

## Effects of Feed-Grade Mannanase Transgenic Maize on Growth Performance, Slaughter Performance, and Serum Physiological and Biochemical Indices in Broiler Chickens: Postprint

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### Abstract

This experiment was conducted to investigate the effects of mannanase transgenic corn on growth performance, slaughter performance, and serum physiological and biochemical indices in broiler chickens. A total of 480 1-day-old Arbor Acres (AA) broiler chickens were randomly allocated into four groups, with six replicates per group and 20 birds per replicate. The four groups were arranged as follows: Group I served as the control group receiving non-transgenic corn without mannanase supplementation; Group II received non-transgenic corn supplemented with low-dose microbial-derived mannanase at an activity of 500 U/kg; Group III received low-dose mannanase transgenic corn with mannanase activity of 500 U/kg; Group IV received high-dose mannanase transgenic corn with mannanase activity of 5,000 U/kg. The experimental period lasted 42 days. The results showed that, compared with the control group, Groups III and IV exhibited significantly higher average daily gain in 42-day-old broilers ( $P < 0.05$ ), and Group IV had a significantly lower feed-to-gain ratio ( $P < 0.05$ ). The dressing percentage and eviscerated yield percentage of 42-day-old broilers in Group IV were significantly higher than those in Group II and the control group ( $P < 0.05$ ). There were no significant differences in blood physiological and biochemical indices of 21- and 42-day-old broilers among Groups II, III, and IV compared with the control group ( $P > 0.05$ ). Therefore, dietary supplementation with mannanase transgenic corn can improve growth performance and slaughter performance in the tested broiler chickens, but has no significant effect on blood physiological and biochemical indices.

## Full Text

# Effects of Feed-Used Mannanase Transgenic Corn on Growth Performance, Carcass Performance and Serum Physiological and Biochemical Indexes of Broilers

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## Abstract

This experiment was conducted to investigate the effects of mannanase transgenic corn on growth performance, carcass performance, and serum physiological and biochemical indexes of broilers. A total of 480 one-day-old Arbor Acres (AA) broilers were randomly allocated to 4 groups, with 6 replicates per group and 20 broilers per replicate. The four groups were as follows: Group 1, a control group fed non-transgenic corn without mannanase supplementation; Group 2, fed non-transgenic corn supplemented with low-dose microbial mannanase at an activity of 500 U/kg; Group 3, fed low-dose mannanase transgenic corn with enzyme activity of 500 U/kg; and Group 4, fed high-dose mannanase transgenic corn with enzyme activity of 5,000 U/kg. The trial lasted for 42 days. The results showed that compared with the control group, Groups 2 and 3 exhibited significantly higher average daily gain in 42-day-old broilers ( $P < 0.05$ ), and Group 4 showed a significantly lower feed-to-gain ratio ( $P < 0.05$ ). The slaughter percentage and eviscerated yield percentage of 42-day-old broilers in Group 4 were significantly higher than those in Group 1 and the control group ( $P < 0.05$ ). No significant differences were observed in blood physiological and biochemical indexes among Groups 2, 3, and 4 at 21 and 42 days of age compared with the control group ( $P > 0.05$ ). These findings indicate that dietary supplementation with mannanase transgenic corn can improve growth and carcass performance in broilers without significantly affecting serum physiological and biochemical indexes.

**Keywords:** mannanase transgenic corn; carcass performance; growth performance; broilers

-Mannan is an antinutritional factor in feed that, as a component of plant cell walls, can bind or trap nutrients within cells along with other non-starch polysaccharides, increasing the viscosity of digestive tract contents and affecting nutrient digestion and absorption. Additionally, -mannan can influence insulin and insulin-like growth factor-1 secretion and affect livestock growth performance. -Mannanase is a widely used feed enzyme preparation. Monogastric animals have limited ability to digest plant cell walls in feed, and supplementation with -mannanase can effectively degrade -mannan, reduce its antinutritional effects, increase glucose absorption rates, promote carbohydrate metabolism, and sig-

