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Effects of Mulberry Leaf Powder and Fermented Mulberry Leaf Powder on Growth Performance, Serum Biochemical Parameters, and Antioxidant Indices in Bearded Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary mulberry leaf meal (MLM) and fermented mulberry leaf meal (FMLM) on growth performance, serum biochemical indices, and antioxidant indices of bearded chickens. A total of 392 Lingnan Yellow III bearded chickens at 95 days of age were selected and randomly divided into 7 groups according to body weight and sex, with 4 replicates per group and 14 chickens per replicate (half male and half female). The control group was fed a basal diet, while the 6 experimental groups were fed diets containing 5%, 10%, and 20% MLM and 5%, 10%, and 20% FMLM in the basal diet, respectively. The pre-trial period was 4 days, and the formal trial period was 28 days.

The results showed that: 1) The growth performance of bearded chickens gradually decreased with increasing dietary MLM levels; compared with the control group, different levels of MLM and FMLM in the diets significantly or extremely significantly reduced average daily gain ($P<0.05$ or $P<0.01$); the average daily feed intake of the 20% MLM group was significantly lower than that of the control group ($P<0.05$); the feed-to-gain ratio of all MLM level groups and the 20% FMLM group was significantly or extremely significantly higher than that of the control group ($P<0.05$ or $P<0.01$).

2) Serum alkaline phosphatase (ALP) activity in bearded chickens of all experimental groups was significantly lower than that of the control group ($P<0.05$), with the 10% MLM group showing the lowest serum ALP activity; serum total protein (TP) and globulin (GLO) contents in all experimental groups were higher than those of the control group, with the 10% MLM and 20% FMLM groups extremely significantly increasing serum TP and GLO contents ($P<0.01$); serum albumin-to-globulin ratio (A/G) in all

experimental groups was significantly lower than that of the control group (P<0.05); compared with the control group, serum total cholesterol (TC) and triglyceride (TG) contents in the 20% MLM group were significantly reduced (P<0.05).

3) Serum and liver total antioxidant capacity (T-AOC) in the 10% MLM group of bearded chickens was significantly higher than that of the control group (P<0.05), while serum and liver catalase (CAT) activity was significantly lower than that of the control group (P<0.05). Serum glutathione peroxidase (GSH-Px) activity in the 20% FMLM group was extremely significantly higher than that of the control group (P<0.01).

In conclusion, dietary MLM reduced the growth performance of bearded chickens, while FMLM showed improved effects on growth performance compared with MLM; both MLM and FMLM had effects such as reducing blood lipid content, enhancing immunity, and improving antioxidant capacity, but the optimal usage levels of both require further investigation.

Full Text

Effects of Mulberry Leaf Meal and Fermented Mulberry Leaf Meal on Growth Performance, Serum Biochemical Indexes and Antioxidant Indexes of Huxu Chickens

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Abstract

This experiment was conducted to investigate the effects of dietary mulberry leaf meal (MLM) and fermented mulberry leaf meal (FMLM) on growth performance, serum biochemical indexes, and antioxidant indexes of Huxu chickens. A total of 392 Lingnan Yellow III Huxu chickens at 95 days of age were randomly assigned to 7 groups based on body weight and sex, with 4 replicates per group and 14 birds per replicate (half male and half female). The control group was fed a basal diet, while the six experimental groups were fed diets containing 5%, 10%, or 20% MLM and 5%, 10%, or 20% FMLM, respectively. The pre-trial period lasted 4 days, and the experimental period lasted 28 days. The results showed: 1) The growth performance of Huxu chickens gradually decreased with increasing dietary MLM levels. Compared with the control group, different levels of MLM and FMLM significantly or extremely significantly reduced average daily gain (P<0.05 or P<0.01). The average daily feed intake in the 20% MLM group was significantly lower than that in the control group (P<0.05).

