry matter                   |                   |     |         |       |           |        |           |
| Crude protein                |                   |     |         |       |           |        |           |
| Ether extract                |                   |     |         |       |           |        |           |
| Finish period (40–42, d)     |                   |     |         |       |           |        |           |
| Dry matter                   |                   |     |         |       |           |        |           |
| Crude protein                |                   |     |         |       |           |        |           |
| Ether extract                |                   |     |         |       |           |        |           |

**Values with different superscripts indicate a significant difference (p < .05).**
quadratic manner at 42 d ($p < .05$), while IL-10 content decreased in a dose-dependent quadratic manner ($p = .0014$). The FCAL groups had higher serum IGF-1 and IgA levels than the CON group ($p = .0013$ and $p = .0025$, respectively). Dietary supplementation with 2 or 4 g/kg FCAL significantly decreased ($p = .0052$) the serum IL-10 level.

### Carcase characteristics

The percentage of breast muscle increased ($p < .05$) in a dose-dependent linear and quadratic manner with FCAL treatment (Table 6). Broilers in the 8 g/kg FCAL group had a higher ($p = .0047$) percentage of breast muscle than the CON group. Dietary supplementation with FCAL had no effect ($p > .05$) on the carcase, eviscerated, leg muscle and abdominal fat percentages of broilers.

### Meat quality

As shown in Table 7, orthogonal polynomial contrast analysis revealed that the drip loss of the breast muscle decreased in response to FCAL dose in a linear and quadratic manner ($p < .05$). The water loss and shear force of the breast muscle also decreased in a dose-dependent linear manner ($p < .05$). Compared
with the CON group, broilers in the 4 and 8 g/kg FCAL groups had decreased breast muscle drip loss (p < .001). The FCAL groups had a lower water loss rate and shear force of breast muscle (p < .05).

In response to dose, the water loss rate of leg muscle linearly and quadratically decreased (p < .05), and the L* of the leg muscle quadratically decreased (p = .00208) (Table 8). The water loss rate and L* of the leg muscle were lower in the FCAL groups than in the CON group.

Discussion

The growth-promoting effects of HPs on broilers have been extensively reported (Wu et al. 2015; Niu et al. 2017). As a medicinal herbs, the growth-promoting effect of CAL has been reported in rats with mercury-induced oxidative stress (Jahan et al. 2019). Arora et al. (2014) had demonstrated that oral gavage CAL acetone extract significantly prevented the body weight loss of arthritic rats. Similarly, Sikarwar et al. (2017) also proved that feeding CAL aqueous extract to rats with kidney stones could decrease their weight loss. In this study, dietary FCAL supplementation significantly increased the ADG of broilers during the starter, finisher and whole periods. However, there have been no reports to the best of our knowledge on the effects of FCAL on broilers, thus, it is difficult to make any direct comparison. It is suggested that the growth-promoting effects of HPs might partly be attributed to bioactive components, such as polyphenols and polysaccharides (Gopi et al. 2020; Long et al. 2021). Oloruntola et al. (2018, 2020) indicated that polyphenols, flavonoids and saponins extracted from medicinal herbs could scavenge free radicals and maintain intestinal epithelial barrier integrity. It is believed that the structural and functional homeostasis of the gastrointestinal tract is important for nutrient digestion and absorption as well as the growth performance of broilers (Cheled-Shoval et al. 2011; Wang et al. 2020). As reported in a previous study (Rahiminejad and Gornall 2004; Gao 2018; He et al. 2018), CAL contains abundant bioactive ingredients (polysaccharides, polyphenols, flavonoids and γ-amino butyric acid). Furthermore, the polyphenols and polysaccharide levels of CAL were increased by fermentation with compound bacteria cooperating pectinase in this study. Thus, the performance-promoting effects of FCAL could be related to the improved nutrient digestibility, which was confirmed by the increased improved apparent digestibility of DM, CP and EE. Moreover, the increased serum IGF-1 level of broilers fed with FCAL was observed in this study. The positive relationship between growth performance and plasma IGF-1 content has been indicated in a previous study (Kareem et al. 2016).

The determination of nutrients digestibility is a quantitative assessment of digestion and absorption

| Items                        | FCAL levels (g/kg) | SEM | Treatment | Linear | Quadratic |
|-----------------------------|-------------------|-----|-----------|--------|-----------|
| Drip loss, %                | 2.20a             | 0.038 | <.0001    | <.0001 | <.0001    |
| Water loss rate, %          | 16.22a            | 0.359 | <.0001    | .3228  | .0055     |
| Cooking loss, %             | 27.51             | 0.227 | .4476     | .1912  | .2579     |
| Shear force, N              | 59.01a            | 0.207 | .0108     | .4030  | .0093     |
| pH45, min                   | 6.79              | 0.044 | .8647     | .4221  | .6900     |
| Lightness (L*)              | 47.47             | 0.129 | .2717     | .0516  | .1428     |
| Redness (a*)                | 5.26              | 0.100 | .8759     | .5308  | .8064     |
| yellowness (b*)             | 12.00             | 0.166 | .2431     | .0838  | .2021     |

abcValues with different superscripts indicate a significant difference (p < .05).
functions as well as health status of the gastrointestinal tract in broilers. Furthermore, it is regarded as an important evaluation index for feed suitability (Arlinghaus and Niesar 2005). In this study, supplementation with FCAL increased the apparent digestibility of DM, CP and EE of broilers. The beneficial effects of HPs on the nutrient digestibility of broilers have been established in many studies. The apparent CP digestibility of broilers was shown to increase with peppermint essential oil (Emami et al. 2012) and Nigella sativa seed (Rahman and Kim 2016). The improvement in nutrient digestibility may be partly explained by increased secretions of saliva and bile and enhanced enzyme activity, which are stimulated by polyphenols extracted from medicinal herbs (Lee et al. 2014; Amodeo et al. 2019). In this study, the polyphenols of CAL were released after fermentation with compound probiotics and pectinase. Although pectin content was not detected, we speculated that FCAL could enhance the nutrient digestibility of broilers by increased polyphenol levels and decomposed pectin.