nificantly improve dietary energy utilization. This enzyme can promote animal growth, enhance disease resistance, and reduce feed costs. Like other widely used industrial feed enzymes,  $\alpha$ -mannanase currently applied in feed is produced through large-scale fermentation. However, transgenic crops provide a new approach for producing feed-grade  $\alpha$ -mannanase. Introducing exogenous genes into crops to enable natural expression and developing them as feed transgenic crops can avoid microbial fermentation and supplementation processes when used as animal feed, thereby achieving green enzyme production with high application prospects and social, economic, and ecological benefits. As a major feed grain, corn offers greater advantages than other plants as a bioreactor for feed enzymes, and the successful development of phytase corn has provided a basis and example for further development of transgenic feed. Xu et al. obtained mannanase transgenic corn through genetic engineering and found that the exogenous  $\alpha$ -mannanase gene could be efficiently expressed and stably inherited in transgenic corn kernels, demonstrating potential for feed industry applications. Safety evaluation through livestock animals provides an effective basis for determining whether transgenic crops produce unexpected effects when used as feed ingredients. The effects of diets on nutrition-related performance and indicators in test animals are important components of feed safety evaluation for genetically modified organisms. The aforementioned feed safety evaluation of mannanase transgenic corn has not yet been conducted. This study aims to evaluate the effects of mannanase transgenic corn on broiler growth performance, carcass performance, and blood physiological and biochemical indexes to obtain preliminary feed safety evaluation results, which will provide a basis for its industrialization process.

### 1.1 Experimental Materials and Diet Design

Mannanase transgenic corn seeds used in the experiment were provided by the Feed Research Institute of the Chinese Academy of Agricultural Sciences, with mannanase activity of approximately 10,000 U/kg in the seeds. A total of 480 one-day-old Arbor Acres (AA) broilers were randomly divided into 4 groups with 6 replicates per group and 20 broilers per replicate. The four groups were arranged as follows: Group 1 was a control group fed non-transgenic corn without mannanase supplementation; Group 2 was fed non-transgenic corn supplemented with low-dose microbial mannanase at an activity of 500 U/kg; Group 3 was fed low-dose mannanase transgenic corn with enzyme activity of 500 U/kg; and Group 4 was fed high-dose mannanase transgenic corn with enzyme activity of 5,000 U/kg. The experimental period was divided into two phases: 1–21 days and 22–42 days of age. All groups were fed diets with consistent nutrient levels at each stage. Broilers were allowed free access to mash feed and water and were raised in cages. The experiment was conducted at the Nankou Experimental Base of the Feed Research Institute, Chinese Academy of Agricultural Sciences, with other routine management procedures followed according to standard practices.

## 1.2 Experimental Diets

Experimental diets were formulated according to the nutrient requirements for broilers in the Chinese “Feeding Standard of Chickens” (NY/T 33-2004), based on measured nutrient contents in corn. The composition and nutrient levels of experimental diets for 1-21 days and 22-42 days of age are shown in and , respectively.

**Table 1** Composition and nutrient levels of experimental diets at 1-21 days of age (dry matter basis)

Item	Non-transgenic corn group	Transgenic corn group
	Group	Group
Ingredients		
Transgenic corn	-	-
Non-transgenic corn	55.00	55.00
Plant oil	3.00	3.00
Soybean meal	32.00	32.00
Cottonseed meal	3.00	3.00
Rapeseed meal	2.00	2.00
Palm meal	2.00	2.00
NaCl	0.30	0.30
CaHPO	1.20	1.20
Limestone	1.00	1.00
Lys · HCl	0.30	0.30
DL-Met	0.20	0.20
L-Thr	0.10	0.10
Premix <sup>1</sup>	1.00	1.00
<b>Total</b>	<b>100.00</b>	<b>100.00</b>
Nutrient levels <sup>2</sup>		
ME (MJ/kg)	12.50	12.50
CP	21.00	21.00
EE	4.50	4.50
Ca	1.00	1.00
TP	0.68	0.68
AP	0.45	0.45
Lys	1.15	1.15
Met	0.50	0.50
Met+Cys	0.85	0.85