The feed-to-gain ratio in all MLM level groups and the 20% FMLM group was significantly or extremely significantly higher than that in the control group ($P<0.05$ or $P<0.01$). 2) Serum alkaline phosphatase (ALP) activity in all experimental groups was significantly lower than that in the control group ($P<0.05$), with the 10% MLM group showing the lowest serum ALP activity. Serum total protein (TP) and globulin (GLO) contents in all experimental groups were higher than those in the control group, with the 10% MLM and 20% FMLM groups showing extremely significant increases in serum TP and GLO contents ($P<0.01$). The albumin-to-globulin ratio (A/G) in all experimental groups was significantly lower than that in the control group ($P<0.05$). Compared with the control group, the 20% MLM group showed significantly reduced serum total cholesterol (TC) and triglyceride (TG) contents ($P<0.05$). 3) The 10% MLM group exhibited significantly higher total antioxidant capacity (T-AOC) in serum and liver compared with the control group ($P<0.05$), while serum and liver catalase (CAT) activity was significantly lower ($P<0.05$). The 20% FMLM group showed extremely significantly higher serum glutathione peroxidase (GSH-Px) activity compared with the control group ($P<0.01$). In conclusion, dietary MLM reduced the growth performance of Huxu chickens, while FMLM showed improved effects compared with MLM. Both MLM and FMLM reduced blood lipid contents, enhanced immune function, and improved antioxidant capacity, though the optimal inclusion levels require further investigation.

Keywords: Huxu chicken; mulberry leaf meal; fermented mulberry leaf meal; growth performance; serum biochemical indexes; antioxidant indexes

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Mulberry leaves, the foliage of *Morus* species, are traditional medicinal and edible plants rich in nutritional value and bioactive substances such as mulberry polyphenols, flavonoids, and polysaccharides, which possess antimicrobial, anti-inflammatory, sedative, and immune-enhancing properties. China currently has over 800,000 hectares of mulberry plantations, yet approximately half of the mulberry leaf yield is not used for silkworm rearing. According to incomplete statistics, the country' s developing ecological mulberry plantations have exceeded 530,000 hectares, but no rational and efficient utilization method has been identified. If these mulberry leaf resources could be used as livestock feed ingredients, it would not only prevent environmental harm but also alleviate the severe shortage of feed ingredients, particularly protein feed ingredients, in China, while extending the sericulture industry chain and promoting economic recovery in the sector.

Current research on the application of mulberry leaves in diets for pigs, chickens, cattle, and sheep primarily involves direct feeding in the form of mulberry leaf meal (MLM), demonstrating advantages such as improved livestock product quality, enhanced meat flavor, and promotion of animal health. However, mulberry leaves are rarely widely used as feed ingredients in practical production because they contain anti-nutritional factors such as tannins and lectins, which can reduce growth performance when added in large quantities. Therefore, ef-

fective processing methods for mulberry leaves have become a bottleneck for their extensive utilization in animal diets. Solid-state fermentation technology is one of the most effective means for processing feed ingredients in the feed industry. Consequently, this experiment compared the effects of fermented mulberry leaf meal (FMLM) processed through solid-state fermentation with MLM on growth performance, serum biochemical indexes, and serum and liver antioxidant indexes in Huxu chickens, providing a reference for the development and utilization of mulberry leaves and their processed products in animal husbandry.

1.1 Experimental Materials

MLM was prepared from fresh leaves of the "Da 10"variety harvested in May 2014 at Baosang Garden in Huadu, Guangzhou. The leaves were dried at 80°C and pulverized. The measured nutritional composition was: crude protein 18.00%, crude fat 3.10%, crude fiber 10.60%, crude ash 13.60%, calcium 2.68%, total phosphorus 0.29%, lysine 0.79%, and methionine 0.10%. FMLM was produced by mixing 80% MLM, 15% wheat bran, and 5% soybean meal, followed by co-fermentation with *Aspergillus sojae*, *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and anaerobic endophytes from mulberry leaves for 48 hours, then dried at 60°C and pulverized. Its measured nutritional composition was: crude protein 24.47%, crude fat 3.40%, crude fiber 7.80%, crude ash 10.80%, calcium 2.58%, total phosphorus 0.49%, lysine 0.97%, and methionine 0.17%.

