Data comparison of the effects of feeding ginkgo leaf fermentation and Chinese herbal medicine on meat ducks

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Abstract. In order to study the effect of feeding Ginkgo leaf fermentation and Chinese herbal medicine on meat duck, we added Ginkgo leaf fermentation and Chinese herbal medicine additive to the daily feed of meat ducks, and set up a control group (basic feed), a western medicine group (basic feed + 150mg/kg aureomycin), an experimental group 1 (basic feed + Chinese herbal medicine 0.1%) and an experimental group 2 (basic feed + 0.1% Chinese herbal medicine + 2.5% Ginkgo leaf ferment). We fed the ducks for 45 days, and then completed the measurement of production performance and blood index. Based on the measurement method of related data, we used Excel and spss25 software for data statistics and analysis. The results showed that the diet supplemented with 0.1% Chinese herbal medicine and 2.5% Ginkgo leaf fermentation could significantly increase daily growth weight of meat ducks during the whole growth period (P<0.05), at the same time, it could reduce feed conversion ratio, and the content of total protein and albumin in serum could be significantly increased (P<0.05). The value of alkaline phosphatase in the experimental group was lower than that of the control group. The amount of immunoglobulin and complement in the serum were all higher in the experimental group than in the control group. The overall effect of Ginkgo leaf fermentation and Chinese herbal medicine in basic diet was significantly better than that of western medicine group (P<0.01).

1. Introduction

Ginkgo is a unique economic plant resource in China. The results show that, after processing, the content of ginkgolic acid is relatively higher. Although ginkgolic acid has a wide range of biological activities [1], such as anti-tumor, anti-allergy, anti-inflammatory, etc., it also has considerable toxicity to liver, kidney and embryo [2-3]. Experiments and research have proved that, after the components in Ginkgo leaves are decomposed and metabolized by some microorganisms, not only the content of active components such as flavonoids and lactones will increase, but also the decomposition and transformation of ginkgolic acid will be promoted, achieving the purpose of increasing efficiency and reducing toxicity for its components [4].

Domestic medical research and practice show that Chinese herbal medicine also plays a very important role in promoting immunity and animal growth [5]. Studies have shown that using microbial fermentation can promote the separation of effective ingredients in Chinese herbal medicine, and reduce the toxicity and side effects of Chinese herbal medicine [6]. Using
traditional Chinese medicine or traditional Chinese medicine dregs as fermentation substrate and solid fermentation method for fermentation, not only the cost is relatively low, but also the operation is more convenient [7]. Therefore, we consider using solid fermentation method to process Ginkgo biloba leaves and Chinese herbal medicines, and make feed additives to test whether it has a favorable impact on the growth performance and serum indicators of ducks.

2. Materials and methods

2.1. Main test animals and materials
The test animal materials: Suyou No.2 meat duck [9], which is from Jiangsu Fengda waterfowl breeding farm. Gaoyou duck and wild duck were used as parents. After crossbreeding, cross fixation and generation selection, a new breed of meat duck with good meat production performance was obtained.

The test materials: Ginkgo leaves collected from May to August. Chinese herbal medicines were mainly (Fleece-flower root: Astragalus: Rhizoma atractylodis =1:1:1). The kit was purchased from Jiangsu Nanjing Jiancheng Bioengineering Research Institute.

2.2. Test methods
Ferment the ginkgo leaves for 0, 24, 36 and 48 hours respectively. After fermentation, add it to basic feed according to 2.5% and add Chinese herbal medicine to basic feed according to 0.1%. Based on the basic feed as the control, determine the nutrient composition, and finally select the ginkgo leaf fermented for 24 hours as the additive. Set up western medicine group: add 150mg of chlortetracycline per kilogram of feed; test group 1: basic feed + Chinese herbal medicine 0.1%; test group 2: basic feed + 0.1% Chinese herbal medicine + 2.5% Ginkgo leaf fermentation; control group: only basic feed. Select seven-day old healthy ducks and randomly divide them into four groups, 20 in each group. Then we carried out a 45-day experiment and determine the initial relevant indicators.

2.2.1 Analysis of feed composition
We use conventional methods to determine water, crude ash, crude fat, crude fiber and crude protein. Determination of flavonoids: take 4g of each group of samples and then extract it by Soxhlet extraction by using 70% ethanol for 4h, and then evaporate to obtain Ginkgo biloba extract, anddissolve it with 70% ethanol, take 1ml each, and then add 0.3ml sodium nitrite solution first, mix well, and let it stand for 6min. Then add 0.3ml aluminum nitrate solution, mix well, and let it stand for 6min. Then add 4ml sodium hydroxide solution, mix well, and let it stand for 6min. Finally, use 70% ethanol to determine the volume. After making rutin standard solution, use spectrophotometer to measure it and draw standard curve. Then, determine the sample extract and calculate the flavone content. Determination of amino acid [8]: weigh a same amount of feed sample and dissolve it in 100mL 0.1mol/L HCl, filter it with 0.22μm filter membrane, inject 10μL, and finally get the result.