<sup>1</sup>Premix provided per kg of diet: VA 10,000 IU, VD 2,000 IU, VE 10 IU, VB 1.8 mg, VB 4.0 mg, VB 0.71 mg, VK 2.5 mg, pantothenate 11.0 mg, folic acid 0.5 mg, biotin 0.12 mg, choline 1,000 mg, Zn 40.0 mg, Mn 60.0 mg, Fe 80.0 mg, Cu 8.0 mg, I 0.35 mg, Se 0.15 mg, aureomycin 50 mg.

<sup>2</sup>Nutrient levels were calculated values. The same as Table 2.

**Table 2** Composition and nutrient levels of experimental diets at 22-42 days of age (dry matter basis)

Item	Non-transgenic corn group	Transgenic corn group
	Group	Group
Ingredients		
Transgenic corn	-	-
Non-transgenic corn	62.00	62.00
Plant oil	4.00	4.00
Soybean meal	26.00	26.00
Cottonseed meal	3.00	3.00
Rapeseed meal	2.00	2.00
Palm meal	1.00	1.00
NaCl	0.30	0.30
CaHPO	1.00	1.00
Limestone	0.90	0.90
Lys · HCl	0.25	0.25
DL-Met	0.15	0.15
L-Thr	0.05	0.05
Premix	1.00	1.00
<b>Total</b>	<b>100.00</b>	<b>100.00</b>
Nutrient levels		
ME (MJ/kg)	12.90	12.90
CP	19.00	19.00
EE	5.00	5.00
Ca	0.90	0.90
TP	0.65	0.65
AP	0.40	0.40
Lys	1.00	1.00
Met	0.38	0.38
Met+Cys	0.70	0.70

Premix provided per kg of diet: VA 8,000 IU, VD 1,500 IU, VE 8 IU, VB 1.5 mg, VB 3.0 mg, VB 0.35 mg, VK 2.0 mg, pantothenate 10.0 mg, folic acid 0.3 mg, biotin 0.10 mg, choline 750 mg, Zn 40.0 mg, Mn 60.0 mg, Fe 80.0 mg, Cu 8.0 mg, I 0.35 mg, Se 0.15 mg, aureomycin 50 mg.

### 1.3 Growth Performance and Carcass Performance Measurement

**Growth performance:** During the experiment, feed intake and mortality were recorded in detail by replicate. At 21 and 42 days of age, after 12 hours of fasting, broilers were weighed by replicate to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

**Carcass performance:** At 42 days of age, 3 broilers with body weight close to

the replicate average were randomly selected from each replicate for slaughter and sampling to determine slaughter percentage, eviscerated yield percentage, breast muscle percentage, leg muscle percentage, and abdominal fat percentage. Measurements were conducted according to “Poultry Production Performance Terminology and Measurement Methods” (NY/T 823-2004).

#### 1.4 Serum Physiological and Biochemical Index Detection

At 21 and 42 days of age after 12 hours of fasting, 3 broilers with body weight close to the replicate average were selected from each replicate cage. Blood was collected from the wing vein using heparinized anticoagulant tubes for whole blood and coagulant tubes for serum. A KX-21 automatic hematology analyzer (SYSMEX, Japan) was used to determine white blood cell count (WBC), red blood cell count (RBC), hemoglobin content (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). For serum samples, blood in coagulant tubes was centrifuged at 3,000 r/min for 6 minutes, and a HITACHI 7600 automatic biochemical analyzer (HITACHI, Japan) was used to determine aspartate aminotransferase, glutamyltransferase, cholinesterase, and lactate dehydrogenase activities, as well as total phosphorus, albumin, urea nitrogen, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, calcium, and glucose concentrations.

#### 1.5 Statistical Analysis

Data were analyzed using one-way ANOVA procedure in SPSS 16.0 software for significance testing. Data are expressed as “mean  $\pm$  standard error.” Differences were considered significant at  $P < 0.05$ .