1.2 Experimental Design and Management

A total of 392 healthy 95-day-old Lingnan Yellow III Huxu chickens were randomly allocated to 7 groups based on body weight and sex, with 4 replicates per group and 14 birds per replicate (half male and half female). The control group received a corn-soybean meal basal diet, while the six experimental groups received diets containing 5%, 10%, or 20% MLM and 5%, 10%, or 20% FMLM, respectively. Experimental diets were formulated according to the "Lingnan Yellow III Huxu Chicken Finisher Feed Nutritional Levels" recommended by the Institute of Animal Husbandry, Guangdong Academy of Agricultural Sciences, and the "China Agricultural Industry Standard (NY/T 33-2004) Nutritional Requirements for Yellow-Feathered Broiler Chickens." All experimental diets were in powder form. Diet composition and nutrient levels are shown in Table 1 . The pre-trial period lasted 4 days, and the experimental period lasted 28 days. Chickens were raised on the floor under conventional management with free access to feed and water.

Table 1 Diet Composition and Nutrient Levels (Air-Dry Basis)

Item	MLM Level/%	FMLM Level/%	Control Group
Ingredients			
Corn			
Soybean meal			

Item	MLM Level/%	FMLM Level/%	Control Group
Corn protein meal			
MLM/FMLM			
Soybean oil			
Ca(H PO)			
Limestone			
L-Lys · HCl			
DL-Met			
L-Thr			
Premix[1])			
Total			
Nutrient Levels[2])			
ME/(MJ/kg)			
CP			
CF			
Ca			
AP			
Lys			
Met			
Thr			
Met+Cys			

[1]) The premix provided per kilogram of diet: phytase 200 mg, nicarbazin+maduramycin 600 mg, antioxidant 200 mg, colistin 300 mg, 8% flavomycin 350 mg, AB enzyme (yellow powder) 400 mg, Kefulai 500 mg, multi-vitamins 250 mg, lysine 2 g, methionine 1.7 g, multi-minerals 1.5 g, choline 1.3 g, NaCl 2 g, NaHCO 1.5 g, Carophyll red (yellow powder) 400 mg, yellow powder 6.8 g.

[2]) Nutrient levels were all calculated values.

1.3 Measurement Indicators and Methods

Growth Performance: On a replicate basis, chickens were weighed after fasting at the beginning and end of the experiment, and feed intake was recorded throughout the experimental period to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

Serum Biochemical Indexes: At the end of the experiment, one male and one female chicken close to the average body weight were selected from each replicate (8 birds per group). Blood samples were collected from wing veins and centrifuged at 3,000 r/min for 15 min at 4°C. Serum was separated and stored at -20°C for analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities, and total protein (TP), albumin (ALB), globulin (GLO), glucose (GLU), urea nitrogen

(UN), uric acid (UA), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) contents were determined using an automatic biochemical analyzer with kits purchased from Shanghai Kehua Bioengineering Co., Ltd.

Antioxidant Indexes: After blood collection, the 8 chickens from each group were immediately slaughtered, and liver samples were weighed, packaged, frozen in liquid nitrogen, and stored at -80°C. Serum and liver total antioxidant capacity (T-AOC), malondialdehyde (MDA) content, and glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) activities were measured using a Multiskan GO full-wavelength microplate reader (Thermo) with kits purchased from Nanjing Jiancheng Bioengineering Institute, following the manufacturer' s instructions.

1.4 Data Processing and Analysis

Experimental data were analyzed using SPSS 19.0 software for analysis of variance, with LSD method for multiple comparisons. Results are expressed as means ± standard error. $P<0.01$ indicated extremely significant difference, and $P<0.05$ indicated significant difference.