Cytokines are regulators of host responses to infection, immune response and inflammation. Some cytokines (IL-1β, IL-8 and TNF-α) promote inflammation and are called proinflammatory cytokines, whereas others cytokines (IL-10 and IFN-γ) suppress the activity of proinflammatory cytokines and are called anti-inflammatory cytokines (Berger 2000). In this study, the levels of IL-1β and IL-8 of broilers were decreased by dietary FCAL supplementation. It is suggested that FCAL could reduce the likelihood of inflammation in broilers. Previous studies have pointed out the anti-inflammatory effects of CAL in rats with arthritis (Arora et al. 2014; Amodeo et al. 2019) and ethanol-induced gastric lesion rats (Kim and Jeong 2011). Similarly, Yao et al. (2014) found that saponin extracted from Quinoa belonging to the family Chenopodiaceae could suppress the production of TNF-α and IL-6 in LPS-induced RAW264.7 macrophage cells. Furthermore, the extracts of CAL could inhibit NF-κB expression (Arora et al. 2014) and NO production (Amodeo et al. 2019), which consequently suppresses inflammation (Lee et al. 2009). In this study, the serum IL-10 content also decreased in the 2 and 4 g/kg FCAL groups. As a kind of anti-inflammatory cytokine, IL-10 plays a crucial role in suppressing pro-inflammatory processes in CD4+ T cells (Bedke et al. 2019). The lower serum IL-10 concentration of broilers in this study might be attributed to the low degradation of the inflammatory condition.

Igs produced by B cells are the key components of humoral immunity (Mast et al. 2000). The positive correlation between antibody contents (IgA, IgM and IgG), and immunity and disease resistance of animals has been well-demonstrated (Bi et al. 2020; Gong et al. 2020). In this study, dietary supplementation with FCAL promoted the humoral immune responses of broilers, which was confirmed by increased serum IgA and IgM concentrations at 21 d and serum IgA concentration at 42 d. Similar results were reported by Estrada et al (1998), who found that saponins from Quinoa could enhance specific Igs (IgG and IgA), which responded to the antigens in the serum, and in the intestinal and lung secretions of mice. The saponins from Quinoa also remarkably increased the serum IgG content of the immunised mice (Verza et al. 2012). Furthermore, previous studies have shown that the bioactive substance in HPs could enhance humoral immune response of animals by stimulating B cell proliferation and Ig production (Han et al. 2003; Kong et al. 2007; Wu 2018; Long et al. 2020; Long et al. 2021).

In this study, the breast muscle percentage was significantly increased by 8 g/kg FCAL. The better carcase quality of the broilers might be due to improved nutrient deposition (Li et al. 2019). The positive effects of FCAL on ADFI, FCR and nutrient digestibility may be responsible for the increased breast meat content. In agreement with our results, previous studies have reported that supplementation with HPs, such as lycopene and fermented ginkgo biloba leaves, in the diet improves the growth and carcase yield of broilers (Sahin et al. 2006; Niu et al. 2017).

Drip, cooking, and water loss rates are important indicators to measure the water-holding capacity of breast and leg muscles (Cheng et al. 2019). A low water-holding capacity in muscles can increase the liquid outflow and lead to the loss of soluble nutrients and flavour (Xing et al. 2020). The results of this study showed that the addition of FCAL decreased the drip loss of the breast muscle and water loss rate of the breast and leg muscles, which was consistent with the results of Wang et al. (2015), who reported that the drip loss of broiler breast and thigh meat was decreased by turmeric rhizome extract supplementation. The manner in which the supplementation of
FCAL improved the water-holding capacity in the muscles of broilers might be related to the increase in polyphenols levels, which exhibited strong free radicals scavenging activity and reduced oxidative-induced conformational alterations and fragmentation of myofibrillar proteins (Patel et al. 2010). Moreover, shear force is an important variable that reflects the tenderness of meat products (Pascual Guzmán et al. 2021). The results of this study indicated that FCAL addition to the diet of the broilers decreased the shear force of the breast muscle, which is in accordance with the findings of Rahman and Kim (2016), who reported that dietary supplementation with *Nigella sativa* seeds decreased the shear force of thigh muscle. It is suggested that the decreased shear force might be caused by the improved water-holding capacity (Webb and Agbeniga 2020). The colour of meat affects the meat-purchasing decisions of consumers, thus is a useful criterion for meat quality assessments (Barbut S 1993). In this study, the inclusion of 2–8 g/kg of FCAL in the diet also decreased the value of L* in the leg muscle. The changes in the L* value can be attributed to the higher water-holding capacity, resulting in lower surface light reflectivity (Hughes et al. 2014). However, desirable changes in the pH value at 45 min and 24 h post-slaughter were not observed, and this requires further investigation.

**Conclusions**

In conclusion, dietary supplementation with a 2–8 g/kg FCAL basal diet significantly improved the growth performance, nutrient digestibility, immunity and meat quality of broilers. Furthermore, the dietary addition of 8 g/kg FCAL could significantly increase the breast muscle percentage. Overall, FCAL may be used as a reliable phytogenic feed additive to promote growth and maintain the health of broilers.

**Ethics statement**

All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in China (The State Science and Technology Commission of China, 1988). Animal care, slaughter and sampling of broiler chickens used in this study passed through the process of Inner Mongolia University Animal Science Care and Use Committee.

**Software and data repository resources**

None of the data were deposited in an official repository.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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