2.2.2 Determination of production performance
Record the beginning weight and end weight and daily gain of ducks, and calculate the feed conversion ratio. Determine the content of crude protein and crude fiber in feces.
2.2.3 Determination of the content of crude protein and crude fiber in feces
Collection and preparation of fecal samples: fecal samples were taken three times a day at 8:30 in the morning, 2:30 in the afternoon and 8:30 in the evening. Take about 5-10g of fresh feces each time, add 2.5ml of 10% tartaric acid solution to every 10g of fresh feces and mix it well, and after mixing the three samples, dry them at 75 ℃. We took samples for 3 consecutive days, and we took the sample mixture as the fecal sample.

2.2.4 Determination method for immune index
Collect 5 ml blood from the vein under the armpit of duck. The blood sample was centrifuged at 3000r/min for 15min. Take the supernatant, sub-pack it and label it, and store it at -20 ℃.

(1) Determination of total protein by biuret method
Sample: take 50μl serum for determination.
Calculation formula:

\[
\text{Total protein content} \ (g/L) = \frac{\text{measured OD value} - \text{blank OD value}}{\text{standard OD value} - \text{blank OD value}} \times \text{Concentration of total protein standard} (g/L) \times \text{Dilution ratio before sample determination}
\]

Note: the concentration of protein standard is 52.4g/L. OD is optical density

(2) Determination of albumin by bromocresol green colorimetry
Sample: take 10μl serum for determination.
Calculation formula:

\[
\text{ALB content} \ (g/L) = \frac{\text{measured OD} - \text{blank OD}}{\text{standard OD} - \text{blank OD}} \times \text{Concentration of standard} (g/L) \times \text{Dilution ratio before sample determination}
\]

Note: the concentration of the standard is 28.9g/L.

(3) Determination of alkaline phosphatase (AKP)
Sample: take 50μl serum for determination (2-time dilution)
Calculation formula:

\[
\text{Alkaline phosphatase (king unit / 100ml)} = \frac{\text{measured tube OD value} - \text{blank tube OD value}}{\text{standard tube OD value} - \text{blank tube OD value}} \times \text{Phenol content of standard tube} (0.005mg) \times \frac{100ml}{0.05ml}
\]

(4) Determination of uric acid
Sample: take 0.1ml serum for determination
Calculation formula:

\[
\text{Serum uric acid} \ (mg/L) = \frac{\text{measured tube absorbance value}}{\text{standard tube absorbance value}} \times \text{Standard tube content} (50mg/L)
\]

(5) Determination of urea
Sample: take 0.02ml serum for determination
Calculation formula:

\[
\text{Blood urea nitrogen content} \ (mmol/L) = \frac{\text{measured tube OD value} - \text{blank tube OD value}}{\text{standard tube OD value} - \text{blank tube OD value}} \times \text{Concentration of standard} (mmol/L)
\]

(6) IgA, IgM, IgG, complement C3, C4, IL-2
Sample: take 50μl serum for determination
Kit: Duck Immunoglobulin A (IgA) Elisa; Duck Immunoglobulin G (IgG) Elisa; Duck Immunoglobulin M (IgM) Elisa; Duck Complement Component 3 (C3) Elisa; Duck Complement Fragment 4 (C4) Elisa; Duck Interleukin 2 (IL-2) Elisa.

2.3. Data analysis
Use Excel and spss25 software to carry out data statistics and analysis, and use One-Way ANOVA and t-test (test level $\alpha=0.05$) for the comparison between groups.