2.1 Effects of Dietary MLM and FMLM Levels on Growth Performance of Huxu Chickens

As shown in Table 2 , compared with the control group, dietary inclusion of different MLM levels extremely significantly reduced the average daily gain of Huxu chickens ($P<0.01$), with average daily gain gradually decreasing as MLM inclusion increased. The effects of dietary FMLM on average daily gain were similar to those of MLM but showed improvement compared with MLM. The 10% and 20% FMLM groups showed extremely significant differences in average daily gain compared with the control group ($P<0.01$) and also compared with the 10% and 20% MLM groups ($P<0.01$). The 5% FMLM group showed a significant difference in average daily gain compared with the control group ($P<0.05$). As dietary MLM levels increased, average daily feed intake showed a gradual decreasing trend, with the 20% MLM group showing significantly lower average daily feed intake than the control group ($P<0.05$). Dietary FMLM had no significant effect on average daily feed intake compared with the control group ($P>0.05$). Dietary MLM and FMLM increased the feed-to-gain ratio, with the 10% and 20% MLM groups showing extremely significantly higher ratios than the control group ($P<0.01$), the 5% MLM and 20% FMLM groups showing significantly higher ratios ($P<0.05$), and the 10% and 20% MLM groups showing extremely significantly higher ratios than all other groups ($P<0.01$).

Table 2 Effects of Dietary MLM and FMLM Levels on Growth Performance of Huxu Chickens

Item	MLM Level/%	FMLM Level/%	Control Group
ADG/g	14.27±0.77	11.73±0.80	8.68±0.42
	7.73±0.46	12.14±0.49	11.95±0.62
	11.80±0.34		
ADFI/g	88.63±1.42	86.05±1.97	83.92±3.26
	81.63±1.56	88.67±0.96	84.11±2.32
	86.94±1.34		
F/G	6.27±0.36	7.40±0.34	9.68±0.27
	10.69±0.72	7.33±0.25	7.07±0.23
	7.38±0.12		

In the same row, values with different capital letter superscripts indicate extremely significant differences (P<0.01), different small letter superscripts indicate significant differences (P<0.05), and the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Dietary MLM and FMLM Levels on Serum Biochemical Indexes of Huxu Chickens

As shown in Table 3 , serum ALT activity in all experimental groups showed no significant difference compared with the control group (P>0.05), though the 5% MLM group showed significantly higher serum ALT activity than the 20% MLM and 10% FMLM groups (P<0.05). No significant differences in serum AST activity were observed among groups (P>0.05). Serum ALP activity in all experimental groups was significantly lower than that in the control group (P<0.05), with the 10% MLM group showing the lowest serum ALP activity, representing a 58.6% reduction compared with the control group.

Serum TP and GLO contents in all experimental groups were higher than those in the control group, with the 10% MLM and 20% FMLM groups showing extremely significant increases in serum TP and GLO contents (P<0.01). The 10% FMLM group showed a significant increase in serum GLO content (P<0.05). Serum TP content in the 10% MLM group was extremely significantly different from that in the 20% MLM group (P<0.01) and significantly different from that in the 5% MLM and 5% FMLM groups (P<0.05). Serum GLO content in the 10% MLM group was extremely significantly different from that in the 5% and 20% MLM groups and the 5% FMLM group (P<0.01) and significantly different from that in the 10% FMLM group (P<0.05). The GLO content in the 20% FMLM group was significantly different from that in the 20% MLM group (P<0.05). Serum ALB content in all experimental groups showed no significant difference compared with the control group (P>0.05), though the 20% FMLM group showed the highest serum ALB content, representing an 11.2% increase compared with the control group. Serum ALB content in the 20% FMLM group was significantly different from that in the 20% MLM and 5% FMLM groups (P<0.05). The serum A/G in the 5% MLM group was significantly lower than

that in the control group (P<0.05), while other experimental groups showed extremely significantly lower A/G than the control group (P<0.01). The serum A/G in the 10% MLM group was extremely significantly lower than that in all other groups (P<0.01).

Serum GLU content in all experimental groups showed no significant difference compared with the control group (P>0.05), with the 10% FMLM group showing the highest serum GLU content and the 10% MLM group showing the lowest, with a significant difference between these two groups (P<0.05). No significant differences in serum UN and UA contents were observed among groups (P>0.05).

Compared with the control group, the 20% MLM group showed significantly reduced serum TC content (P<0.05). The 5% and 20% MLM groups showed significantly lower serum TG content than the control group (P<0.05), while the 5% FMLM group showed significantly higher serum TG content than other experimental groups (P<0.05). Dietary MLM and FMLM tended to increase serum HDL-C content, though no significant differences were observed among groups (P>0.05).