## 2 Results and Analysis

### 2.1 Effects of Mannanase from Different Sources on Broiler Growth Performance

As shown in , at 21 days of age, no significant differences were observed among the four groups in ADG, ADFI, or F/G ( $P > 0.05$ ). At 42 days of age, ADG in Groups , , and was significantly higher than in the control group ( $P < 0.05$ ), with Group showing a 12% increase compared to the control and also differing significantly from Groups and ( $P < 0.05$ ). Groups and increased ADG by approximately 7% compared to the control. For ADFI, no significant difference was observed between Groups and ( $P > 0.05$ ), but both were significantly higher than the control and Group . Regarding F/G, Group was significantly superior to all other groups ( $P < 0.05$ ), showing a 12.3% difference from the control and 7.9% and 8.9% differences from Groups and , respectively. No significant difference in F/G was observed between Groups and ( $P > 0.05$ ), but Group reduced F/G by 4.1% compared to the control ( $P < 0.05$ ). Overall,

supplementation with both microbial and transgenic mannanase significantly improved growth performance.

**Table 3** Effects of mannanase addition from different sources on broiler growth performance

Item	Group	Group	Group	Group	P-value
<b>1-21 days of age</b>					
ADG (g/bird)	30.13±0.21	31.05±0.31	30.23±0.12	30.29±0.35	
ADFI (g/bird)	52.59±0.25	53.02±0.26	52.82±0.23	52.91±0.66	
F/G	1.75±0.03	1.71±0.02	1.75±0.04	1.75±0.03	
<b>1-42 days of age</b>					
ADG (g/bird)	55.35±0.32c	59.26±0.87b	58.99±0.76b	62.01±0.38a	
ADFI (g/bird)	126.34±1.05b	129.78±1.57a	130.37±1.81a	125.88±0.47b	
F/G	2.28±0.04a	2.19±0.05b	2.21±0.05ab	2.03±0.04c	

In the same row, values with different lowercase letter superscripts indicate significant differences ( $P < 0.05$ ), while values with no or the same letter superscripts indicate no significant difference ( $P > 0.05$ ). The same applies to Table 4.

## 2.2 Effects of Mannanase from Different Sources on Broiler Carcass Performance

No lesions or obvious damage were observed in carcasses of 42-day-old broilers. As shown in , Group exhibited significantly higher slaughter percentage and eviscerated yield percentage than Group and the control group ( $P < 0.05$ ), increasing by 1.3% and 2.7% compared to the control, respectively. The control group showed significantly lower slaughter and eviscerated yield percentages than other groups ( $P < 0.05$ ). However, no significant differences were observed between the control group and other groups in eviscerated yield or carcass percentage ( $P > 0.05$ ). For abdominal fat percentage, Group was significantly higher than all other groups ( $P < 0.05$ ), while no significant differences were observed among the control, Group , and Group ( $P > 0.05$ ). Group was 23.8% higher than the control and 19.7% and 19.0% higher than Groups and , respectively. For breast muscle percentage, Groups and increased by 11.5% and 12.3% compared to the control ( $P < 0.05$ ), and by 6.6% and 7.3% compared

to Group (P<0.05). For leg muscle percentage, Groups and increased by 10.8% and 14.5% compared to the control (P<0.05), with no significant differences among Groups , , and (P>0.05).

**Table 4** Effects of mannanase addition from different sources on carcass performance of 42-day-old broilers

Item	Group	Group	Group	Group	P-value
Slaughter percentage (%)	90.15±0.11c	90.88±0.19b	91.33±0.13a	91.35±0.29a	
Carcass percentage (%)	90.58±0.30	88.08±9.00	91.09±0.88	91.68±1.97	
Eviscerated yield (g)	1,626±155	1,500±154	1,447±112	1,585±231	
Eviscerated yield percentage (%)	70.67±0.18c	71.92±0.62b	72.1±0.16b	72.61±0.43a	
Abdominal fat percentage (%)	1.47±0.16b	1.82±0.15a	1.52±1.17b	1.53±0.23b	
Breast muscle percentage (%)	24.46±0.54b	27.28±0.41a	25.59±0.63b	27.46±0.99a	
Leg muscle percentage (%)	20.16±0.45b	22.34±0.36a	21.71±0.57ab	23.10±0.96a	