3. Result analysis

3.1. Analysis results of feed nutrients

| Grouping of experiments | Water content | Crude ash | Crude fat | Crude fiber | Crude protein | Total flavonoids% |
|-------------------------|---------------|-----------|-----------|-------------|---------------|------------------|
| Basic feed              | 12.60         | 5.26      | 3.42      | 4.49        | 15.31         |                  |
| Chinese herbal medicine | 10.96         | 19.97     | 0.84      | 15.40       | 5.40          |                  |
| 0.1% 2.5% Ginkgo leaves |               |           |           |             |               |                  |
| (fermented for 24 hours)| 9.66          | 13.30     | 8.59      | 8.86        | 13.50         | 5.56             |
| (fermented for 36 hours)| 9.42          | 13.60     | 7.35      | 11.87       | 13.70         | 3.33             |
| (fermented for 48 hours)| 10.09         | 14.03     | 9.26      | 15.40       | 14.05         | 3.26             |

According to the analysis results of feed nutrients in Table 1 and Table 2, the Chinese herbal medicine group and ginkgo leaf fermentation group are more comprehensive in nutrients than the basic feed. The total content of flavonoids and amino acids are significantly higher than that of the non-fermented ginkgo leaves.
3.2. Test results of production performance

Table 3. Daily gain and feed conversion ratio of meat ducks

| Group              | Average initial weight / g | Average final weight / g | Average daily gain / g | Feed conversion ratio |
|--------------------|----------------------------|--------------------------|------------------------|-----------------------|
| Control group      | 176.43±5.42a               | 1489.47±59.79a           | 29.18±1.28a            | 5.93a                 |
| Western medicine   | 197.50±8.73b               | 1802.63±8.21a            | 35.66±0.17b            | 5.61b                 |
| Experimental group 1| 203.13±4.13b              | 2383.97±152.14b          | 48.46±3.38c            | 5.34b                 |
| Experimental group 2| 196.25±5.00c              | 2470.15±115.45c          | 50.53±2.59c            | 5.18c                 |

Note: the different letters in the upper right corner of the data in the same row in the table indicate that the difference is significant (P<0.05).

For the daily weight gain effect, test group 2 > test group 1 > western medicine group, that is to say, the feed with basic feed + 0.1% Chinese herbal medicine + 2.5% gingko leaf ferment as formula is better than the basic feed with single addition of 0.1% Chinese herbal medicine, which can effectively reduce the feed conversion ratio (Table 3).

3.3. The content of crude protein in duck feces

From Figure 1, we can see that adding western medicine, Chinese herbal medicine and Ginkgo feed additives can promote the digestion and absorption ability of ducks, and the promotion effect of experimental group 2 > experimental group 1 > western medicine group, that is to say, when the duck is fed with the diet with basic feed + 0.1% Chinese herbal medicine + 2.5% Ginkgo leaf ferment as the formula, the digestion and absorption ability of meat ducks is the best.
3.4. Test results of immune indexes

Table 4. Index results of duck serum

| Grouping                              | Control group            | Western medicine group | Experimental group 1 | Experimental group 2 |
|---------------------------------------|--------------------------|------------------------|----------------------|----------------------|
| Total protein (g/L)                   | 27.93±0.31<sup>a</sup>  | 32.05±0.42<sup>b</sup> | 35.15±0.35<sup>bc</sup> | 38.49±0.36<sup>c</sup> |
| Albumin (ALB) (g/L)                   | 10.57±0.22<sup>a</sup>  | 10.83±0.25<sup>a</sup> | 11.17±0.28<sup>b</sup> | 11.40±0.32<sup>b</sup> |
| Alkaline phosphatase (AKP) (King unit/100ml) | 47.96±0.75<sup>d</sup> | 42.34±0.73<sup>c</sup> | 26.85±0.72<sup>a</sup> | 25.39±0.67<sup>a</sup> |
| Uric acid (mg/L)                      | 49.02±0.02<sup>a</sup>  | 44.61±0.03<sup>b</sup> | 40.69±0.02<sup>b</sup> | 32.35±0.02<sup>c</sup> |
| Urea nitrogen (BUN) (mmol/L)          | 1.70±0.21<sup>a</sup>   | 1.39±0.24<sup>b</sup>  | 1.11±0.35<sup>c</sup> | 0.98±0.03<sup>d</sup>  |
| IgA (mg/ml)                           | 0.8165±0.02<sup>a</sup> | 0.9552±0.11<sup>ab</sup> | 1.1107±0.13<sup>b</sup> | 1.2796±0.15<sup>c</sup> |
| IgG (mg/ml)                           | 4.6399±0.41<sup>a</sup> | 5.531±0.29<sup>b</sup>  | 7.1393±0.19<sup>d</sup> | 7.6786±0.16<sup>de</sup> |
| IgM (mg/ml)                           | 0.5993±0.02<sup>a</sup> | 0.7498±0.01<sup>b</sup> | 0.859±0.01<sup>c</sup> | 0.9039±0.02<sup>d</sup> |
| C3 (ng/L)                             | 196.848±1.32<sup>a</sup> | 220.27±0.57<sup>b</sup> | 256.1904±1.29<sup>c</sup> | 408.8624±1.98<sup>e</sup> |
| C4 (ng/L)                             | 102.7504±0.98<sup>a</sup> | 113.9385±0.86<sup>b</sup> | 147.285±0.69<sup>cd</sup> | 159.5384±0.39<sup>d</sup> |
| IL-2 (ng/L)                           | 15.7699±0.03<sup>a</sup> | 21.0471±0.04<sup>b</sup> | 30.4405±0.04<sup>c</sup> | 33.6055±0.02<sup>d</sup> |