Table 3 Effects of Dietary MLM and FMLM Levels on Serum Biochemical Indexes of Huxu Chickens

Item	MLM Level/%	FMLM Level/%	Control Group
ALT/(U/L)	25.25±0.85	28.00±1.73	25.25±1.49
	23.25±0.63	26.75±2.59	22.75±0.25
	25.50±1.85		
AST/(U/L)	170.00±11.20	178.50±4.35	188.75±23.77
	173.25±5.53	183.50±18.34	181.00±15.72
	190.00±8.36		
ALP/(U/L)	869.50±82.74	415.25±68.99	360.00±13.06
	489.25±70.67	653.00±103.09	416.50±75.09
	520.50±82.54		
TP/(g/L)	40.58±1.48	43.98±1.11	50.98±3.12
	41.93±1.69	43.28±0.86	44.90±1.36
	49.78±3.40		
ALB/(g/L)	16.48±0.38	16.55±0.38	16.90±1.08
	15.63±0.46	15.85±0.52	16.53±0.68
	18.33±1.35		
GLO/(g/L)	24.10±1.12	27.43±0.84	34.08±2.05
	26.30±1.28	27.43±0.49	28.38±0.85
	31.45±2.41		
A/G	0.69±0.02	0.61±0.02	0.50±0.01
	0.60±0.020	0.58±0.02	0.58±0.02
	0.59±0.04		
GLU/(mmol/L)	13.89±0.36	13.60±0.49	12.67±0.15
	13.54±0.24	13.97±0.60	14.64±0.79
	13.62±0.58		

Item	MLM Level/%	FMLM Level/%	Control Group
UN/(mmol/L)	0.33±0.03	0.27±0.02	0.33±0.04
	0.27±0.02	0.33±0.04	0.34±0.04
	0.30±0.03		
UA/(μmol/L)	172.00±45.50	163.50±13.00	161.75±11.91
	178.25±32.75	177.25±26.70	176.00±14.01
	160.75±4.01		
TC/(mmol/L)	3.73±0.13	3.22±0.30	3.59±0.18
	3.06±0.20	3.26±0.25	3.41±0.30
	3.32±0.13		
TG/(mmol/L)	1.19±0.36	0.46±0.01	0.52±0.17
	0.44±0.03	1.24±0.49	0.51±0.04
	0.51±0.05		
HDL-C/(mmol/L)	2.74±0.63	3.34±0.22	3.10±0.14
	3.12±0.15	2.72±0.46	3.23±0.10
	3.26±0.35		

2.3 Effects of Dietary MLM and FMLM Levels on Antioxidant Indexes of Huxu Chickens

2.3.1 Effects on Serum Antioxidant Indexes As shown in Table 4 , dietary MLM and FMLM tended to increase serum T-AOC in Huxu chickens, with the 10% MLM group showing significantly higher serum T-AOC than other groups ($P<0.05$). Compared with the control group, all experimental groups showed increased serum GSH-Px activity, with the 20% FMLM group showing the highest activity, extremely significantly different from the control group ($P<0.01$) and representing an 18.3% increase. Serum GSH-Px activity in the 20% FMLM group was significantly higher than that in the 10% and 20% MLM groups and the 5% and 10% FMLM groups ($P<0.05$). Serum CAT activity in the 10% MLM group was extremely significantly lower than that in the control group ($P<0.01$), while other MLM and FMLM level groups showed no significant differences ($P>0.05$). Serum CAT activity in the 10% MLM group was extremely significantly lower than that in the 5% MLM and 10% FMLM groups ($P<0.01$) and significantly lower than that in the 5% and 20% FMLM groups ($P<0.05$). Serum SOD activity in the 5%, 10%, and 20% MLM groups increased by 11.0%, 7.9%, and 9.1%, respectively, compared with the control group, while serum SOD activity in the 10% FMLM group increased by 3.6%, though no significant differences were observed among groups ($P>0.05$). No significant differences in serum MDA content were observed among experimental groups compared with the control group ($P>0.05$), though serum MDA content in the 5% FMLM group was significantly higher than that in the 10% FMLM group ($P<0.05$).