### 2.3 Effects of Mannanase from Different Sources on Broiler Blood Physiological Indexes

As shown in , supplementation with either microbial or transgenic corn-derived mannanase did not significantly affect WBC, RBC, HGB, HCT, MCV, MCH, or MCHC in broilers at 21 and 42 days of age (P>0.05).

**Table 5** Effects of mannanase addition from different sources on blood physiological indexes of broilers

Item	Group	Group	Group	Group	P-value
<b>21</b>					
<b>days</b>					
<b>of age</b>					
WBC	276.33±3.67	292.20±2.24	294.75±2.05	285.90±2.19	
(×10 <sup>9</sup> /L)					
RBC	2.27±0.05	3.02±0.17	2.85±0.08	2.93±0.17	
(×10 <sup>12</sup> /L)					
HGB	111±4	143±7	136±6	135±8	
(g/dL)					
HCT	0.35±0.01	0.39±0.02	0.37±0.01	0.37±0.01	
(%)					
MCV	131.77±2.39	129.50±1.04	130.50±0.20	127.43±1.94	
(fL)					
MCH	48.30±1.58	47.30±0.55	47.85±0.95	46.20±0.26	
(pg)					
MCHC	366.67±5.69	365.33±3.18	366.50±6.50	362.67±5.04	
(g/L)					
<b>42</b>					
<b>days</b>					
<b>of age</b>					
WBC	259.63±2.18	269.17±6.76	260.85±6.05	254.88±8.72	
(×10 <sup>9</sup> /L)					
RBC	2.48±0.11	2.39±0.14	2.14±0.16	2.61±0.11	
(×10 <sup>12</sup> /L)					
HGB	115±1	119±4	117±1	119±1	
(g/dL)					
HCT	0.33±0.01	0.33±0.01	0.28±0.03	0.33±0.03	
(%)					
MCV	133.73±2.53	138.03±2.29	132.60±5.20	137.45±0.45	
(fL)					
MCH	46.80±1.18	49.87±1.33	48.10±1.80	49.55±0.65	
(pg)					
MCHC	350.00±2.64	361.33±3.75	357.00±4.50	360.50±3.50	
(g/L)					

In the same column, values with different lowercase letter superscripts indicate significant differences ( $P < 0.05$ ), while values with no or the same letter superscripts indicate no significant difference ( $P > 0.05$ ). The same applies to the table below.

## 2.4 Effects of Mannanase from Different Sources on Broiler Serum Biochemical Indexes

As shown in , supplementation with either microbial or transgenic corn-derived mannanase did not significantly affect aspartate aminotransferase, glutamyltransferase, cholinesterase, or lactate dehydrogenase activities, nor total phosphorus, albumin, urea nitrogen, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, calcium, or glucose concentrations in broiler serum at 21 and 42 days of age ( $P>0.05$ ).