Note: the different letters in the upper right corner of the data in the same row in the table indicate that the difference is significant (P<0.05)

Compared with the control group, the test group has a increasing function in the total protein, albumin, immunoglobulin, complement and interleukin, and has a decreasing funktion in AKP, uric acid and urea nitrogen. The effect of the test group 2 > the test group 1 > the western medicine group (Table 4).

4. Discussion

4.1. The effect of fermentation on the nutritional components of Ginkgo leaves

Ginkgo leaves is a kind of abundant biological resources in China, and it has gradually produced scale in modern production. At present, fermentation is the most effective and practical method for the development of Ginkgo resources. The experiment results show that microbial fermentation can increase the content of vitamin, enzyme and growth factor [10]. In this experiment, we also found that the total flavonoids of Ginkgo leaves treated by solid-state fermentation method, especially those fermented for 24 hours, were significantly better than those before fermentation. And the amino acid content of the fermented Ginkgo leaves was higher than that of the non-fermented ginkgo leaves.

4.2. Effects of feeding of fermented Ginkgo leaves and Chinese herbal medicine on growth performance of ducks

The results of this experiment show that feeding ducks with 0.1% Chinese herbal medicine and 2.5% Ginkgo leaf ferment can significantly increase the daily growth of ducks during the whole growth period, and at the same time reduce the feed conversion ratio, which is directly related to the rich active ingredients in the extracts of Ginkgo leaf and Chinese herbal medicine, which can promote the growth and protein synthesis of ducks. This can be concluded from the digestibility of crude protein.
Because Ginkgo leaves are rich in flavonoid compounds, flavonoid of traditional Chinese medicine has always had highly-effective and low-toxic functions in actual animal production, and it has a broad development prospect [11]. At the same time, after 24 hours of fermentation, the flavonoids extracted were 90.08% higher than that of the unfermented ginkgo leaves. It is known that flavonoids can promote the growth of animals, and the content of extract increased after fermentation increases the promotion effect, which further proves that Ginkgo leaf fermentation products can be used as a new green and safe feed additive.

4.3. Effects of feeding of fermented Ginkgo leaves and Chinese herbal medicine on serum biochemical indexes of meat ducks

The results showed that the content of total protein and albumin in serum of ducks fed with basic feed supplemented with Ginkgo leaf ferment and Chinese herbal medicine could be significantly increased, and compared with the group only added with Chinese herbal medicine, the total protein increased nearly 9.5%. These two values can reflect the body’s digestion and metabolism of protein, so it can be concluded that adding Ginkgo leaf fermentation and Chinese herbal medicine in daily feed can promote the growth of ducks. Alkaline phosphatase, which can reflect the body skeleton and hepatobiliary system disease, was lower in the test group than in the control group, and the indexes of uric acid and urea nitrogen, which can reflect the function of kidney, were also lower. It indicated that ginkgo leaf ferment and Chinese herbal medicine added to the feed had the function of health care for the duck, liver and kidney. At the same time, the amount of immunoglobulin and complement in serum were higher in the test group than in the control group, which significantly improved the antioxidant capacity and immune capacity of ducks. And through the comparison between the test group 1 and the western medicine group, the overall effect of the test group 1 is better than that of the western medicine group, but the difference is not significant, while the overall effect of the test group 2 and western medicine group is better than that of other groups, and the difference is significant (p<0.05). It can be concluded that Ginkgo leaf fermentation has a great influence on the growth performance and blood index of ducks.

5. Conclusion

The combination of Ginkgo leaf fermentation and traditional Chinese medicine can not only accelerate the digestion and absorption, but also accelerate the muscle growth, increase the antibacterial power of animals, and improve the immunity of the body. Because the traditional Chinese medicine is low toxic and has no side effects, it can make meat quality of meat ducks greener and healthier, and reduce people’s worry about too much antibiotic intake. The combination feeding of Ginkgo leaf fermentation and traditional Chinese medicine compound can play a great role in the modern green cultivation. There is a great development space for the promotion of the overall growth performance of meat ducks. At the same time, it has significant effect on improving serum biochemical indicators, and can effectively improve immunity and regulate liver and kidney functions of the body.

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