Table 4 Effects of Dietary MLM and FMLM Levels on Serum Antioxidant Indexes of Huxu Chickens

Item	MLM Level/%	FMLM Level/%	Control Group
T-AOC (U/mL)	7.23±0.93 10.85±1.54 9.23±0.66	8.39±0.80 10.48±2.33	14.93±1.20 7.19±1.32
GSH-Px (U/mL)	412.63±14.66 432.65±5.84 488.25±9.72	449.26±10.87 432.27±25.32	431.66±13.72 439.16±23.32
CAT (U/mL)	6.62±0.34 5.30±0.57 5.89±0.24	6.84±0.67 5.43±0.85	3.80±0.44 6.64±0.49
SOD (U/mL)	111.25±7.11 121.34±3.29 111.60±7.40	123.51±5.80 108.02±8.12	120.00±1.79 115.23±4.51
MDA (nmol/mL)	7.98±0.05 7.72±0.18 8.34±0.32	7.82±0.86 8.89±0.65	7.47±0.75 7.01±0.66

2.3.2 Effects on Liver Antioxidant Indexes As shown in Table 5 , liver T-AOC in the 10% MLM group was significantly increased compared with the control group ($P<0.05$). Liver T-AOC in the 10% MLM group was significantly different from that in the 10% and 20% FMLM groups and the 5% MLM group ($P<0.05$). Compared with the control group, liver CAT activity was reduced in all experimental groups, with the 10% MLM group showing significantly lower liver CAT activity ($P<0.05$). Liver CAT activity in the 10% MLM group was significantly different from that in the 5% MLM and 10% FMLM groups ($P<0.05$). No significant differences were observed in liver GSH-Px and SOD activities or MDA content among groups ($P>0.05$).

Table 5 Effects of Dietary MLM and FMLM Levels on Liver Antioxidant Indexes of Huxu Chickens

Item	MLM Level/%	FMLM Level/%	Control Group
T-AOC (U/mg)	0.98±0.13 1.30±0.21 1.08±0.12	0.84±0.16 1.17±0.21	1.64±0.16 0.88±0.17
GSH-Px (U/mg)	149.92±8.62 150.67±9.97 162.60±13.97	153.3±5.02 141.39±8.41	147.31±13.83 142.47±10.52
CAT (U/mg)	23.82±2.21 17.98±1.93 19.19±1.29	23.24±3.00 17.51±3.04	12.76±2.18 21.26±2.11
SOD (U/mg)	210.66±17.83 218.77±16.31 191.00±7.58	217.27±4.84 184.53±15.05	211.84±18.23 193.74±10.97

Item	MLM Level/%	FMLM Level/%	Control Group
MDA (nmol/mg)	1.95±0.17 1.82±0.18 1.84±0.13	1.80±0.18 1.92±0.13	1.70±0.14 1.58±0.22

3.1 Effects of Dietary MLM and FMLM Levels on Growth Performance of Huxu Chickens

Research findings on the effects of dietary MLM on broiler growth performance have been inconsistent. Wu et al. reported that dietary inclusion of 2%-6% MLM significantly improved daily gain and survival rate in yellow-feathered broilers. Simol et al. found that dietary inclusion of 30% MLM had no adverse effects on feed intake, growth performance, or mortality. However, Fan et al. reported that dietary inclusion of 5% or 10% MLM significantly reduced broiler body weight, with greater effects at higher inclusion levels, consistent with our results. Compared with MLM, dietary FMLM improved the effects on growth performance. Has et al. reported that dietary inclusion of 10% or 20% MLM and 10% or 20% FMLM fermented with rumen liquor resulted in lower final body weight with increasing inclusion levels, consistent with our results. However, their 10% rumen liquor FMLM group showed a trend toward lower final body weight compared with the 10% MLM group, which differs from our finding that 10% FMLM significantly improved average daily gain compared with 10% MLM. This discrepancy may be because the FMLM in our experiment was produced through strain selection and fermentation process optimization, resulting in better fermentation effects and degradation of anti-nutritional factors than direct rumen liquor fermentation.