**Table 6** Effects of mannanase addition from different sources on serum biochemical indexes of broilers

Item	Group	Group	Group	Group	P-value
<b>21</b>					
<b>days</b>					
<b>of age</b>					
TP (mmol/L)	15.20±1.96	14.83±1.85	15.25±1.65	14.33±0.71	
ALB (g/L)	24.94±1.33	24.58±2.87	23.50±0.64	23.43±0.47	
UN (mmol/L)	13.88±0.82	14.93±1.18	13.23±0.17	13.65±0.83	
CHO (mmol/L)	1.04±0.18	1.15±0.18	1.03±0.09	0.84±0.08	
TG (mmol/L)	2.84±0.29	3.03±0.21	2.78±0.17	2.73±0.24	
HDL (mmol/L)	0.52±0.14	0.54±0.07	0.53±0.57	0.55±0.11	
LDL (mmol/L)	0.41±0.07	0.38±0.04	0.41±0.06	0.38±0.03	
LDH (U/L)	2,056.8±297.02	2,507.0±336.62	2,737.7±627.22	2,035.3±222.2	
GGT (U/L)	775.00±41.04	808.17±86.45	790.75±58.92	785.83±47.62	
<b>42</b>					
<b>days</b>					
<b>of age</b>					
TP (mmol/L)	18.33±1.40	20.67±1.02	21.17±1.40	24.33±2.20	
ALB (g/L)	33.90±1.35	34.73±1.45	36.36±0.82	33.31±1.51	
UN (mmol/L)	15.43±0.45	16.70±0.33	16.03±0.48	16.16±0.87	
CHO (mmol/L)	0.85±0.10	0.67±0.06	0.71±0.07	0.85±0.08	

Item	Group	Group	Group	Group	P-value
TG (mmol/L)	3.08±0.14	3.26±0.26	3.45±0.18	3.23±0.19	
HDL (mmol/L)	0.60±0.06	0.70±0.05	0.68±0.07	0.58±0.07	
LDL (mmol/L)	0.69±0.06	0.82±0.09	0.81±0.07	0.79±0.09	
LDH (U/L)	1,872.7±214.3	2,145.8±175.6	1,861.8±155.7	2,038.2±150.0	
GGT (U/L)	807.00±69.80	740.33±46.82	773.50±57.81	874.67±98.73	

## Discussion

Mannan is present at high levels in plant-based feed ingredients. Feed formulations in northern China are primarily corn-soybean meal and wheat-soybean meal types, with meal-based diets containing relatively high levels of mannan antinutritional factors at 1.5%-1.6%.  $\alpha$ -Mannanase primarily functions as an exogenous enzyme in livestock activities. Previous reports have shown that mannanase supplementation can significantly improve dietary energy utilization, promote animal growth performance, and reduce feed costs, making it a novel feed additive. Expressing feed enzymes in corn represents a new approach for developing nutritionally enhanced feed ingredients through transgenic technology, and feed safety evaluation of transgenic corn is an important foundation for its practical application. Previous research comparing mannanase transgenic corn with the elite inbred line Zheng 58 showed no significant differences in moisture, crude protein, crude fat, crude fiber, crude ash, nitrogen-free extract, or 14 amino acids in the kernels. This study further compared the effects of  $\alpha$ -mannanase from transgenic corn versus microbial fermentation sources on broiler growth performance, carcass performance, and blood physiological and biochemical indexes, providing a preliminary evaluation of the feeding effects and safety of mannanase transgenic corn.

Regarding growth performance, results showed that at 1-21 days of age, no significant differences were observed in ADG, ADFI, or F/G among groups. However, at 1-42 days of age, both transgenic corn-derived and microbial mannanase significantly improved broiler growth performance. Furthermore, the two groups with equivalent mannanase activity (500 U/kg) showed no significant differences in growth performance, indicating comparable growth-promoting effects between transgenic corn-derived and microbial mannanase at this dosage. The study also found that high-dose transgenic corn (5,000 U/kg) promoted ADG more significantly than the low-dose group (500 U/kg), increasing by 12% compared to the control. For ADFI, no significant difference was observed between the low-dose microbial and low-dose transgenic corn groups, but both were significantly higher than the control and high-dose transgenic corn groups, with no

significant difference between the control and high-dose group. This suggests that low-dose supplementation of both enzyme sources achieved optimal effects. For F/G, the high-dose transgenic corn group was significantly superior to all other groups, showing a 12.3% difference from the control and 7.9% and 8.9% differences from the low-dose microbial and low-dose transgenic corn groups, respectively. No significant difference in F/G was observed between the low-dose microbial and low-dose transgenic corn groups, but the low-dose microbial group was significantly lower than the control. Overall, supplementation with both microbial and transgenic mannanase improved growth performance, with the high-dose group showing more significant effects on ADG and F/G.