The negative effects of dietary MLM on growth performance in this experiment were related to the high content of crude fiber and anti-nutritional factors such as tannins and lectins in mulberry leaves. As shown in Table 1, dietary crude fiber content increased with increasing MLM and FMLM levels. Excessive crude fiber is difficult for animals to digest and absorb, reducing nutrient concentration and feed digestibility, increasing satiety, and decreasing feed intake, thereby affecting growth performance. After fermentation, microbial enzymes decomposed cellulose, protein, and other indigestible macromolecules in mulberry leaves into easily absorbable small molecules, and may have transformed anti-nutritional factors such as tannins, thereby reducing their effects on animal growth performance. Jiang et al. reported that yeast significantly reduced trypsin inhibitors and phytic acid in soybean meal, while *Trichoderma* significantly reduced crude fiber content. Fu et al. used mixed solid-state fermentation of rapeseed cake with *Bacillus subtilis*, *Aspergillus niger*, and *Geotrichum candidum*, resulting in more than threefold increases in small peptides and free amino acids and a 93.4% reduction in total glucosinolate content, with complete degradation of isothiocyanates.

3.2 Effects of Dietary MLM and FMLM Levels on Serum Biochemical Indexes of Huxu Chickens

Serum biochemical indexes are important indicators for assessing animal health status, oxidative metabolism, and physiological functions. Under normal conditions, ALT and AST are abundant in liver cells and diffuse into serum in large quantities when liver cells are damaged, causing increased serum ALT and AST activities. Serum ALP is an enzyme with high activity under alkaline conditions, synthesized and secreted by liver cells and excreted through bile, with a half-life of 3 days. Under normal conditions, serum ALP activity is very low, but increases significantly when liver and gallbladder diseases occur. This study found that dietary MLM and FMLM enhanced liver function in Huxu chickens, primarily due to active components such as flavonoids and -aminobutyric acid in mulberry leaves that improve liver function.

Serum TP is the sum of serum ALB and GLO. Serum TP and ALB contents reflect nutritional status and protein absorption and metabolism, with increased contents indicating enhanced protein anabolism. GLO is a protein involved in immune responses, and its content is an important indicator for evaluating immune status, particularly humoral immunity. Our results showed that ALB content in all experimental groups was not significantly different from that in the control group, but GLO content increased, resulting in higher serum TP content and significantly or extremely significantly lower A/G. Increased GLO content indicates elevated antibody levels, suggesting that dietary MLM and FMLM enhanced immune function in Huxu chickens. The 20% FMLM group showed an 11.2% increase in ALB content compared with the control group, indicating that high-dose FMLM may facilitate protein utilization.

Most energy required for animal life activities is derived from GLU, which is normally maintained at certain levels to ensure energy supply. Both excessively high and low GLU levels affect the body. Our results showed no significant differences in serum GLU content among experimental groups compared with the control group, indicating that dietary MLM and FMLM had little effect on blood glucose levels in Huxu chickens, consistent with Zhou et al.' s findings that 8% ensiled mulberry leaves had no significant effect on serum GLU content in finishing steers. The significantly higher serum GLU content in the 10% FMLM group compared with the 10% MLM group may be due to increased digestible carbohydrates and other nutrients after fermentation, though the lack of further increase in the 20% FMLM group may be influenced by other components in FMLM and absorption metabolism.

UN is the end product of protein hydrolysis and amino acid metabolism, while UA is primarily generated from protein and nucleic acid degradation. Reduced serum UN or UA content indicates slowed protein catabolism and improved efficiency of amino acid synthesis into protein. Serum UN and UA contents are negatively correlated with dietary protein level and amino acid balance and are important indicators for evaluating dietary protein status and amino acid

balance, directly reflecting nutritional status and metabolic levels in avian diets. This study found that serum UA content in the 10% MLM and 20% FMLM groups was 6.0% and 6.5% lower than that in the control group, respectively, with higher serum TP and ALB contents, indicating that dietary inclusion of 10% MLM and 20% FMLM promoted protein synthesis and deposition.