For carcass performance, the high-dose transgenic corn group showed significantly higher slaughter and eviscerated yield percentages than other groups, while the low-dose transgenic corn and low-dose microbial groups were significantly higher than the control. This demonstrates that mannanase supplementation in broiler diets can improve carcass performance, with high-dose (5,000 U/kg) showing greater impact and the most significant effect, differing from the control by 1.3%. These results are similar to Qiao et al., who reported that adding 0.06% feed-grade mannanase to broiler basal diets increased slaughter percentage by 1.44% compared to the control. Both low-dose microbial and high-dose transgenic corn groups significantly improved breast muscle percentage, though with some differences in effect. Compared to the control, high-dose (5,000 U/kg) transgenic corn increased breast muscle percentage by 12.3%, while low-dose (500 U/kg) microbial enzyme effectively increased it by 11.5%. However, the low-dose transgenic corn group with equivalent enzyme activity showed no difference from the control. For leg muscle percentage, both high-dose transgenic corn and low-dose microbial groups significantly improved it, but the low-dose transgenic corn group showed no significant difference from the control and also no significant difference from the high-dose transgenic corn or low-dose microbial groups. These results indicate that despite equivalent enzyme activity, low-dose transgenic corn and low-dose microbial mannanase have different effects on muscle growth. Microbial fermentation-derived pure enzyme preparation can effectively promote breast and leg muscle growth, but transgenic corn may not exert the same muscle-promoting effect as microbial enzyme due to other components, requiring further mechanistic study. For abdominal fat percentage, microbial mannanase significantly increased it, while both low-dose (500 U/kg) and high-dose (5,000 U/kg) transgenic corn groups showed no significant difference from the control. Whether this is due to other components in transgenic corn that are unfavorable for abdominal fat accumulation also requires further research. Overall, except for the increased abdominal fat percentage with microbial mannanase, supplementation with either microbial or transgenic corn-derived mannanase significantly improved or did not adversely affect broiler carcass performance, indicating that transgenic corn-derived mannanase can effectively degrade various mannan antinutritional factors in meal-based ingredients, thereby promoting broiler growth and development with effects similar to microbial enzyme. In other words, mannanase expressed in corn

kernels can effectively exert its biological activity in broilers without causing adverse effects.

Physiological and biochemical activities are fundamental life processes in organisms, and the normality of blood physiological and biochemical indexes in broilers reflects their health status. Liu et al. studied the feeding safety of glyphosate-resistant transgenic soybean meal and found that except for significant differences in alanine aminotransferase and alkaline phosphatase at 42 days of age, no significant differences were observed in other serum biochemical indexes. Domestic research on mannanase supplementation in broiler diets has mainly focused on growth performance, carcass performance, and immune function, with no reports on effects on physiological and biochemical indexes. This study demonstrated that neither low-dose microbial, low-dose transgenic corn, nor high-dose transgenic corn groups significantly affected the tested blood physiological and biochemical indexes, indicating that dietary supplementation with transgenic corn-derived mannanase does not impact broiler growth and exhibits good feeding safety.

Safety issues of transgenic foods produced from transgenic crops remain highly controversial. Similar to controversies over transgenic food safety, uncertainty persists regarding whether transgenic crops used as feed affect animal and human health. Research on feed safety evaluation of transgenic organisms will strongly promote their application in safe feed production. Overall, dietary supplementation with different doses of mannanase from transgenic corn improved broiler growth and carcass performance while not affecting blood physiological and biochemical indexes. These results undoubtedly provide valuable reference for the industrialization of mannanase transgenic corn as a functional feed ingredient.

In summary: (1) Low-dose transgenic corn-derived and microbial fermentation-derived mannanase showed similar promotion and improvement of broiler growth and carcass performance, with no adverse effects on unimproved indexes. (2) High-dose transgenic corn supplementation was more effective than low-dose, and neither high- nor low-dose transgenic corn significantly affected broiler blood physiological and biochemical indexes. Therefore, mannanase transgenic corn developed for nutritional functionality demonstrates good feeding safety.

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