Serum cholesterol (CHO), TC, and HDL-C are major components of blood lipids, and their contents reflect lipid metabolism status. This study demonstrated that dietary MLM and FMLM effectively reduced serum TC and TG contents and increased serum HDL-C content, consistent with results from Park et al., Panja, and Islam in broiler studies. The effects of MLM and FMLM on blood lipids in Huxu chickens are primarily related to various lipid-lowering bioactive substances in mulberry leaves. In addition to polysaccharides and flavonoids with lipid-lowering functions, plant sterols in mulberry leaves competitively inhibit CHO in chylomicrons, preventing absorption and reducing serum CHO content. Stigmasterol and sitosterol in mulberry leaves also effectively inhibit intestinal CHO absorption. Additionally, linoleic acid, an unsaturated fatty acid in mulberry leaves, promotes CHO and bile acid excretion, thereby reducing serum CHO content. Furthermore, increased dietary crude fiber levels from MLM and FMLM inclusion contributed to reduced blood lipid levels.

3.3 Effects of Dietary MLM and FMLM Levels on Antioxidant Indexes of Huxu Chickens

Animals continuously produce free radicals during oxidative metabolism, which are highly oxidizing. Free radical scavenging primarily depends on a complete antioxidant defense system that provides protective or blocking control to protect tissues and cells from oxidative damage. Antioxidant substances in the body mainly include T-AOC, GSH-Px, SOD, CAT, and MDA.

T-AOC is a comprehensive indicator measuring antioxidant system functional status, reflecting total antioxidant capacity. SOD is the primary substance for scavenging free radicals, the main scavenger of superoxide anion radicals (O), catalyzing O to generate hydrogen peroxide (H O), which can then be decomposed by CAT into O and H O. MDA is the end product of lipid peroxidation after free radical attack on lipids and is an important indicator of lipid peroxidation intensity and membrane system damage. GSH-Px specifically catalyzes the reduction of H O and other hydroperoxides by reduced glutathione, thereby eliminating intracellular H O and lipid free radicals to protect cell membrane structure and function.

This study demonstrated that dietary MLM and FMLM regulated antioxidant capacity, with the 10% MLM group significantly increasing serum and liver T-AOC and significantly reducing serum and liver CAT activity, while the 20% FMLM group extremely significantly increased serum GSH-Px activity. Li reported that dietary MLM increased serum T-AOC and SOD activity and reduced MDA content in meat sheep, similar to our results. Andallu et al. added

25% MLM to the diet of streptozotocin-induced diabetic mice for 8 weeks, finding that erythrocyte GSH-Px and SOD activities increased by 151% and 106%, respectively, while CAT activity significantly decreased, consistent with our findings.

The antioxidant capacity enhancement by mulberry leaves is attributed to various bioactive substances that exert antioxidant effects through two primary mechanisms: increasing endogenous antioxidant enzyme activities and possessing intrinsic antioxidant properties. Chen et al. found that mulberry leaf flavonoids increased liver SOD activity and reduced liver MDA content in diabetic mice. Li et al. reported that mulberry leaf total flavonoids increased heart SOD activity and reduced MDA content in mice after exhaustive exercise, thereby reducing oxygen radical-induced myocardial cell damage. Numerous in vitro antioxidant studies have demonstrated that flavonoids and polysaccharides in mulberry leaves are natural strong antioxidants that can scavenge superoxide anion radicals, oxygen radicals, lipid peroxides, H O , and hydroxyl radicals that cannot be eliminated by enzymes. However, the antioxidant capacity of MLM decreased to some extent after fermentation, possibly because microbial action transformed some antioxidant functional substances during fermentation, such as decomposition of mulberry leaf polysaccharides into small molecules that lost antioxidant activity. Our preliminary fermentation process optimization results also indicated that flavonoid content decreased after MLM fermentation.

Conclusions

1. Dietary inclusion of MLM and FMLM reduced growth performance in Huxu chickens, with performance decreasing as inclusion levels increased, though FMLM showed improved effects compared with MLM.
2. Dietary MLM extremely significantly reduced serum ALP activity, increased serum TP and GLO contents, significantly increased A/G, reduced serum TC and TG contents, and improved serum and liver antioxidant capacity. FMLM showed similar effects.

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